VOLUME ONE

THOMAS' HEMATOPOIETIC CELL TRANSPLANTATION

5TH EDITION

Edited by
STEPHEN J. **FORMAN**
ROBERT S. **NEGRIN**
JOSEPH H. **ANTIN**
FREDERICK R. **APPELBAUM**

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Thomas' Hematopoietic Cell Transplantation

This book is dedicated to our patients and their families whose courage and trust enabled them to choose a difficult, dangerous, and sometimes unproven therapy which offered the only chance in their fight against a fatal disease. Today, many thousands of patients are alive after a successful hematopoietic cell transplantation, which would have been impossible without the fortitude of our patients, who took risks and thereby paved the way for the increasing application of this therapy. The progress in the area of clinical transplantation of hematopoietic cells would not have been possible without the dedicated, tireless work of the nurses and the supporting staff in the many transplant units around the world.

Thomas' Hematopoietic Cell Transplantation

Stem Cell Transplantation

VOLUME I

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Preface to the First Edition

The widespread application of bone marrow transplantation (BMT) to the treatment of a steadily increasing number of life-threatening hematological, oncological, hereditary, and immunological disorders is the culmination of more than four decades of research by many investigators. Early attempts in the 1950s to transplant living cells from one individual to another were carried out in the face of considerable skepticism. It was generally accepted as axiomatic that the immunological barrier to "foreign tissue" could never be overcome.

The horrors of Nagasaki and Hiroshima spurred interest in studies of the lethal effects of irradiation. It was discovered that mice given total body irradiation in doses lethal to the marrow could be protected from death by shielding the spleen or by an infusion of marrow, and that the marrow of such animals contained living cells of donor origin. These observations suggested that patients with leukemia might be given a lethal exposure of total body irradiation, which would destroy the malignant cells along with remaining normal marrow. The exposure would also destroy the immune system, making it possible to protect against lethality by a transplant of normal marrow cells.

The theory was correct, but results were disappointing. Because the procedure was both unproved and dangerous, only those patients who had no other options were considered. Except for a few patients with an identical twin donor, there were no survivors beyond a few months. Understanding of the human leukocyte antigen (HLA) system was not yet available, and little was known about the complication we now call graft-versus-host disease (GVHD). Thus, after a brief period of enthusiasm, most investigators abandoned this seemingly hopeless pursuit. Fortunately, work in animal models continued. Studies in inbred rodents defined the genetics of the major histocompatibility system and the fundamental rules of transplantation biology. Immunosuppressive drugs were developed to limit the severity of the immune reactions between donor and host. Demonstration of successful marrow transplants in the canine model using littermates matched for the major histocompatibility complex set the stage for successful transplantation of marrow between human siblings. Thus, it is clear that a long series of experimental studies in animals ultimately made human marrow transplantation possible.

By the late 1960s, much was known about the HLA system, more effective antibiotics were available, and platelet transfusions were becoming routine. Thus began the modern era of human BMT. The past 25 years have witnessed an almost exponential growth in the number of transplants being performed and the number of diseases being considered for BMT. Initially, most grafts employed marrow from an HLA-identical sibling. Autologous marrow, long known to be effective in animal systems, is now being used with increasing frequency following intensive cancer chemotherapy. Hematopoietic progenitor cells from the peripheral blood are now being used for BMT, either alone or to supplement marrow. As a result of increasing national and international cooperation, large panels of volunteer marrow donors of known HLA type are becoming available to patients whose own marrow cannot be used or who do not have a family donor.

Currently, thousands of transplants are being performed each year world-wide. With the demonstration that marrow could be transplanted and that the cure rate would be substantial, the logical step was taken to treat patients early during the course of their respective disease (i.e. in leukemia when the burden of malignant cells was relatively low and when the patient was in excellent clinical condition). With improved patient selection, development of improved tissue typing methods, availability of potent antimicrobial agents, advances in supportive care, and improved prevention of GVHD, the results of BMT have continued to improve.

Marrow transplantation is now being applied to a long list of diseases with a wide range of results depending on the disease, the type of transplant, and the stage of the disease. For some of the diseases, BMT has already proven to be the most effective therapy (e.g., some leukemias and severe aplastic anemia), whereas for others it is the only available curative treatment (e.g., thalassemia). In very rare genetic disorders, one successful BMT may establish the success of the treatment. For other more common disorders, controlled trials are necessary to define the proper role of allogeneic or autologous BMT, or therapy not involving BMT.

Only through rigorous study and long-term follow-up can novel approaches be confirmed as effective (or ineffective). For those working in the field of marrow transplantation, a source of intellectual satisfaction has been the interdisciplinary nature of the studies. A view of the wide-ranging disciplines involved can be gleaned by reading the chapter titles for this book. A successful BMT program is always a team effort. There must be cooperation between blood banks, referring physicians, radiation oncologists, immunologists, and physicians from many subspecialties. A dedicated support staff of technicians, data managers, and, above all, nurses, is crucial. The nursing team in particular is responsible for the day-to-day care of patients. Nurses not only provide the bedside management of complex protocol studies, but also bear the burden of emotional support through the difficult hospital period. They are the most readily available source of information for the patients and families day and night. Without a strong nursing team, the entire BMT program is jeopardized.

Most important are the patients who come to the transplant center with the courage to accept days, weeks, and sometimes months of discomfort in the hope of surviving a fatal disease. We must ensure that we acknowledge and respect the dignity and individuality of each patient, that we provide adequate information for informed decision making and then include patients and families in the decision process. The greatest reward for clinical investigators is to see patients reintegrated into their personal, social, and professional lives, free of their disease and its complications.

Stephen J. Forman
Karl G. Blume
E. Donnall Thomas
Summer, 1993

Preface to the Fifth Edition

Twenty-one years ago after the first edition and six years following the fourth edition of this textbook, the editors and publisher, Wiley-Blackwell, are proud to present the fifth edition of *Thomas' Hematopoietic Cell Transplantation*. The inspiration for the development of this fifth edition came from the rapid expansion of knowledge underlying transplantation biology, increased understanding of the complications of treatment, and new information related to the pathogenesis and treatment of diseases for which transplantation is utilized. The book also covers the expansion of stem-cell donor options and the genetic manipulation of cells of the hematopoietic and immune system.

In comparing this edition with the previous one, readers will find that there are new chapters focused on contributions to the field provided from basic science experiments in stem-cell biology, immunology, and tolerance. Along with these new chapters, previous contributions have been revised to reflect the deepening of our understanding of the benefits and challenges of transplantation and the impact of new therapies in the treatment of diseases for which transplant is a potentially curative therapy. In order to expand the information in each chapter, we have limited references to include only those that are most relevant.

New to the fifth edition is a companion digital edition replacing the CD ROM. The digital edition contains the entire text of the book in a searchable form and includes all the figures in a convenient downloadable format. The availability of the digital edition reflects changes in the way readers, including basic science and clinical investigators, clinicians, and nurses, access new information for both their research work and clinical care of patients.

We, the editors, also greatly appreciate the assistance of the staff at Wiley-Blackwell, who have been extremely helpful in the process of creating this new edition, now in two volumes, with a very different and modern cover presentation. The editors feel very proud to have assembled such a talented group of authors representing our colleagues who are experts in the clinical and basic science of transplantation. In addition, this edition would not have been possible without the dedicated work of our assistants, Sara E. Clark-Fuentes, Tanya Chiatovich, and, most importantly, Kimberlie Laramie. Finally, this edition honors the memory of Dr. Karl G. Blume and Dr. E. Donnall Thomas, who passed away in 2012. Their laboratory and clinical contributions helped begin and expand the field of transplantation as a curative therapy for patients suffering from leukemia and, over time, many other disorders. Both Dr. Thomas and Dr. Blume were the first two editors of the book and this edition is dedicated to them, and also to our patients and their families whose trusting partnership makes it possible to make progress and extend hope. We also honor the memory of Dr. Thomas' wife, Dottie, who we warmly consider to be the "mother" of hematopoietic cell transplantation, for her own contributions in helping Dr. Thomas and the field and for her support and editing of the first four editions of the book. They were our colleagues, mentors, and friends, and their impact will be felt in the field for many years to come.

Stephen J. Forman, MD
Robert S. Negrin, MD
Joseph H. Antin, MD
Frederick R. Appelbaum, MD

Tribute

Our textbook honors Dr. E. Donnall Thomas, the single individual most responsible for creating the subject of this book, and Karl G. Blume, the Founder of the Transplant Programs both at City of Hope and Stanford University. Don and Karl were the first editors of this textbook and both passed away in the years following the publication of the fourth edition. We include this tribute as a remembrance of each of them, given the work that they did in their rich, professional lives to develop stem cell transplantation as a curative therapy for so many patients around the world.

E. DONNALL THOMAS, M.D.
March 15, 1920–October 20, 2012

Don was born on March 15, 1920, and was the son of a solo general practitioner in a small Texas village. As a child he often recalled accompanying his father to his small office and to patients' homes and between he and his father, they spanned the period from horse and buggy house calls to our modern high-tech medicine. Don received his B.A. and M.D. from the University of Texas where he met his wife, Dottie (September 18, 1922–January 9, 2015). Besides raising three children and eight grandchildren together, Dottie was Don's partner in every aspect of his professional life, from working in the laboratory to editing manuscripts and administering grants. Anyone who has been fortunate enough to work with Don knows that if he was the father of marrow transplantation, then Dottie was, without question, the mother, and we honor her memory as our friend and colleague in this tribute, too.

Don graduated from Harvard Medical School in 1947 and completed his internship and residency at Peter Bent Brigham Hospital in Boston. It was while in medical school that Don first became interested in normal and malignant hematopoiesis. During those years, Sidney Farber was initiating his first studies of the use of antifolates to treat children with acute leukemia, and Don witnessed the very first patient to achieve a remission with this approach. He was exposed to the pioneering work of Allan Erslev and his search for erythropoietin, and most importantly, he learned from Leon Jacobson and his studies showing that shielding the spleen protected mice from the otherwise lethal effects of total body irradiation. As data emerged that a similar irradiation protection effect could be achieved by transferring bone marrow from a nonirradiated to an irradiated mouse, Don became convinced of the clinical potential of marrow transplantation.

In 1955, Don moved to Cooperstown, New York, and the Mary Imogene Bassett Hospital, a Columbia University affiliate, where he began working on marrow transplantation both in the canine model and in humans with Dr. Joseph Ferrebee. In 1957, he published the first report on human patients, showing that complete remissions of leukemia could be achieved using total body irradiation followed by infusion of marrow from an identical twin. At that time, there was little understanding of the principles of human histocompatibility and so attempts to expand these studies to patients without identical twins were uniformly unsuccessful. These failures were the stimulus for a long series of experiments conducted by Don in the canine model, showing that it was possible to expose dogs to supralethal doses of irradiation and rescue them by reinfusing their own marrow, that the marrow could be cryopreserved, and that large doses of peripheral blood could substitute for marrow. However attempts at allogeneic transplantation in this outbred species continued to fail because of graft-versus-host disease or graft rejection.

In 1963, Don moved to Seattle and the University of Washington to become the first head of the Division of Oncology. There he developed techniques for rudimentary histocompatibility typing in the dog and, by the mid-1960s, showed that by selecting matched donors and using methotrexate posttransplant, it was possible to successfully transplant marrow between matched littermates in almost every case. At the same time, based on the work of Dausset, Payne, Amos, and others, understanding of human histocompatibility dramatically increased and so, in the late 1960s, Don made the decision to return to the subject of allogeneic transplantation in humans. He began to assemble a team of physicians, nurses, and support personnel (many of whom are still part of the current Seattle Transplant Program) and obtained a program project grant from the National Cancer Institute. In November 1968, Dr. Robert Good and his colleagues carried out the first marrow transplant from a matched sibling for an infant with immunodeficiency, and in March 1969, Don performed the first matched sibling donor transplant for a patient with leukemia.

The Seattle Transplant Program was originally housed at the Seattle Public Health Hospital, but in 1972, when the hospital was faced with closure by the federal government, the Program moved to Providence Hospital. In 1975, Don and his team moved to the newly created Fred Hutchinson Cancer Research Center, a move that provided Don with increased space, resources, and scientific collaborations. That same year, he and his colleagues published their classic *New England Journal of Medicine* paper summarizing the field of allogeneic transplantation and particularly the early Seattle experience. These results demonstrated not only the feasibility of the procedure but also that there was a plateau on the survival curves following transplantation, suggesting that some of these patients were cured with this novel technique. Don continued to lead the Clinical Research Division of the Center and its transplant program until his partial retirement in 1989. He continued writing manuscripts, delivering lectures, and participating in research discussions at the Center.

Appropriately, Don received almost every possible award for his work, including the American Society of Hematology's Henry M. Stratton Award, the General Motor's Kettering Prize, the American Society of Oncology's Karnofsky Award, the Presidential Medal of Science, and, of course, the 1990 Nobel Prize in Medicine which he shared with Joseph Murray. With each award, Don always emphasized how much of his work was a team effort. He invariably mentioned the contributions of Rainer Storb, Dean Buckner, Reg Clift, Paul Neiman, Alex Fefer, and Bob Epstein, who helped form the original Seattle Transplant Team, and of Ted Graham, who moved with Don from Cooperstown to help in the animal research. Don never failed to credit the nursing and support staff who played such a critical role in these efforts and, like Karl, always acknowledged the patients and their families who were true partners in his work.

KARL G. BLUME, M.D.
April 10, 1937–January 9, 2013

Karl G. Blume was born in Germany on April 10, 1937; he received his medical education under the mentorship of Professor Georg W. Löhr at the University of Freiburg and graduated in 1963. After graduation he undertook his residency at Marburg and then held two fellowships: one at Freiburg and the other at City of Hope in 1970 and 1971, respectively. His early work was in red cell biochemistry, and he pursued his postdoctoral fellowship under Dr. Ernest Beutler.

In 1975, after discussions with Dr. E. Donnall Thomas in Seattle and with his encouragement, Dr. Beutler decided to develop an allogeneic bone marrow transplantation (BMT) program at City of Hope and recruited Karl to return to City of Hope to establish the program. The program at City of Hope was, in many ways, derived from work and discussions that Karl had with colleagues at the Fred Hutchinson Cancer Research Center and resulted in lifelong friends from those early days of the development of transplantation to treat diseases of bone marrow and immune system origin. The first transplantation at City of Hope was performed on May 18, 1976.

Karl's clinical and research work focused on the problems that confront patients undergoing transplantation, namely, prevention of relapse, understanding the process of immune reconstitution, the treatment and prevention of graft-versus-host disease and cytomegalovirus infection management, and, among his early insights, issues related to patients' quality of life after transplantation and their long-term health. In 1981, Karl was the principal investigator for City of Hope's National Cancer Institute's first approved program project grant in transplantation, which contained projects that focused on those problems, and he organized a talented group of young laboratory and clinical scientists to work together in understanding and solving these challenges. He published the first results from City of Hope on the transplants for acute leukemia in the *New England Journal of Medicine* in 1981, showing the impact of remission status on outcome.

Based on his success at City of Hope, in 1987 Karl was recruited by Stanley Schrier and Ron Levy to begin a new transplant program at Stanford University. Thus, he again developed from scratch his second successful transplantation program, mentoring numerous young physicians and inspiring colleagues and staff to work with him in a common quest to cure patients with hematologic malignancy and return them to their normal lives. Just as he had done at City of Hope, he developed and led another successful program project grant application. He also established the Division of Blood and Marrow Transplantation at Stanford University, taking a personal interest in the lives of each of his patients, incorporating all aspects of the care team into their treatment, including nursing, social work, dietary, and physical therapy, and confirming again a model for patient care and research in use at transplant programs around the country. During that time, he trained both Rob Negrin and Nelson Chao, who went on to direct transplantation programs at Stanford University and at Duke University, respectively. Therefore, Karl was responsible for at least three of the major BMT programs in the United States, not to mention the numerous fellows he trained and mentored at City of Hope and Stanford University.

In 2000, Karl attained emeritus status at Stanford University and stepped down from the role of chief of the BMT division to develop the Stanford Cancer Institute. He continued to serve Stanford University as associate director of research in the Department of Medicine. In 2003, he directed his energies toward developing and achieving the coveted National Cancer Institute-designated cancer center status for the Stanford Cancer Institute, a first for the university.

Karl was also a driving force in 1994 in the founding of the American Society of Blood and Marrow Transplantation, now an international organization for physicians and researchers committed to serving patients with a variety of blood diseases. He was also a leader in the American Society of Hematology and, for many years, was in charge of the organization's Career Development Program. Early on, he recognized that our responsibilities were not only to our patients now but also to those who come to us in the future. He became the first honorary member of ASBMT and, with Richard O'Reilly, was the first coeditor of *The Biology of Blood and Marrow Transplantation*, the major journal in the field. Karl worked tirelessly to make it the central journal in the field, and when there were questions about the cost of the first issues, he made a personal financial donation to ensure the journal's successful launch.

Karl won many honors, both in the United States and Germany, but his major legacy is the impact he had on the thousands of patients he treated and the others he inspired to follow in his footsteps. All of us who knew and loved him knew that his highest priorities remained the quality of life of his patients and their families and the well-being of his transplantation team and those of his colleagues around the country and world. He held us all to high standards of excellence, but he maintained it with wonderful warmth, humor, and sensitivity over the decades and across continents. All of us who worked with him benefited from his respect and could always count on him to extend a helping hand and open ears, as he wanted nothing more than to help, to give of himself, and to be a force for good in this world.

In addition to his interest in music, sports – especially Stanford athletics – and the politics of the world and its impact on all of us, he was an exemplary family man. His children, Philipp and Caroline, could always rely on him for advice, affection, and guidance. In his last years, the lives and activities of his grandchildren, Adrian, Katie, Laura, Kevin, and David, were his focus and, of course, they adored him. Vera, his wife of 45 years, was his partner in his life and his work, as Dottie was to Don, and, at difficult times, always his source of strength and calm.

Although Don and Karl are most noted for their leadership and pioneering scientific achievements, for those of us who have been fortunate enough to work with them, they are equally admired for the way in which they achieved their success. They were always focused, hardworking, and uncompromising in their laboratories and especially in clinical research. They could both be demanding critics and held others accountable for their actions, yet at the same time, they were always generous with their ideas, loyal to their colleagues, and quick to deflect praise to their coworkers and help younger colleagues who chose this career.

For many of us in the field who had the privilege of knowing and working with both Don and Karl and for the many patients who are alive because of their work and dedication, the sentiment of a Hebrew proverb seems appropriate for each of them: "Say not in grief he is no more, but live in thankfulness that he was."

List of Abbreviations

AA	Amyloid A
AA	Aplastic anemia
AABB	American Association of Blood Banks
aaIPI	Age-adjusted International Prognostic Index
AAV	Adeno-associated virus
ABC	Activated B-cell-like
ABL	Abelson leukemia virus
aBMT	Autologous bone marrow transplantation
ABMTR	Autologous Blood and Marrow Transplant Registry – North America
ABVD	Doxorubicin, bleomycin, vinblastine, dacarbazine
ABW	Actual body weight
AC	Adriamycin (doxorubicin), cyclophosphamide
ACE	Adriamycin (doxorubicin), cyclophosphamide, etoposide
ACEI	Angiotensin-converting enzyme inhibitor
ACIF	Anticomplement immunofluorescence
ACIP	Advisory Committee on Immunization Practices
ACOR	Association of Online Cancer Resources
ACP	Advanced care planning
ACR	Albumin-to-urine creatinine ratio
ACS	Acute chest syndrome
ACTH	Adrenocorticotropic hormone
ACVBP	Adriamycin (doxorubicin), cyclophosphamide, vindesine, bleomycin, prednisone
AD	Autoimmune disease
ADA	Adenosine deaminase
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADH	Antidiuretic hormone
ADL	Activities of daily living
AdV	Adenovirus
AEL	Acute erythroleukemia
AFP	α-Fetoprotein
AGM	Aorta–gonad–mesonephros (region)
aGVHD	Acute graft-versus-host disease
AH	Ancestral haplotype
aHCT	Autologous hematopoietic cell transplantation
AHNMD	Associated clonal hematologic non-mast-cell lineage disease
AHPCS	Autologous hematopoietic progenitor-cell support
aHR	Adjusted hazard ratio
AHRQ	Agency for Health Care Research and Quality
aHUS	Atypical hemolytic uremic syndrome
AIDS	Acquired immune deficiency syndrome
AIF	Apoptosis-inducing factor
AIHA	Autoimmune hemolytic anemia
AIRE	Autoimmune regulator
AITD	Autoimmune thyroid disease
AITL	Angioimmunoblastic T-cell lymphoma
AKI	Acute kidney injury
AKIN	Acute Kidney Injury Network
aKIR	Activating killer immunoglobulin-like receptor
AL	Amyloid light-chain
ALA	α-Lipoic acid
ALC	Absolute lymphocyte count
ALCL	Anaplastic large-cell lymphoma
ALDH	Aldehyde dehydrogenase
ALG	Antilymphocyte globulin
ALK	Anaplastic lymphoma kinase
ALL	Acute lymphoblastic leukemia
AlloHCT	Allogeneic hematopoietic cell transplantation
AlloSCT	Allogeneic stem-cell transplantation
ALP	Alkaline phosphatase
ALPS	Autoimmune lymphoproliferative syndrome
ALT	Alanine aminotransferase
AMA	Antimitochondrial antibody
AMKL	Acute megakaryoblastic leukemia
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
ANKL	Aggressive natural killer cell leukemia
AOTONJ	Antiosteoclastic therapy-associated osteonecrosis of the jaws
AP	Accelerated phase
AP-1	Activator protein-1
APACHE	Acute Physiology, Age, Chronic Health Evaluation
APBMT	Asia Pacific Blood and Marrow Transplantation
APC	Antigen-presenting cell
APECED	Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy
API	4′,6-Diamidino-2-phenylindole
APL	Acute promyelocytic leukemia
APMF	Acute panmyelosis with myelofibrosis
Ara-C	Cytosine arabinoside
ARB	Angiotensin receptor blocker
ARDS	Acute or adult respiratory distress syndrome
ARF	Acute renal failure
ARL	AIDS-related lymphoma
ARS	Acute radiation syndrome
ARSA	Arylsulfatase A
ART	Assisted reproductive technology
ASBMT	American Society for Blood and Marrow Transplantation
ASCT	Autologous stem-cell transplantation
ASO	Allele-specific oligonucleotide
ASO-PCR	Allele-specific oligonucleotide polymerase chain reaction
AST	Aspartate aminotransferase
AT	Alkyltransferase
ATG	Antithymocyte globulin
ATL	Adult T-cell leukemia
ATLL	Adult T-cell leukemia/lymphoma
ATM	Ataxia Telangiectasia Mutated
ATRA	All-*trans*-retinoic acid
AUC	Area under the curve
AutoHCT	Autologous hematopoietic cell transplantation
AVP	Arginine vasopressin
AYA	Adolescent and young adult
AZT	Zidovudine
BAEP	Brainstem auditory evoked potentials
BAFF	B-cell-activating factor
BAIAP3	Brain-specific angiogenesis inhibitor 1-associated protein 3
BAL	Bronchoalveolar lavage
BC	Blast crisis

BCC	Basal cell carcinoma
BCMA	B-cell maturation antigen
BCNA	Bicyclic nucleoside analog
BCNU	1,3-Bis(2-chloroethyl)-1-nitrosourea (carmustine)
BCR	B-cell receptor
BCR	Breakpoint cluster region
BD	Bortezomib, dexamethasone
BDP	Beclomethasone 17,21-dipropionate
BEAC	BCNU, etoposide, Ara-C (cytosine arabinoside), cyclophosphamide
BEAM	BCNU, etoposide, Ara-C (cytosine arabinoside), melphalan
BED	Biologically effective dose
BEP	Bleomycin, etoposide, cisplatin
BER	Base excision repair
BFI	Brief Fatigue Inventory
BFM	Berlin–Frankfurt–Münster
BFU-E	Burst-forming units – erythroid
BIW	Twice weekly
BL	Burkitt lymphoma
BLA	Biologics license application
BL-CFC	Blast colony-forming cell
BLS	Bare lymphocyte syndrome
BLyS	B-lymphocyte stimulator
BM	Bone marrow
BMC	Bone marrow cell
BMD	Bone mineral density
BMDW	Bone Marrow Donors Worldwide
BMFS	Bone marrow failure syndromes
BMI	Body mass index
BMSC	Bone marrow stem cell
BMSCT	Blood and Marrow Stem Cell Transplant
BMT CTN	Blood and Marrow Transplant Clinical Trials Network
BMT	Bone marrow transplantation
BMTCN	Blood and Marrow Transplantation Certified Nurse
BMTinfonet	Blood and Marrow Transplant Information Network
BMTSS	Bone Marrow Transplant Survivor Study
BNLI	British National Lymphoma Investigation
BNP	Brain natriuretic peptide
BO	Bronchiolitis obliterans
BOOP	Bronchiolitis obliterans organizing pneumonia
BPDCN	Blastic plasmacytoid dendritic cell neoplasm
BPI	Bactericidal/permeability-increasing
BPI	Brief Pain Inventory
BR	Bendamustine, rituximab
Breg	Regulatory B (cell)
BSE	Bovine spongiform encephalopathy
BSI	Brief symptom inventory
BTK	Bruton's tyrosine kinase
BTLA	B- and T-lymphocyte attenuator
BU	Busulfan
BuMel	Busulfan, melphalan
BUN	Blood urea nitrogen
C1P	Ceramide-1 phosphate
CABSI	Catheter-associated bloodstream infection
CAEBV	Chronic active Epstein–Barr virus
CALGB	Cancer and Acute Leukemia Group B
CALLA	Common acute lymphoblastic leukemia antigen
CAR	Chimeric antigen receptor
CAR	Cocksackie and adenovirus receptor
CAR	CXCL12 adventitial reticular cell
CARES	Cancer Rehabilitation Evaluation System
cART	Combination antiretroviral therapy
CB	Cord blood
CBC	Cord blood cell
CBF	Core binding factor
CBG	Corticosteroid-binding globulin
CBHC	Cord blood hemapoietic cell
CBSC	Cord blood stem cell
CBT	Cognitive–behavioral therapy
CBT	Cord blood transplantation
CBV	Cyclophosphamide, BCNU, etoposide
CC	Complete chimerism
CCG	Children's Cancer Group
CCI	Charlson Comorbidity Index
CCI	Corrected count increment
CCNU	1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea
CCR	Cytologic complete remission
CCSG	Children's Cancer Study Group
CCyR	Complete cytogenetic remission
CDA	Cytidine deaminase
CDAD	*Clostridium difficile*-associated diarrhea
CDAI	Crohn Disease Activity Index
CDC	Centers for Disease Control and Prevention
CDC	Complement-dependent cytotoxicity
CDF	Cumulative distribution function
CDK	Cyclin/cyclin-dependent kinase
CE	Carboplatin, etoposide
CEA	Carcinoembryonic antigen
CEACAM	Carcinoembryonic-antigen-related cell-adhesion molecule
CEC	Carboplatin, etoposide, cyclophosphamide
CEL-NOS	Chronic eosinophilic leukemia-not otherwise specified
CEM	Carboplatin, etoposide, melphalan
CESD	Center for Epidemiologic Studies – Depression
CFAR	Cyclophosphamide, fludarabine, alemtuzumab, rituximab
CFH	Complement factor H
CFU	Colony-forming unit
CFU-B	Colony-forming unit – B-lymphocyte
CFU-BLAST	Colony-forming unit – blast
CFU-E	Colony-forming unit – erythroid
CFU-F	Colony-forming unit – fibrolast
CFU-GEMM	Colony-forming unit – granulocyte/erythroid/macrophage/megakaryocyte
CFU-GM	Colony-forming unit – granulocyte–macrophage
CFU-MEG	Colony-forming unit – megakaryocytic
CFU-Mix	Colony-forming unit – mixed
CFU-S	Colony-forming unit – spleen
CFU-T	Colony-forming unit – T-lymphocyte
CGD	Chronic granulomatous disease
CGH	Comparative genomic hybridization
cGVHD	Chronic graft-versus-host disease
CHD	Coronary heart disease
CHF	Congestive heart failure
CHOEP-14	Doxorubicin, vincristine, etoposide, prednisone
CHOP	Cyclophosphamide, hydroxydaunomycin, vincristine (Oncovin), prednisone
CHR	Complete hematologic response
CHRIs	Child Health Ratings Inventory
CHS	Chediak–Higashi syndrome
CI	Comorbidity index
CI	Confidence interval
CI	Cumulative incidence
CIA	Collagen-induced arthritis
CIBMTR	Center for International Blood and Marrow Transplant Research
CID	Combined immunodeficiency syndrome
CIDP	Chronic inflammatory demyelinating polyneuropathy
CIF	Cumulative incidence function
CIHHV-6	Chromosomal integration of human herpesvirus 6
CIPN	Critical illness polyneuropathy
CJD	Creutzfeldt–Jakob disease
CKD	Chronic kidney disease
CLIA	Clinical Laboratory Approved Amendments
CLL	Chronic lymphoid/lymphocytic leukemia
CLO	Clofarabine

CLP	Common lymphoid progenitor
CLR	C-type lectin receptor
CMF	Cyclophosphamide, methotrexate, fluorouracil
CMI	Cellular mediated immunity
CML	Chronic myeloid/myelogenous leukemia
CMML	Chronic myelomonocytic leukemia
CMN	Chronic myeloid neoplasm
CMP	Common myeloid progenitor
CMR	Complete molecular response
CMV	Cytomegalovirus
CMV-Ig	Cytomegalovirus antibody-enriched intravenous immunoglobulin
CMV-IP	Cytomegalovirus-associated interstitial pneumonia
CNI	Calcineurin inhibitor
CNL	Chronic neutrophilic leukemia
CNS	Central nervous system
COG	Children's Oncology Group
ConA	Concanavalin A
COP	Cryptogenic organizing pneumonia
COPE	Coping Orientations to Problems Experienced Scale
COX-2	Cyclooxygenase-2 (inhibitor)
CP	Chronic phase
CR	Complete remission
CRBSI	Catheter-related bloodstream infection
CREG	Cross-reactive group
CRM	Continual reassessment method
CRS	Cytokine release syndrome
CRVT	Catheter-related venous thrombosis
CSA	Cyclosporine A
CSF	Cerebrospinal fluid
CSF	Colony-stimulating factor
CSP	Cyclosporine
CT	Computed tomography
CT	Cyclophosphamide, thioTEPA
CTC	Cyclophosphamide, thioTEPA, carboplatin
CTC	Cytotoxic T cell
CTCL	Cutaneous T-cell lymphoma
CTD	Cyclophosphamide, thalidomide, dexamethsone
CTL	Cytotoxic T-lymphocyte
CTLA	Cytotoxic T-lymphocyte antigen
CTLP	Cytotoxic T-lymphocyte precursor
CTN	Clinical Trials Network
CTX	Cyclophosphamide
CTXD	Cancer and Treatment Distress
CTZ	Chemotactic trigger zone
CVAD	Fractionated cyclophosphamide, vincristine, adriamycin, dexamethasone
CVC	Central venous catheter
CVD	Cardiovascular disease
CVID	Common variable immune deficiency
CVRF	Cardiovascular risk factor
CVVH	Continuous veno-venous hemofiltration
CVVHDF	Continuous veno-venous hemodiafiltration
CY	Cyclophosphamide
CyBorD	Cyclophosphamide, bortezomib, dexamethasone
CYP	Cytochrome P
DAD	Direct antigen detection
DAF	Decay-accelerating factor
DAG	Diacylglycerol
DAH	Diffuse alveolar hemorrhage
DAMP	Damage-associated molecular pattern
DAS	Dasatinib
DC	Dendritic cell
DCEP	Dexamethasone, cyclophosphamide, etoposide, cisplatin
DCK	Deoxycytidine kinase
DDR	Discoidin domain receptor
DFA	Direct fluorescent antibody (test)
DFCI	Dana–Farber Cancer Institute
DFS	Disease-free survival
DGK	Deoxyguanosine kinase
DH	Double hit
DHAP	Dexamethasone, high-dose cytarabine, cisplatin
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
DHFR	Dihydrofolate reductase
DHPG	9-(1,3-Dihydroxy-2-propoxymethyl)guanine (ganciclovir)
DIC	Disseminated intravascular coagulation
DILI	Drug-induced liver injury
DIPSS	Dynamic International Prognostic Scoring System
DKA	Diabetic ketoacidosis
DLA	Dog leukocyte antigen
DLBCL	Diffuse large B-cell lymphoma
DLCL	Diffuse large cell lymphoma
DLCO	Diffusion capacity of the lung for carbon monoxide
DLI	Donor lymphocyte infusion
DLT	Dose-limiting toxicity
DM	Diabetes mellitus
DMSO	Dimethyl sulfoxide
DN	Double negative
DNA	Deoxyribonucleic acid
DNAX	DNA accessory molecule
DNR	Do not resuscitate
DOTA	1,4,7,10-Tetraazacyclododecane-*N*,*N*′,*N*′′,*N*′′′-tetraacetic acid
DOTMP	1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid
DPC	Days post-coitum
DPTS	Delayed pulmonary toxicity syndrome
DR	Death receptor
DRG	Dorsal root ganglion
DSA	Donor-specific antibody
DSB	Double-strand break
DSII	Disease-Specific Impairment Inventory
DSRCT	Desmoplastic small round cell tumor
dsRNA	Double-stranded RNA
DST	Donor-specific transfusion
DTH	Delayed type hypersensitivity
DTI	Diffusion-tensor MRI
DTPA	Diethylenetriaminepentaacetic acid
DVT	Deep venous thrombosis
DXA	Dual-energy X-ray absorptiometry
EAE	Experimental allergic encephalomyelitis
EAE	Experimental autoimmune encephalomyelitis
EATL	Enteropathy-associated T-cell lymphoma
EB	Embryoid body
EBMT	European Group for Blood and Marrow Transplantation
EBMTR	European Bone Marrow Transplant Registry
EBNA	Epstein–Barr (virus) nuclear antigen
EBV	Epstein–Barr virus
EBV-LDP	Epstein–Barr virus-associated lymphoproliferative disorder
EC	Endothelial cell
EC	Epirubicin, cyclophosphamide
ECAT	Extracorporal adsorption therapy
ECFC	Endothelial colony-forming cells
ECG	Electrocardiogram
ECIL	European Conference on Infections in Leukaemia
ECM	Extracellular matrix
ECP	Extracorporeal photopheresis
ED	Erectile dysfunction
EDSS	Expanded Disability Status Scale
EDTMP	Ethylenediaminetetramethylenephosphonic acid
EEG	Electroencephalogram
EFS	Event-free survival
EGBMT	European Group for Blood and Marrow Transplantation
ELISA	Enzyme-linked immunosorbent assay

ELN	European LeukemiaNet
EM	Erythema multiforme
EMLA	Eutectic Mixture of Local Anesthetic
EN	Enteral nutrition
ENKL	Extranodal NK/T-cell nasal type leukemia
ENSG	European Neuroblastoma Study Group
ENU	Ethyl-*N*-nitrosourea
EORTC	European Organization for Research and Treatment of Cancer
EPC	Endothelial progenitor cell
EPO	Erythropoietin
EPOCH	Etoposide, prednisone, vincristine (Oncovin), doxorubicin hydrochloride
ER	Endoplasmic reticulum
ER	Estrogen receptor
ERIC	European Research Initiative in chronic lymphocytic leukemia
ERT	Enzyme replacement therapy
ESBL	Extended-spectrum β-lactamase
ESC	Embryonic stem cell
ESFT	Ewing sarcoma family of tumors
ET	Essential thrombocythemia
ETP	Early thymic progenitor
ETS	Euthyroid sick syndrome
EWOG	European Working Group
EWOG-MDS	European Working Group of Myelodysplastic Syndromes in Childhood
FA	Fanconi anemia
FAB	French–American–British Classification
FACIT-SP	Functional Assessment of Chronic Illness Therapy – Spirituality and Wellbeing Scale
FACS	Fluorescence-activated cell sorter
FACT	Foundation for the Accreditation of Cellular Therapy
FACT	Functional Assessment of Chronic Illness Therapies
FACT-BMT	Functional Assessment of Cancer Therapy – BMT Module
FAMA	Fluorescent-antibody staining of membrane antigen
FasL	Fas ligand
FC	Facilitator cell
FCH	Fibrosing cholestatic hepatitis
FCR	Fludarabine, cyclophosphamide, rituximab (RTX)
FDA	Food and Drug Administration
FDG	[^{18}F]Fluorodeoxyglucose
FDR	False discovery rate
FEC	Fluorouracil, epirubicin, cyclophosphamide
FEV$_1$	Forced expiratory ventilation in 1 second
FFP	Fresh frozen plasma
FFS	Failure-free survival
FHCRC	Fred Hutchinson Cancer Research Center
FISH	Fluorescence in situ hybridization
FKBP	Tacrolimus (FK)-binding protein
FL	Follicular lymphoma
FLACC	Face, Legs, Activity, Cry, Consolability (pain assessment tool)
FLAIR	Fluid attenuated inversion recovery
FLC	Free light chain
FLIPI	Follicular Lymphoma International Prognostic Index
FLU	Fludarabine
FN	Febrile neutropenia
FNA	Fine-needle aspiration
FNH	Focal nodular hyperplasia
FoxP3	Forkhead box P3
FSH	Follicle-stimulating hormone
FSI	Fatigue Symptom Inventory
FTBI	Fractionated total body irradiation
FTBI	Full total body irradiation
FTI	Farnesyltransferase inhibitor
FTOC	Fetal thymic organ culture
5-FU	5-Fluorouracil
FVC	Forced vital capacity

GA	Glatiramer acetate
GABG	German Autologous Bone Marrow Transplant Group
GALT	Gut-associated lymphoid tissue
GAP	GTPase-activating protein
GBM	Glioblastoma multiforme
G-BM	G-PBHC and/or G-CSF primed bone marrow
gc	Genome copies
GCB	Germinal center B-cell-like
G-CSF	Granulocyte colony-stimulating factor
GCT	Germ-cell tumor
GDP	Guanosine diphosphate
GEBDIS	German Breast Cancer Dose Intensity Study
GEE	Generalized estimating equation
GEL/TAMO	Grupo Español de Linfomas/Trasplante Autólogo de Médula Ósea
GEM	Gemcitabine
GEM	Grid Enabled Measures
GEP	Gene expression profiling
GF	Graft failure
GFP	Green fluorescent protein
GFR	Glomerular filtration rate
GGT	γ-Glutamyl transferase
GH	Growth hormone
GHRH	Growth hormone-releasing hormone
GHSG	German Hodgkin Study Group
GI	Gastrointestinal (tract)
GICAT	Gruppo Italiano Collaborativo AIDS e Tumori (Italian Cooperative Group on AIDS and Tumors)
GISL	Gruppo Italiano per lo Studio dei Linfomi (Italian Lymphoma Study Group)
GITMO	Gruppo Italiano per il Trapianto di Midollo Osseo
GM	Galactomannan
GM-CSF	Granulocyte–macrophage colony-stimulating factor
GMP	Granulocyte macrophage progenitor (cell)
GMP	Guanosine monophosphate
GNB	Gram-negative bacilli
GnRH	Gonadotropin-releasing hormone
gp	Glycoprotein
G-PBHC	Granulocyte colony-stimulating factor-mobilized peripheral blood hematopoietic cell
GPI	Glycosylphosphatidylinositol
GPI-AP	Glycosylphosphatidylinositol-anchored protein
GRE	Glucocorticoid response element
GSH	Glutathione (reduced)
GST	Glutathione-*S*-transferase
GTP	Good tissue practice
GTP	Guanosine triphosphate
GVD	Gemcitabine, vinorelbine, liposomal doxorubicin
GVH	Graft-versus-host
GVHD	Graft-versus-host disease
GVHL	Graft-versus-Hodgkin disease (lymphoma)
GVL	Graft-versus-leukemia
GVLE	Graft-versus-leukemia effect
GVT	Graft-versus-tumor
GWAS	Genome-wide association study
H&E	Hematoxylin and eosin (stain)
HA	Hemagglutinins
HAART	Highly active antiretroviral therapy
HADS	Hospital Anxiety and Depression Scale
HAMA	Human antimouse antibody
HA-MRSA	Healthcare-associated MRSA
HAV	Hepatitis A virus
HBoV	Human bocavirus
HBV	Hepatitis B virus
HC	Hematopoietic cell
HC	Hemorrhagic cystitis
HC	Histocompatibility complex

4-HC	4-Hydroperoxycyclophosphamide
HCG	Human chorion gonadotropin
HCoV	Human coronavirus
HCT	Hematopoietic cell transplantation
HCT-CI	HCT-Comorbidity Index
HCV	Hepatitis C virus
HD	Hodgkin disease
HDAC	High-dose Ara-C
HDC	High-dose chemotherapy
HDIT	High-dose immunosuppressive therapy
HDT	High-dose therapy
HEPA	High-efficiency particulate air
HES	Hydroxyethyl starch
HES	Hypereosinophilic syndrome
hESC	Human embryonic stem cell
HEV	Hepatitis E virus
HFTBI	Hyperfractionated total body irradiation
HGF	Hepatocyte growth factor
HGNC	HUGO Genome Nomenclature Committee
HHS	Human and Health Services
HHV	Human herpesvirus
HI	Hemagglutination inhibition
HIB	*Haemophilus influenzae* type B
HIF	Hypoxia-inducible transcription factor
HIV	Human immunodeficiency virus
HL	Hodgkin lymphoma
HLA	Human leukocyte antigen
HLH	Hemophagocytic lymphohistiocytosis
hMPV	Human metapneumovirus
HOVON	Hemato-Oncologie voor Volwassenen Nederland
HPA	Hypothalamic–piuitary–adrenal
HPC	Hematopoietic progenitor cell
HPI	Helicase–primase inhibitor
HPV	Human papillomavirus
HR	Hazard ratio
HR	Homologous recombination
HRCT	High-resolution chest tomography
HRE	Hypoxia-inducible transcription factor response element
HRhV	Human rhinovirus
HRPBC	High-risk primary breast cancer
HRQOL	Health-related quality of life
HRSA	Health Resources and Services Administration
HRT	Hormone replacement therapy
HSC	Hematopoietic stem cell
HSC	Hepatic stellate cell
HSCT	Hematopoietic stem-cell transplantation
HSDD	Hypoactive sexual desire disorder
HSPC	Hematopoietic stem/progenitor cell
HSTCL	Hepatosplenic T-cell lymphoma
HSV	Herpes simplex virus
HSV-TK	Herpes simplex virus thymidine kinase
HT	Histologic transformation
5-HT	5-Hydroxytryptamine (serotonin)
hTERT	Human telomerase reverse transcriptase
HTLV	Human T-cell lymphotropic virus
HTS	High-throughput sequencing
HU	Hydroxyurea
HUS	Hemolytic uremic syndrome
HVEM	Herpes virus entry mediator
HVG	Host-versus-graft (reaction)
IA	Invasive aspergillosis (infection)
IBCSG	International Breast Cancer Study Group
IBDIS	International Breast Cancer Dose Intensity Study
IBMTR	International Bone Marrow Transplant Registry
IBW	Ideal body weight
ICAM	Intracellular adhesion molecule
ICE	Ifosfamide, carboplatin, etoposide
ICOS	Inducible costimulator (protein)
ICOS-L	Inducible costimulator ligand
ICSI	Intracytoplasmic sperm injection
ICU	Intensive care unit
ID	Intermediate dose
iDC	Immature dendritic cells
IDO	Indoleamine 2,3-dioxygenase
IDOX	4-Iodo-4-deoxydoxorubicin
IDSA	Infectious Diseases Society of America
IE	Immediate early (antigen expression)
IEM	Inborn errors of metabolism
IFI	Invasive fungal infection
IFN	Interferon
IFRT	Involved field radiotherapy
Ig	Immunoglobulin
IGCCCG	International Germ Cell Cancer Collaborative Group
IGEV	Ifosfamide, gemcitabine, vinorelbine, prednisone
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
IgG	Immunoglobulin G
IgH	Immunoglobulin heavy chain
IGRT	Image-guided radiation therapy
IHC	Immunohistochemistry
IHWG in HCT	International Histocompatibility Working Group in Hematopoietic Cell Transplantation
iKIR	Inhibitory killer immunoglobulin-like receptor
IL	Interleukin
IM	Imatinib
IMGT	International ImMunoGenetics
IMID	Immunomodulatory drug
IMPDH	Inosine monophosphate dehydrogenase
IMRT	Intensity-modulated radiation therapy
IMWG	International Myeloma Working Group
IND	Improvised nuclear device
IND	Investigational new drug
iNK-T	Invariant natural killer T (cell)
iNOS	Inducible NO synthase
INR	International normalized ratio
INRG	International Neuroblastoma Risk Group
INRGSS	International Neuroblastoma Risk Group Staging System
INSS	International Neuroblastoma Staging System
INSTI	Integrase strand transfer inhibitor
IORT	Intraoperative radiotherapy
IP	Interstitial pneumonia
IP$_3$	Inositol-1,4,5-triphosphate
IPD	Invasive pneumococcal disease
IPEX	Immunodysregulation polyendocrinopathy enteropathy X-linked (syndrome)
IPFSG	International Prognostic Factor Study Group
IPI	International Prognostic Index
iPS	Induced pluripotent stem
IPS	Idiopathic pneumonia syndrome
IPS	Interstitial pneumonia syndrome
IPSS	International Prognostic Scoring System
IPV	Inactivated polio vaccine
IRB	Institutional Review Board
IRF	Interferon regulatory factor
IRSG	Intergroup Rhabdomyosarcoma Study Group
IS	Immune synapse
IS	Immunosuppressive
IS	International Scale
ISC	Intestinal stem cell
ISO	Isohemagglutinin
IST	Immunosuppressive therapy
ISW	Immune suppression withdrawal
ITAM	Immunoreceptor tyrosine-binding activation motif
ITCC	Innovative Therapies for Children with Cancer

ITD	Internal tandem duplication
ITIM	Immunoreceptor tyrosine-binding inhibition motif
iTreg	Induced regulatory T (cell)
ITT	Intent-to-treat
IUPM	Infectious units per million
IV	Intravenous
IVF	In vitro fertilization
IVF	Intravenous fluid
IVIg	Intravenous immunoglobulin
IκB	Inhibitor I kappa B
JACIE	Joint Accreditation Committee of ISCT-Europe and EBMT
JAK	Janus kinase
JALSG	Japan Adult Leukemia Study Group
JCBBN	Japan Cord Blood Bank Network
JCV	JC virus
JIA	Juvenile idiopathic arthritis
JMDP	Japan Marrow Donor Program
JMML	Juvenile myelomonocytic leukemia
K15	Cytokeratin 15
KD	Kinase domain
KGF	Keratinocyte growth factor
KIR	Killer immunoglobulin-like receptor
KM	Kaplan–Meier
KPC	*Klebsiella pneumoniae* carbapenemase
KPS	Karnofsky Performance Score
KREC	Kappa-deleting recombination excision circle
LAIV	Live attenuated influenza vaccine
LAMP	Loop-mediated isothermal amplification
LAVV	Live attenuated varicella vaccine
LBL	Lymphoblastic lymphoma
LC	Langerhans cell
LCH	Langerhans cell histiocytosis
LCL	Lymphoblastic cell lines
LD	Linkage disequilibrium
LDH	Lactate dehydrogenase
LEDGF	Lens epithelium-derived growth factor
LenDex	Lenalidomide, dexamethasone
LET	Linear energy transfer
LFA	Leukocyte function-associated antigen
LFR	Limited field radiation
LFS	Leukemia-free survival
LFS	Lung function score
LGL	Large granular lymphocyte
LH	Luteinizing hormone
LHRH	Luteinizing hormone-releasing hormone
LIF	Leukemia inhibitory factor
LMD	Laser microdissection
LMP	Latency membrane protein
LMWH	Low molecular weight heparin
LN	Lymph node
LOH	Loss of heterozygosity
LPHD	Lymphocyte-predominant Hodgkin disease
LPS	Lipopolysaccharide
LQ	Linear quadratic
LRD	Lower respiratory tract disease
LRP	Lipoprotein receptor-related protein
LRP	Lung resistance protein
LRR	Leucine-rich-repeat
LSD	Lyosomal storage disorders
LTC-IC	Long-term culture initiating cell
LTFU	Long-term follow-up
LT-HSC	Long-term hematopoietic stem cell
LUTS	Lower urinary tract syndrome
LV	Lenti virus
LVEF	Left ventricular ejection fraction
M protein	Monoclonal protein
MA	Myeloablative
mAb	Monoclonal antibody
MAC	Membrane attack complex
MAC	Myeloablative conditioning
MAFIA	Macrophage Fas-induced apoptosis
MALDI-TOFMS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MAP	High-dose methotrexate, cisplatin, and doxorubicin
MAPK	Mitogen-activated protein kinase
MBC	Metastatic breast cancer
MBL	Monoclonal B-cell lymphocytosis
MBP	Myelin basic protein
MCD	Minimal change disease
MCL	Mantle cell lymphoma
MCL	Mast cell leukemia
mCMV	Murine cytomegalovirus
M-CSF	Macrophage colony-stimulating factor
MCyR	Major cytogenetic response
MDASI	M.D. Anderson Symptom Inventory
mDC	Mature dendritic cells
MDex	Melphalan, dexamethasone
MDRD	Modification of Diet in Renal Disease (Study)
MDS	Myelodysplastic syndrome
MDSC	Myeloid-derived suppressor cell
MEAC	Minimum effective analgesic concentration
MEL	Melphalan
MEP	Megakaryocyte erythrocyte progenitor
mESC	Mouse embryonic stem cell
mesna	Sodium 2-mercaptoethanesulfonate
MetaEICESS	Meta European Intergroup Cooperative Ewing Sarcoma Study
MF	Mycosis fungoides
MF	Myelofibrosis
MFC	"Multiparametric" flow cytometric
MFC	Multidimensional flow cytometry
MFD	Matched family donor
MG	Myasthenia gravis
MGDF	Megakaryocyte growth and development factor
MGH	Massachusetts General Hospital
MGMT	Methylguanine methyltransferase
MGUS	Monoclonal gammopathy of undetermined significance
mHA	Minor histocompatibility antigen
mHAQ	Modified Health Assessment Questionnaire
MHC	Major histocompatibility complex
MHV	Mouse hepatitis virus
MIC	Minimum inhibitory concentration
MICA	MHC class I chain-related gene A
MIP-1	Macrophage inflammatory protein 1
MIPI	Mantle Cell Prognostic Index
MIRD	Medical internal radiation dose
MIRL	Membrane inhibitor of reactive lysis
miRNA	micro-RNA
mITT	Modified intent-to-treat
MJM	Muscle and Joint Measure
MKP	Megakaryocyte progenitor
MLC	Mixed leukocyte culture
MLD	Metachromatic leukodystrophy
MLL	Mixed leukemia lymphoma gene
MLL	Mixed lineage leukemia
MLR	Mixed leukocyte reaction
MLS	Maroteaux–Lamy syndrome
MM	Multiple myeloma
MMF	Mycophenolate mofetil
MMR	Major molecular response
MMR	Measles/mumps/rubella
MMRD	Mismatched related donor
MMUD	Mismatched unrelated donor

MNC	Mononuclear cell
MODS	Multiple-organ dysfunction syndrome
MOG	Myelin oligodendrocyte glycoprotein
MOPP	Mechlorethamine, vincristine (Oncovin), procarbazine, prednisone
MP	Melphalan, prednisone
MP	Methylprednisolone
MPA	Mycophenolic acid
MPAG	7-*O*-Mycophenolic acid glucuronide
MPAL	Mixed phenotype acute leukemia
MPB	Mobilized peripheral blood (cells)
MPFC	Multiparameter flow cytometry
MPN	Myeloproliferative neoplasm
MPP	Multipotent progenitor
MPT	Melphalan, prednisone, thalidomide
MPV	melphalan, prednisone, bortezomib (Velcade)
MRC	Medical Research Council
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
m-RNA	Messenger RNA
MRP	Multidrug resistance-associated protein
MRS	Magnetic resonance spectroscopy
MRSA	Methicillin-resistant *Staphylococcus aureus*
mRSS	Modified Rodman skin score
MS	Mass spectrometry
MS	Multiple sclerosis
MSC	Mesenchymal stem cell
MSC	Mesenchymal stromal cell
MSCH	Mouse spinal cord homogenate
MSFC	Multiple sclerosis functional composite
MSI	Microsatellite instability
MSKCC	Memorial Sloan Kettering Cancer Center
MS-LCH	Multi-system Langerhans cell histiocytosis
MSSA	Methicillin-susceptible *Staphylococcus aureus*
MTD	Maximum tolerated dose
MTOR	Mammalian target of rapamycin
Mtvs	Mouse mammary tumor virus
MTX	Methotrexate
MUD	Matched unrelated donor
MV	Measles virus
MVA	Multivariate analysis
MZ	Marginal zone
NA	Nucleoside analog
NA-DP	Nucleoside analog diphosphate
NANT	New Approaches to Neuroblastoma Therapy
NAP1	North American Pulsed Field Type 1
NASH	Non-alchoholic steatohepatitis
NAT	Nucleic acid amplification test
NA-TP	Nucleoside analog triphosphate
nbmtLINK	National Bone Marrow Transplant link
NC	Nucleated cell
NCAM	Neural cell adhesion molecule
NCCN	National Comprehensive Cancer Network
NCHS	National Center for Health Statistics
NCI	National Cancer Institute
NCIC	National Cancer Institute of Canada
NCI-WG	National Cancer Institute – Working Group
nCR	Near complete response
NCR	Natural cytotoxicity receptor
NDK	Nucleotide diphosphate kinase
NE	Norepinephrine
NEMO	Nuclear factor-kappa B essential modulator
NER	Nucleotide excision repair
NF-AT	Nuclear factor of activated T cells
NFκB	Nuclear factor-kappa B
NGS	Next-generation sequencing
NHANES	National Health and Nutrition Examination Surveys
NHEJ	Non-homologous end-joining
NHL	Non-Hodgkin lymphoma
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institutes of Health
NIL	Nilotinib
NIMA	Non-inherited maternal antigen
NIPA	Non-inherited paternal antigen
NK	Natural killer (cell)
NK	Normal karyotype
NK-T	Natural killer T (cell)
NLR	Nucleotide-binding oligomerization domain leucine-rich-repeat-containing receptor
NM	Non-myeloablative
NMA	Non-myeloablative
NMDA	*N*-Methyl-D-aspartate
NMDP	National Marrow Donor Program
NMHCT	Non-myeloablative hematopoietic cell transplantation
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NO	Nitric oxide
NOD	Non-obese diabetic (mouse)
NOD	Nucleotide-binding oligomerization domain
NPC	Nasopharyngeal carcinoma
NRM	Non-relapse mortality
NRS	Neurological Rating Scale
NRTI	Nucleoside reverse transcriptase inhibitor
NS	Nephrotic syndrome
NS	Noonan syndrome
NSAID	Non-steroidal anti-inflammatory drug
N-SH2	N-Terminal src-homology 2
NST	Non-myeloablative stem-cell transplantation
NT-proBNP	N-Terminal fragment of pro-brain natriuretic peptide
nTreg	Naturally occurring regulatory T (cell)
NWTS	National Wilms Tumor Study
NZB	New Zealand black
NZW	New Zealand white
OATP	Organic anion-transporting polypeptide
OCTP	Organic cation-transporting polypeptide
OFAR	Oxaliplatin, fludarabine, cytarabine, rituximab
OLD	Obstructive lung disease
ONJ	Osteonecrosis of the jaw
ONS	Oncology Nursing Society
OPA	Office of Patient Advocacy
OPG	Osteoprotegerin
OPM	Oropharyngeal mucositis
O-PRISM	Oncologic Pediatric Risk of Mortality
OR	Odds ratio
OS	Overall survival
PACT	Psychological Assessment of Candidates for Transplantation Scale
PAD	Bortezomib, adriamycin, dexamethasone
PALE	Post-transplant acute limbic encephalitis
PAM	Pretransplant assessment of mortality
PAMP	Pathogen-associated molecular pattern
PAS	Periodic acid–Schiff
PB	Peripheral blood
PBHC	Peripheral blood hematopoietic cell
PBHCT	Peripheral blood hematopoietic cell transplantation
PBL	Peripheral blood lymphocyte
PBMC	Peripheral blood mononuclear cell
PBMTC	Pediatric Blood and Marrow Transplant Consortium
PBPC	Peripheral blood progenitor cell
PBSC	Peripheral blood stem cell
PCA	Patient-controlled analgesia
PCNSL	Primary central nervous system lymphoma
PCORI	Patient-Centered Outcomes Research Institute
PCR	Polymerase chain reaction
PCR	Programmed cell renewal

PCT	Pulmonary cytolytic thrombi
PCV	Pneumococcal conjugate vaccine
PCV	Protein conjugate vaccine
PD	Programmed death
PD	Progressive disease
PDC	Plasmacytoid dendritic cell
PDE	Phosphodiesterase
PDGFA	Platelet-derived growth factor A chain
PDGFR	Platelet-derived growth factor receptor
pDLI	Preemptive donor lymphocyte infusion
PDQ	Physician Data Query
PedsQL	Pediatric Quality of Life Inventory
PEGS	Platinum, etoposide, gemcitabine, methylprednisolone
PERDS	Peri-engraftment respiratory distress syndrome
PET	Positron emission tomography
PETHEMA	Programa para el Tratamiento de Hemopatías Malignas
PFS	Progression-free survival
PFT	Pulmonary function test
PGD	Pre-implantation genetic diagnosis
P-gp	P-glycoprotein
Ph	Philadelphia (chromosome)
PH	Pulmonary hypertension
PHA	Phytohemagglutinin A
PHN	Post-herpetic neuralgia
PHQ-9	Patient Health Questionnaire-9
PI	Protease inhibitor
PI3K	Phosphatidylinositol 3-kinase
PICC	Peripherally inserted central venous catheter
PID	Primary immunodeficiency disease
PIG-A	Phosphatidylinositol glycan-A
PIP$_2$	Phosphatidylinositol 4,5-diphosphate
PIP$_3$	Phosphatidylinositol 3,4,5-triphosphate
PIQ	Performance IQ
PJP	*Pneumocystis jirovecii* pneumonia
PK	Pharmacokinetic
PKC	Protein kinase C
PlGF	Placental growth factor
PLL	Prolymphocytic leukemia
PLP	Proteolipid protein
PLPHA	Postlumbar puncture headache
PMC	Persistent mixed chimerism
PMF	Primary myelofibrosis
PMGCT	Primary mediastinal germ-cell tumor
PML	Progressive multifocal leukoencephalopathy
PMN	Polymorphonuclear neutrophil
PN	Parenteral nutrition
PN	Peripheral neuropathy
PNET	Primitive neuroectodermal tumor
PNH	Paroxysmal nocturnal hemoglobinuria
PNH-sc	Subclinical paroxysmal nocturnal hemoglobinuria
POEMS	Polyneuropathy, organomegaly, endocrinopathy, M protein, skin changes
POF	Premature ovarian failure
POG	Pediatric Oncology Group
POLST	Physician Orders for Life Sustaining Treatment
PomDex	Pomalidomide, dexamethasone
PORN	Progressive outer retinal necrosis
PORT	Patient Outcomes Assessment Research Team
PPI	Proton pump inhibitor
PR	Partial remission or partial response
PR	Progesterone receptor
PR3	Proteinase 3
PRCA	Pure red cell aplasia
PRES	Posterior reversible encephalopathy syndrome
PRO	Patient-reported outcome
PROMIS	Patient-Reported Outcomes Measurement Information System
PRR	Pathogen recognition receptor
PRST	Pediatric Registry for Stem Cell Transplantations
PSA	Prostate-specific antigen
PSC	Pluripotent stem cell
PSE	Prednisone
PSQI	Pittsburgh Sleep Quality Index
PT	Pertussis toxoid
PTA	Peripheral tissue antigen
PTCL	Peripheral T-cell lymphoma
PTCL-nos	Peripheral T-cell lymphoma not otherwise specified
PTH	Parathyroid hormone
PTLD	Post-transplant lymphoproliferative disorder
PTPase	Protein tyrosine phosphatase
PUVA	Psoralen–ultraviolet A irradiation (treatment)
PV	Polycythemia vera
PVR	Polio virus receptor
PyVAN	Polyomavirus-associated nephropathy
PyVHC	Polyomavirus-associated hemorrhagic cystitis
QALY	Quality-adjusted life-year
QLQ	Quality of Life Questionnaire
QOL	Quality of life
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
QTL	Quantitative trait loci
QW	Once weekly
RA	Refractory anemia
RA	Retinoic acid
RA	Rheumatoid arthritis
RA	Right atrium
RAb	Radiolabeled antibody
RAEB	Refractory anemia with excess blasts
RAEB-T	Refractory anemia with excess blasts in transformation
RANKL	Receptor activator of nuclear factor **k**appa B ligand
RARS	Refractory anemia with ringed sideroblasts
RBC	Red blood cell
RCC	Ratio of costs to charges
RCC	Refractory cytopenia of childhood
RCC	Renal cell carcinoma
R-CHOP	Rituximab, cyclophosphamide, hydroxydaunomycin, vincristine (Oncovin), prednisone
RCMD	Refractory cytopenia with multilineage dysplasia
RCR	Replication competent retrovirus
RCT	Randomized controlled trial
REAL	Revised European–American Lymphoma
R-EPOCH	Rituximab, etoposide, prednisone, vincristine (Oncovin), doxorubicin hydrochloride
RES	Reticuloendothelial system
R-ESHAP	Rituximab, etoposide, cytarabine, cisplatin, methylprednisolone
RFS	Relapse-free survival
Rh	Rhesus
rHBV	Recombinant hepatitis B vaccine
RHC	Residual host cells
rhGH	Recombinant human growth hormone
r-hIL-7	Recombinant human interleukin-7
rHuEPO	Recombinant human erythropoietin
R-hyperCVAD	RTX (rituximab), vincristine, doxorubicin, dexamethasone
RI	Relapse incidence
RIC	Reduced-intensity conditioning
RICR	Reduced-intensity conditioning regimen
RIFLE	Risk, injury, failure, loss, end-stage
RISC	RNAi-induced silencing complex
RIT	Radioimmunotherapy
RLD	Restrictive lung disease
RLR	Rig-1-like receptor
RN	Registered nurse

RNAi	RNA interference
RNR	Ribonucleotide reductase
ROC	Receiver-operating characteristic
ROS	Reactive oxygen species
RR	Relapse rate
RR	Relative risk
RR	Response rate
RRE	Rev response element
RRE	Risk ratio estimate
RRMS	Relapsing–remitting multiple sclerosis
RRT	Regimen-related toxicity
RSCH	Rat spinal cord homogenate
RSCL	Rotterdam Symptom Checklist
RSV	Respiratory syncytial virus
RT	Reverse transcriptase
RTE	Recent thymic emigrant
RTID	Respiratory tract infectious disease
RT-PCR	Real-time polymerase chain reaction
RTQ	Real-time quantitative
RTX	Rituximab
RVD	Revlimid/lenalidomide, Velcade/bortezomib, dexamethasone
S1P	Sphingosine-1 phosphate
SAA	Serum amyloid A
SAA	Severe aplastic anemia
SAAG	Serum-ascites albumin gradient
SAHA	Suberoylanilide hydroxamic acid
SAP	Serum amyloid protein P
SARS	Severe acute respiratory syndrome
SAS-SR	Social Adjustment Scale – Self Report
SBT	Sequence-based typing
SCC	Squamous cell carcinoma
SCD	Sickle cell disease
SCF	Stem-cell factor
scFv	Single-chain variable fragment
SCID	Severe combined immunodeficiency syndrome
SCID-hu	Severe combined immunodeficiency syndrome/human (mouse)
SCL	Stem-cell leukemia
SCT	Stem-cell transplantation
SCTOD	Stem Cell Therapeutics Outcomes Database
SD	Stable disease
SD	Standard deviation
SDC	Standard-dose chemotherapy
SDF	Stroma cell-derived factor
SDS	Shwachman–Diamond syndrome
SE	Standard error
SEC	Sinusoidal endothelial cell
SEER	Surveillance Epidemiology and End Results
Sema	Semaphorin
SEREX	Serological identification of antigens by recombinant expression cloning
SERM	Selective estrogen response modulator
SF-36	Medical Outcomes Study Short Form 36
SFGM	Société Française de Greffe de Moelle
SFK	SRC-family kinase
SFOP	Société Française de l'Oncologie Pédiatrique, French Society of Pediatric Oncology
SFQ	Sexual Function Questionnaire
SGOT	Serum glutamic-oxaloacetic transaminase
SHBG	Sex hormone-binding globulin
SHEA	Society for Healthcare Epidemiology of America
Shh	Sonic hedgehog
SHM	Somatically hypermutated
SI	Stimulation index
SIADH	Syndrome of inappropriate antidiuretic hormone
SIG	Special Interest Group
sIgm	Cell surface immunoglobulin M
siRNA	Short interfering RNA

SJS	Stevens–Johnson syndrome
SKD	Severe keratinocyte dysplasia
SLAM	Signaling lymphocyte activation molecule
SLD	Sublethal damage
SLE	Systemic lupus erythematosus
SLEDAI	SLE Disease Activity Index
SM	Systemic mastocytosis
SMILE	Ddexamethasone, methotrexate, ifosfamide, L-asparaginase, etoposide
SMN	Subsequent malignant neoplasm
SMR	Standardized mortality ratio
SNP	Single nucleotide polymorphism
SNRI	Serotonin–norepinephrine reuptake inhibitor
SNS	Sympathetic nervous system
SOFA	Sequential (or sepsis-related) Organ Failure Score
SOP	Standard operating procedure
SOS	Sinusoidal obstruction syndrome
SOT	Solid organ transplantation
SP	Surfactant protein
SPECT	Single photon emission computed tomography
SR	Sensitive relapse
SR	Standard risk
SS	Sézary syndrome
SSA	Sex steroid ablation
SSA	Single-strand annealing
SSc	Systemic sclerosis
SSD	Source-to-skin distance
SSOP	Sequence-specific oligonucleotide probe
SSP	Sequence-specific primer
SSRI	Selective serotonin reuptake inhibitor
SST	Short Syntachten Test
STAMP-I	Cyclophosphamide, cisplatin, BCNU
STAMP-V	Cyclophosphamide, carboplatin, thioTEPA
STATs	Signal transducers and activators of transcription
STBI	Single-fraction total body irradiation
ST-HSC	Short-term hematopoietic stem cell
STING	Stimulator of interferon genes
STR	Short tandem repeat
SVC	Superior vena cava
SWOG	Southwest Oncology Group
T1DM	Type 1 diabetes mellitus
T3	Triiodothyronine
T4	Thyroxine
TAA	Tumor-associated antigen
TAAM	Time-averaged mean of the maximum
TACL	Therapeutic Advances in Childhood Leukemia
TAD	Thalidomide, adriamycin, dexamethasone
TA-GVHD	Transfusion-associated graft-versus-host disease
TAI	Thoracoabdominal irradiation
TAM	Transplant-associated microangiopathy
t-AML	Therapy-related acute myeloid leukemia
TAP	Transporter associated with antigen processing
TAR	Tat response element
TBI	Total body irradiation
TCA	Tricyclic antidepressant
TCD	T-cell depletion
TCD	Transcranial Doppler (ultrasonography)
TCE	T-cell epitope
T_{CM}	Central memory T (cells)
Tcon	Conventional T
TCR	T-cell receptor
TD	Thalidomide, dexamethasone
TDM	Therapeutic drug monitoring
TdT	Terminal deoxynucleotidyl transferase
TED	Thromboembolic disease
T_{EM}	Effector memory T (cell)
TEN	Toxic epidermal necrolysis

TEPA	*N*-Triethylenephosphoramide
TEPC	Thymic epithelial progenitor cell
TERS	Transplant Evaluation Rating Scale
TERT	Telomerase reverse transcriptase
TF	Tissue factor
T_{FH}	T follicular helper
TFIIH	Transcription factor IIH complex
TFPI	Tissue factor pathway inhibitor
TG	Triglyceride
TGF-β	Transforming growth factor beta
T-GVHD	Transfusion-associated graft-versus-host disease
TH	Helper T-lymphocytes
T_H	T helper
thioTEPA	*N*-Triethylenethiophosphoramide
TICE	Paclitaxel, ifosfamide followed by high-dose carboplatin, etoposide
TIL	Tumor-infiltrating lymphocytes
TIP	Paclitaxel, ifosfamide, cisplatin
TIV	Trivalent influenza vaccine
TK	Thymidine kinase
TKI	Tyrosine kinase inhibitor
TLC	Total lung capacity
TLI	Total lymphoid irradiation
TLR	Toll-like receptor
TLS	Tumor lysis syndrome
TM	Thalassemia major
TMA	Thrombotic microangiopathy
TMD	Temporomandibular dysfunction
t-MDS	Therapy-related myelodysplastic syndrome
TMEV	Theiler's murine encephalomyelitis virus
TMI	Total marrow irradiation
TMLI	Total marrow and lymphoid irradiation
TMP–SMX	Trimethoprim–sulfamethoxazole
TM–SLF	Trimethoprim–sulfamethoxazole
TNC	Total nucleated cell
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TNF-α	Tumor necrosis factor α
TOI	Trial Outcome Index
TPA	Tissue plasminogen activator
T-PLL	T-cell prolymphocytic leukemia
TPN	Total parenteral nutrition
TPO	Thrombopoietin
TR	Tandem repeat
TRAIL	TNF-related apoptosis-inducing ligand
TRALI	Transfusion-related acute lung injury
TREC	T-cell receptor excision circles
Treg	Regulatory T (cell)
Treo	Treosulfan
TRM	Transplant-related mortality
TRT	Testosterone replacement therapy
TRV	Tricuspid regurgitation velocity
TSH	Thyroid-stimulating hormone
TSLP	Thymic stromal lymphoietin
TT	*N*-Triethylenethiophosphoramide, thioTEPA
TTP	Thrombotic thrombocytopenic purpura
TTR	Transthyretin

UBC	Umbilical cord
UCB	Umbilical cord blood
UCB	Unrelated cord blood
UCBT	Umbilical cord blood transplant
UGT	Uridine 5′-diphosphate glucuronosyltransferase
UPD	Uniparental disomy
URD	Unrelated donor
URI	Upper respiratory tract infection
UV	Ultraviolet
VAD	Vincristine, adriamycin, dexamethasone
VATS	Video-assisted thoracoscopic surgery
VBAD	Vincristine, BCNU, doxorubicin, dexamethasone
VBMCP	Vincristine, BCNU, melphalan, cyclophosphamide, prednisone
VCD	Velcade/bortezomib, cyclophosphamide, dexamethasone
VDCR	Velcade/bortezomib, dexamethasone, cyclophosphamide, Revlimid/lenalidomide
VDR	Vitamin D receptor
VE	Vascular endothelial
VEGF	Vascular endothelial growth factor
VeIP	Vinblastine, ifosfamide, cisplatin
VF	Ventricular fibrillation
VGPR	Very good partial remission
VIC	Virus isolation by cell culture
VIP	Etoposide, ifosfamide, cisplatin
VIP	Vasoactive intestinal peptide
VISA	Vancomycin-intermediate *Staphylococcus aureus*
VLCFA	Very long-chain fatty acid
VNTR	Variable number of tandem repeat
VOD	Veno-occlusive disease
VRE	Vancomycin-resistant *Enterococcus*
VSAA	Very severe aplastic anemia
VT	Ventricular tachycardia
VTD	Velcade/bortezomib, thalidomide, dexamethasone
vWF	von Willebrand factor
VWMD	Vanishing white matter disease
VZIG	Varicella zoster immune globulin
VZV	Varicella zoster virus
WAS	Wiskott–Aldrich syndrome
WBC	White blood cell
WBM	Whole bone marrow
WBMT	Worldwide Network for Blood & Marrow Transplantation
WBRT	Whole-brain radiotherapy
WES	Whole exome sequencing
WGA	Wheat germ agglutinin
WGS	Whole genome sequencing
WMDA	World Marrow Donor Association
WNV	West Nile virus
WPSS	WHO classification-based Prognostic Scoring System
WSG	West German Study Group
wt	Wild-type
X-ALD	X-linked adrenoleukodystrophy
XRT	External-beam radiotherapy
YFC	[^{90}Y]ibritumomab tiuxetan, fludarabine, cyclophosphamide
ZAP-70	Zeta-associated protein
ZFN	Zinc finger nuclease
ZIG	Zoster immune globulin
ZIP	Zoster immune plasma

CHAPTER 1

A History of Allogeneic and Autologous Hematopoietic Cell Transplantation

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How it all began

In the Fall of 1868, two experimental pathologists reported their important observations that blood cells of mammals are produced inside the bone marrow and that the mature cells leave the marrow cavity through small blood vessels that penetrate the bone cortex. Neumann [1] from the University at Königsberg in Prussia (this town is now known as Kaliningrad and is part of Russia) had made his observations in rabbits, and Bizzozero [2] had worked at the University of Pavia in Italy with mice and rabbits. Both reports from these two investigators appeared less than 6 weeks apart and both men deserve to be mentioned as the original contributors of this critical finding.

Soon, physicians of leukemia patients eagerly used the exciting news from Neumann and Bizzozero and began treatments that appear rather bizarre to the current reader. Prescriptions detailed that patients should swallow fresh bone marrow from young cattle mixed with equal amounts of orange juice or that they should ingest one dessert spoon full of ox bone marrow three times daily [3]. Apparently, these "therapies" were employed based on the assumption that leukemia was a deficiency disease rather than with the expectation of cellular engraftment. Successes were not reported and for the next seven decades there was little progress in the promise of therapies using bone marrow.

In 1939, an attempt was made to inject a few milliliters of ABO matched bone marrow cells into the sternum of a patient with marrow aplasia, but a response was not achieved [4]. During the same decade, the immunologic basis for tumor transplants was established through experiments in mice [5] and led to the recognition of the H2 transplantation antigen system [6].

The explosion of the two atomic bombs at the end of World War II and the consequences of radiation on the bone marrow function of survivors of the nuclear exposure stimulated the interest in marrow transplantation again. When Medawar [7] established the immunologic basis for graft rejection and demonstrated neonatal tolerance in mice, the stage was set for the development of hematopoietic cell transplantation (HCT), and enhanced the level of interest of the medical scientific community in this subject.

History of allogeneic HCT

1949–1956: The humoral versus the cellular hypotheses

In 1949, experiments by Jacobson et al. [8] demonstrated that mice could survive lethal irradiation exposure if their spleens were shielded by lead foils. The investigators attributed their observations to a humoral or hormonal factor. In 1951, Lorenz and colleagues [9] reported that a similar protective effect could be attained by the infusion of cells from the spleen or bone marrow of mice. These two observations became the subject of a scientific debate, that is, whether the marrow recovery was possibly the consequence of humoral or of cellular mechanisms, respectively. In 1954, experiments by Barnes and Loutit [10] indicated that the recovery conferred by spleen and marrow cell infusion might be due to living cells, supporting the cellular hypothesis. In the following year, Main and Prehn [11] described studies of lethally irradiated mice being protected by marrow infusion. When their transplanted animals were given skin grafts from their respective marrow donors, skin implants from the donor animal were not rejected, providing proof of acquired tolerance that could only be explained by the transfer of living donor cells. Final proof for the cellular hypothesis came in 1956 when Ford and colleagues [12] showed that the marrow of transplanted mice displayed the cytogenetic characteristics of the donor. In this article by Ford et al. [12], the term "radiation chimera" was introduced into the scientific transplant literature. A most exciting observation was contributed also in 1956, when Barnes and colleagues [13] reported on the treatment of murine leukemia with high-dose irradiation followed by marrow graft infusion.

1956–1959: Early efforts in clinical marrow transplantation

The promising pre-clinical studies described above created considerable enthusiasm on the part of clinical investigators. First attempts to treat patients with total body irradiation and chemotherapy with subsequent marrow grafting were reported in 1957 by Thomas et al. [14]. In 1959, the same group [15] described their experience with two patients with advanced leukemia who were exposed to high-dose irradiation followed by the infusion of marrow from their identical twins. Prompt hematologic recovery was observed and the two patients survived for several months; however, then both succumbed to disease recurrence. Worldwide, approximately 200 clinical transplant procedures were performed between the mid-1950s and the

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Thomas' Hematopoietic Cell Transplantation, Fifth Edition. Edited by
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early 1960s. A retrospective review (published 1970) [16] indicated that none of the transplant recipients had become long-term survivors, and the future for HCT was generally considered bleak. Reasons for treatment failure and the resulting pessimism included the lack of reliable tissue typing methods to identify suitable donors and problems related to the lack of effective supportive care measures such as potent antibiotics and transfusional support with platelets. Also, the phenomenon of graft-versus-host disease (GVHD) which had been first encountered in animal studies, was occurring in human allogeneic HCT recipients.

In 1958, a nuclear accident occurred at the Boris Kidrich Institute in Vinča, Yugoslavia (now in Serbia) during which six laboratory workers were exposed to 2.0 to 4.3 Gy. Following this highly publicized event, the patients were flown to Paris where a team of physicians under the leadership of Dr. Mathé were given human blood-forming tissues such as fetal liver cells, spleen and bone marrow cells. The patient who had been exposed to the highest amount of radiation succumbed after a massive hemorrhage while the remaining five patients eventually recovered their bone marrow function. Although some evidence of transient engraftment was presented, it is uncertain whether the administration of the allogeneic grafts had played a role in the recovery process [17].

1955–1967: Progress in animal models of allogeneic HCT

It was apparent from the initial negative clinical experience that more information in the field of transplantation biology and improvement in the area of supportive care were necessary. Two types of animals seemed especially suitable for such studies, the inbred murine and the out-bred canine models. It was shown in mice that intravenous infusion was the optimal route for administration of bone marrow grafts [18]. Billingham and Brent [19] found that allogeneic marrow cells could cause a "wasting" syndrome, later identified as GVHD. Uphoff [20] reported that genetic factors controlled the immune reactions against the host. She also detected that methotrexate could ameliorate GVHD [21].

A report by Main and Prehn [11] raised great interest in the possibility that a marrow graft followed by a solid organ graft – such as a kidney from the same donor – might facilitate durable engraftment of the solid organ. One such effort was reported in 1959 by Mannick et al. [22] who had administered high-dose irradiation and bone marrow from an unrelated dog followed by a kidney transplant from the same donor animal. The dog eventually died of pneumonia but had a cellular marrow and no signs of rejection of the kidney. It would take an additional four decades until this attractive concept would become a successful approach in human organ transplantation medicine, that is, allowing for the eventual discontinuation of immunosuppressive therapy of the transplant patients. This approach is an intriguing alternative to life-long immunosuppression – another impressive example of successful translational research (see section on reduced intensity regimens later).

Continued studies in the dog model showed the characteristic spectrum of complications, as well as favorable events following allogeneic marrow grafting, including primary graft failure and secondary graft rejection, as well as GVHD but also stable engraftment and survival without GVHD [23]. Another important observation from the early 1960s which would have a major impact on clinical transplantation practices a quarter of a century later indicated that hematopoietic cells for engraftment could not only be harvested from the bone marrow but could also be obtained from the peripheral blood in both mice [24] and dogs [25].

In Europe, Dausset [26], as well as van Rood et al. [27], described in 1958 the human leukocyte antigen (HLA) groups. It was soon recognized that these antigens represent important genetic factors for donor selection in experimental and clinical transplantation medicine.

Studies by Epstein et al. [28] of the canine leukocyte antigen system proved that dog leukocyte antigens (DLA) were crucial in determining the outcome of allogeneic marrow transplantation in the canine model. Storb et al. [29] reported that dogs given irradiation and a marrow graft from DLA mismatched litter mates died due to graft rejection or GVHD, whereas most recipients of DLA matched marrow, especially when given post-transplant methotrexate to suppress the graft-versus-host reaction, became long-term survivors [30,31].

While these important and informative pre-clinical studies were performed in mice and dogs, a few clinical transplants were done at the same time. Mathé and colleagues [32] performed an allogeneic marrow transplantation procedure in a patient with leukemia. However, the transplant recipient eventually died with serious problems that were likely complications of chronic GVHD. Continued success with transplantation between identical twins signaled the potential of marrow grafting from histocompatible donors.

The pre-clinical observations described earlier and the increasing knowledge of the HLA system to identify compatible family members as marrow donors, as well as the improvement of supportive care measures now available, encouraged clinical investigators to plan new attempts of marrow grafting in patients with suitably matched sibling donors.

1968–1975: The beginning of the modern era of human marrow transplantation

In 1968 and 1969, three infants with congenital immunodeficiency disorders received successful marrow transplants from HLA matched siblings [33–35]. All three patients engrafted, promptly restored their previously deficient immune function, and grew up to become healthy adults [36]. It is a startling coincidence that it took exactly a full century from the two observations of Neumann [1] and Bizzozero [2] in 1868, namely that the bone marrow produces all blood cells, until 1968, when the first successful and curative bone marrow transplants were performed.

Marrow transplantation from sibling donors for patients with advanced leukemia or bone marrow failure conditions proved considerably more challenging than transplantation for immunodeficiency diseases. In 1969, the transplant group in Seattle [37] conditioned a patient in blast crisis of chronic myeloid leukemia (CML) with total body irradiation followed by marrow grafting from a histocompatible sibling. The patient engrafted, however, later succumbed to cytomegalovirus (CMV)-associated pneumonia, a complication for which no drugs had yet been developed. Successful transplant efforts for aplastic anemia patients were reported in 1972 [38]. In a seminal report in 1975, the Seattle transplant team [39] described their observations of the first 100 patients, 73 treated for advanced leukemia and 37 for aplastic anemia. Successful outcomes were noted in 50% of the aplastic anemia patients, and an ever increasing number of patients with leukemia were surviving in remission (Figure 1.1).

1976–1989: Widening application of allogeneic marrow grafting for human patients

Long-term disease-free survival of patients with leukemia was first achieved and reported by the group from Seattle in 1977 [40,41]. These patients had – without exception – failed the best available chemotherapy combinations at least once and many of them were

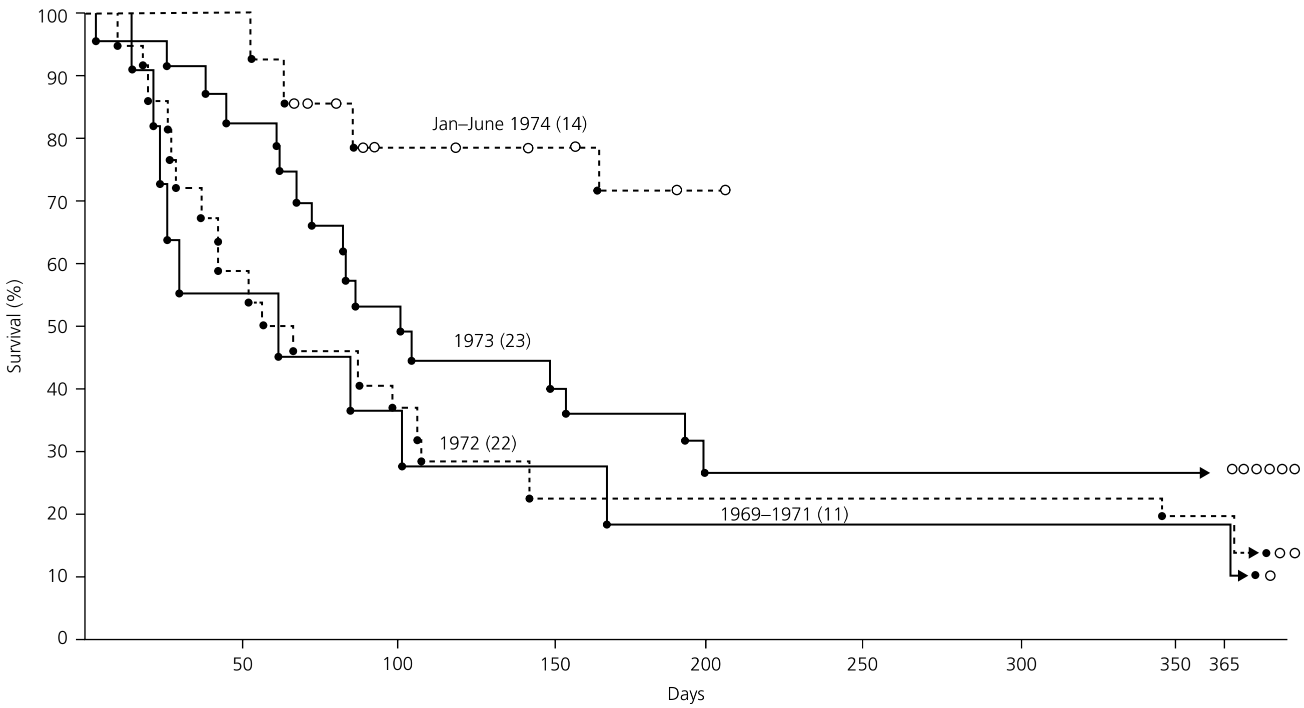

Figure 1.1 Survival curves of 70 patients with acute leukemia transplanted with a marrow graft from a histocompatible sibling in Seattle between 1969 and 1974. Source: Thomas et al. 1975 [39]. Reproduced with permission of the Massachusetts Medical Society.

treated in florid relapse. With a follow-up period of 1 to 4.5 years, 13 of 100 recipients of grafts from their sibling donors were alive and well at the time of the analysis, indicating that even some of such high-risk patients could be cured.

Considering that relapse was a major contributor to post-transplant treatment failure, it was logical to explore HCT as a curative concept in patients with acute leukemia who were in first complete remission, that is, at a time when the burden with malignant cells was relatively low and the abnormal clone was not yet resistant to high-dose radiochemotherapy. In 1979, two centers [42,43] reported their favorable experience with transplant candidates who had received their grafts from matched sibling donors while in first remission. These observations demonstrated greatly improved results compared to patients who were treated in advanced condition. It was demonstrated by further studies that the remission status at the time of HCT had a major influence on the long-term outcome of HCT [44]. The major impact of the burden of malignant cells at the time of HCT has been demonstrated over the past three decades in study after study. A recent report shows again the superior outcome in 49 patients with Philadelphia chromosome positive acute lymphoblastic leukemia transplanted in first remission compared to 30 patients who had relapsed at least once prior to HCT; see Figure 1.2. In this long-term follow-up study, patients were 2–57 years old (median age, 36 years). Age did not have an effect on the outcome of HCT [45], see Figure 1.2.

Another hematologic malignancy for which HCT was explored with curative potential was CML. Initial observations by Fefer et al. [46] indicated the disappearance of the Philadelphia chromosome in four patients after radio-chemotherapy and marrow grafting from their identical twin donors. Subsequently, HCT from matched siblings was tested in two patients with CML and established that the abnormal clone could be abrogated [47,48]. These limited but promising observations led to two larger and successful trials on HCT from matched siblings for CML patients who were still in the chronic phase of their disease [49,50].

The first transplants for thalassemia major [51] and for sickle cell disease [52] were performed during the 1980s in Seattle and opened the field for successful correction of severe non-malignant hematologic disorders.

During this time, new approaches for the prevention of GVHD were developed in animal models and were tested clinically [53,54]. The potent combination of cyclosporine with methotrexate was introduced and became a new standard, while efforts to remove T cells from the marrow inoculum were shown to be also a powerful approach to prevent GVHD [55,56].

Alternatives to the frequently employed preparatory regimen of irradiation and cyclophosphamide were explored in clinical trials, for example, a combination of busulfan with cyclophosphamide [57,58] or total body irradiation in combination with etoposide [59,60].

Another critical event with future consequences occurred in the 1980s: The National Marrow Donor Program was founded in 1987, and succeeded over the next 25 years in recruiting 20 million volunteers from all continents to serve as unrelated HCT donors. This international organization had been preceded in its efforts by the British Anthony Nolan Foundation. Following the first successful transplant procedure [61] using a graft from an unrelated volunteer, HCT from such donors has grown to a point that several cell collections from unrelated donors occur daily and are transported by couriers from donor facilities to the respective transplant centers – with approximately half of the transplants occurring on an international basis.

In 1986, another major nuclear accident occurred at the Chernobyl Power Station (in the former Soviet Union, now Ukraine). Of the 200 people who were exposed to large doses of radiation, 13 severely affected patients whose estimated dose had ranged from 5.6 to 13.4 Gy, received bone marrow transplants by a

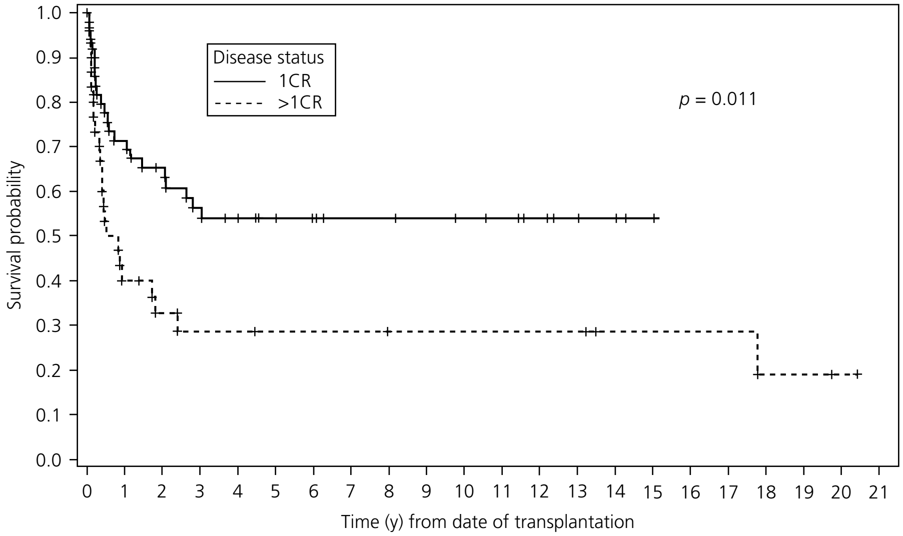

Figure 1.2 Survival of 79 patients with Philadelphia chromosome positive acute lymphoblastic leukemia who received high-dose total body irradiation and etoposide followed by allogeneic HCT from HLA-matched sibling donors. At the time of transplantation, 49 patients were in first complete remission and 30 patients were beyond first remission. The outcome is significantly better for patients who were transplanted earlier compared to those who had suffered at least one relapse prior to HCT. Source: Laport et al. 2008 [45]. Reproduced with permission of the American Society of Hematology.

team of physicians and scientists from the Soviet Union, the United States of America, and Israel. There was hematopoietic recovery in nine patients, six of whom had transient partial engraftment before they recovered their autologous marrow function. Eleven of the transplant recipients died from various causes, including extensive burns, interstitial pneumonitis, GVHD, renal failure, and adult respiratory distress syndrome. Two patients whose radiation doses had been 5.6 and 8.7 Gy, respectively, were alive after 3 years following the radiation accident. It is clear from this experience that bone marrow recovery does not necessarily ensure survival [62].

1989–1999: Progress in the prevention and therapy of CMV disease. New sources of grafts for allogeneic HCT

The introduction of a new antiviral drug, ganciclovir, made it possible to control and reverse infections with CMV, a complication which until then had caused a fatal post-transplant infection in about 15% of HCT recipients. This major accomplishment was demonstrated in two independent, concomitantly performed phase III trials [63,64]. At the same time, new and powerful molecular tests were developed which were based on the polymerase chain reaction principle. These non-invasive tests allowed the rapid and non-invasive monitoring of CMV presence and progression of this viral infection in HCT recipients [65,66].

The knowledge that hematopoietic cells are present in circulating blood, the arrival of cloned growth factors such as the granulocyte colony-stimulating factor (G-CSF) and the availability of apheresis techniques allowed the wide application of collection methods for blood-derived grafts for allogeneic HCT. Three simultaneously published reports documented prompt and durable hematologic reconstitution by such “peripheral” grafts from histocompatible sibling donors [67–69].

Cord blood, another important new source for allogeneic HCT, was introduced by Gluckman et al. [70]. Cord blood registries and banks were developed with national and international participation and have facilitated the use of such grafts into a widely used source for transplantation, especially for pediatric patients.

At the same time, registries of volunteer donors continued to grow, leading to an increased successful use of unrelated donor transplants for patients who were lacking suitably matched related donors [71,72]. This progress was made possible through the continued improvement of molecular tissue typing methods which eventually replaced the conventional serologic typing techniques developed during earlier decades [73–75]. Finally, successful crossing of the HLA barrier became possible allowing HCT from haplotype-matched related donors [76,77].

2000–present: Reduced intensity regimens (RIC) allow transplantation of elderly and medically infirm patients. Hematologic grafts induce tolerance for solid organ transplantation

It had been recognized since the early days of experimental and clinical HCT that a graft-versus-malignancy effect contributes to a major extent to the prevention of post-transplant relapse of the underlying malignancy. Moreover, donor lymphocyte infusions were shown to lead to eradication of recurrent diseases, especially in patients with CML [78,79]. The concept of adoptive immunotherapy was pursued in murine and canine models leading to clinical protocols [80–84]. Countless modifications of reduced intensity regimens have been developed over the past decade, some containing low-dose total body irradiation plus chemotherapy and others consist of combinations of various drugs. The use of these rather non-toxic preparatory regimens has allowed the use of HCT for older patients or those with comorbid conditions who previously had not been considered suitable transplant candidates. It was demonstrated in a large group of 372 uniformly treated patients who were between 60 and 75 years old that age is no longer a barrier to successful allogeneic HCT [85]. These RIC transplant procedures can be performed most often on an outpatient basis and require only infrequent transfusional support. The need for immunosuppressive drugs is similar to that in patients prepared with high-dose preparatory regimens [86].

After several decades of extensive pre-clinical research in mice, investigators were finally able to utilize the experimental principle first described by Main and Prehn [11], and by Mannick et al. [22], that is, the induction of tolerance for solid organ transplantation by the administration of donor hematopoietic cells [87,88]. In a recent series of 16 patients with end-stage renal disease who were conditioned with total lymphoid irradiation and anti-thymocyte globulin, kidney transplants and hematopoietic cells enriched with T cells from HLA-matched donors were administered [89]. Fifteen patients developed multi-lineage chimerism without GVHD. It was possible to discontinue all immunosuppression in eight patients who have now been followed off medications for 16–40 months, four patients have just completed or are in the midst of drug withdrawal, and four patients were not withdrawn due to return of their underlying renal disease or because of rejection episodes. At the last observation point, all 16 patients had an excellent kidney graft function with or without maintenance drugs [89].

History of autologous HCT

1958–1964: From pre-clinical studies to first clinical transplant efforts

The concept of storing autologous hematopoietic cells while patients are being exposed to high doses of irradiation and/or high-dose cancer drugs with subsequent cell reinfusion was developed over half a century ago. The initial studies were performed in canine or murine models [25,90–96]. First, glycerol was used to protect hematopoietic cells from the destructive effects of freezing and thawing, later dimethyl sulfoxide was successfully employed as a cryoprotectant and is still the preferred chemical agent for this purpose. Storage of marrow cells without cryopreservation was also shown to be possible. However, with preparatory regimens becoming more complex and more time-consuming to administer, cryopreservation of hematopoietic cells evolved into the generally accepted storage technology.

In the beginning, preparatory regimens for clinical use contained mostly high-dose irradiation and/or alkylating drugs (nitrogen mustard or cyclophosphamide). The first series of autografted patients were reported between 1959 and 1962 [97–100]. Hematopoietic function was restored in most of these patients and transient tumor responses were frequently observed, mostly in patients with non-Hodgkin or Hodgkin lymphoma.

1978–1995: The beginning of an era of promising results in autologous HCT

With reliable cryopreservation methods for storage of bone marrow cells and an increasing number of drugs becoming available, investigators began to explore combinations of dose-escalated anticancer agents [101]. In 1978, Appelbaum and colleagues [102,103] described their experience with the quadruple combination of carmustine, cytarabine, cyclophosphamide and thioguanine followed by autografting in patients with non-Hodgkin lymphoma. Transplanted patients recovered their blood counts significantly faster than control patients and several of the transplant recipients entered durable remissions and were apparently cured of their lymphoma.

Another drug combination of cyclophosphamide, carmustine, and etoposide [104] was tested successfully in patients with Hodgkin lymphoma and has become a widely employed regimen, sometimes cytarabine is added as a fourth drug. This regimen was modified by replacing cyclophosphamide with melphalan and is now widely used at transplant centers around the world, especially for patients with lymphoid malignancies.

A pivotal clinical study was performed by Philip and colleagues which became known as the PARMA trial. The investigators succeeded in their efforts to compare the outcome of autologous HCT to conventional chemotherapy in non-Hodgkin lymphoma patients: HCT recipients attained significantly superior event-free survival compared to the conventionally treated group [105], see Figure 1.3.

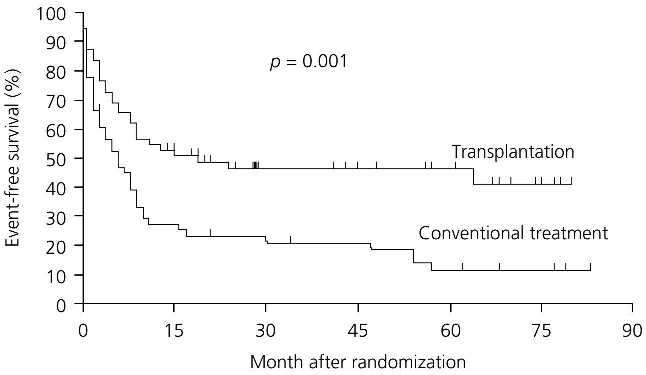

Figure 1.3 Actuarial event-free survival of patients with non-Hodgkin lymphoma treated on the PARMA trial. In this clinical study, the outcome of autologous HCT was compared prospectively to conventional dose chemotherapy. The shown data are based on an intent-to-treat analysis. Source: Philip et al. 1995 [105]. Reproduced with permission of the Massachusetts Medical Society.

Autologous transplantation for the acute leukemias was also actively explored, ultimately leading to a series of prospective clinical trials in which standard dose chemotherapy was compared to high-dose regimens followed by autologous HCT. The data unfortunately show rather inconsistent results with highly positive studies conflicting with negative outcomes data from other trials. New regimens have been developed for patients with acute myeloid leukemia, for example, the high-dose busulfan-etoposide regimen which is well tolerated and has led to promising results that are now being tested under cooperative group conditions [106–108].

1986–2004: Circulating stem cells and cloned hematopoietic growth factors

It was known from earlier studies in mice [24], dogs [109], and baboons [110] that cells from circulating blood could restore bone marrow function in lethally irradiated animals. The use of multiple apheresis procedures allowed the successful clinical use of autologous grafts [111]. Initial approaches to increase the number of stem cells in the peripheral blood included the administration of small amounts of endotoxin [112]. Other investigators exploited the rebound effect on the release of stem cells from bone marrow in patients recovering from chemotherapy [113,114]. However, major progress had to wait for the introduction of cloned hematopoietic growth factors, the granulocyte–macrophage colony-stimulating factor (GM-CSF) and the already mentioned factor, G-CSF [115–117]. Over time, it became a standard approach to administer cyclophosphamide followed by G-CSF or G-CSF alone and apheresis for the collection of autologous hematopoietic grafts. The presence of peripheral blood stem cells can be monitored by measuring $CD34^+$ cells as a surrogate marker. Between 2 and 5 million $CD34^+$ cells/kg body weight of the recipient are considered an adequate number of “stem cells” to proceed to transplantation. The question of which is the preferred source for an autologous graft, bone marrow or peripheral blood, has been answered, at least in the treatment of lymphoma, by two appropriately designed clinical trials which both showed the superiority of the graft collected by apheresis [118,119]. The criteria for speed of restoration were the recovery from white blood cell and platelet nadirs which has resulted in lower morbidity and mortality following autologous HCT. Patients who mobilize "stem cells" poorly can still respond favorably to a new CXCR4 antagonist [120].

1982–2001: Testing for minimal residual disease and efforts to "purge" grafts for autologous HCT

It was – and still is – a serious concern that autologous cell collections aspirated from the marrow cavity or collected from blood by apheresis contain tumor cells which – if clonogenic – may contribute to relapse of the underlying malignancy. The presence of tumor cells in cell collections was demonstrated by immunohistochemistry, bone marrow cultures, and by molecular methods [121–124]. Gene-marking studies performed by Brenner and colleagues [125] indicated that such marked cells were detectable in the patient at the time of relapse after autologous transplantation.

Tumor cell "purging" was attempted and clinically tested in multiple graft engineering laboratories around the world. Methods for *negative* selection employed immunologic, as well as pharmacologic principles: monoclonal antibodies (mAb) with complement, mAbs conjugated to magnetic beads, or toxins or drugs, or drugs only, such as 4-hydroperoxycyclophosphamide or mafosfamide [126–142]. Still other investigators developed and tested devices such as columns to separate tumor cells from marrow aspirates or from blood concentrates collected by apheresis [143–147].

Positive stem cell isolation techniques were pursued in mice and humans [148,149] and such highly purified cell preparations were administered to patients after they had received high-dose irradiation or combination chemotherapy [150–153]. A few stage IV breast cancer patients who were autografted with these cell preparations have become long-term survivors and are in continued complete remission more than a decade after their transplant procedure [154].

The diseases in which the different negative and positive selection methods were used included non-Hodgkin lymphoma, acute myeloid leukemia, acute lymphoblastic leukemia, multiple myeloma, neuroblastoma, and breast cancer. Well-designed, large prospective trials to prove convincingly the superiority of "purged" grafts are unfortunately missing from the clinical scientific literature.

1986–1999: High-dose chemotherapy and transplantation of autologous hematopoietic cells for patients with breast cancer

Initially, high-dose chemotherapy and autologous transplantation was tested in women who had stage IV breast cancer. Tumor responses were attained in some patients, however, these favorable reactions were only transient. During the 1990s, several high-dose regimens were established in large cancer treatment centers and were soon used worldwide in women with stage II or stage III breast cancer [155,156]. This approach to breast cancer was so frequently employed that in 1994–1995, about 40% of all autologous transplant procedures were performed in patients with breast cancer. This rapid increase in use of the procedure was not based on any solid data from appropriately designed phase III trials. Several prospective trials were done concomitantly during the last decade of the twentieth century. In 1999, the first reliable interim analyses of several comparative studies indicated that no survival benefit was to be expected and the use of autologous transplantation for breast cancer plummeted. A subsequent meta-analysis of 15 randomized trials of 6102 patients with high-risk disease detected a significant advantage with respect to event-free survival, but the difference in overall survival at 5 years after transplantation did not reach statistical significance [157]. Earlier, a clinical phase III study had indicated equivalence in outcome of autologous HCT compared to conventional chemotherapy in patients with metastatic breast cancer [158]. Finally, the revelation of scientific fraud [159,160] in the reports on positive clinical trials from one center [161] led to a dramatic reduction of HCT worldwide for the treatment of women with breast cancer.

1993–present: Research to improve preparatory regimens and efforts to consolidate post-HCT remissions

The single purpose of the preparatory regimen in autologous HCT is the permanent eradication of tumor cells: the goal of this step is the induction of a durable remission, that is, cure. However, relapse of the underlying disease is the major cause for treatment failure. Therefore, investigators have attempted to intensify the pretransplant regimens by further dose escalation of drugs already established for this purpose, or by adding new agents to existing high-dose combinations. Alternatively, investigators have explored high-dose sequential therapies with infusion of autologous hematopoietic cells after each treatment cycle [162]. Dose-limiting for all these efforts were toxicities to vital, non-hematologic organs such as liver, lung, heart, and the central nervous system.

Another rather elegant – because more targeted – approach is the incorporation of radio-labeled mAbs into preparatory regimens which has been done successfully for patients with non-Hodgkin lymphoma [163–165]. Unfortunately, these promising technologies are not yet widely used, mostly due to the need for experienced nuclear medicine specialists and rather sophisticated equipment.

Finally, the option to consolidate the transplant-induced complete remission with immunologic techniques has been actively pursued. For this purpose, cytokines (interleukin-2 or interferon) mAbs, irradiation to areas of prior "bulk" disease, idiotype vaccines or "educated" autologous cells such as cytokine-induced killer cells have been employed. These studies were mostly performed as clinical feasibility trials. However, the data following the use of rituximab after autologous HCT for non-Hodgkin lymphoma are impressive [166] and long-term disease-free survival at a high rate has been attained [167], see Figure 1.4.

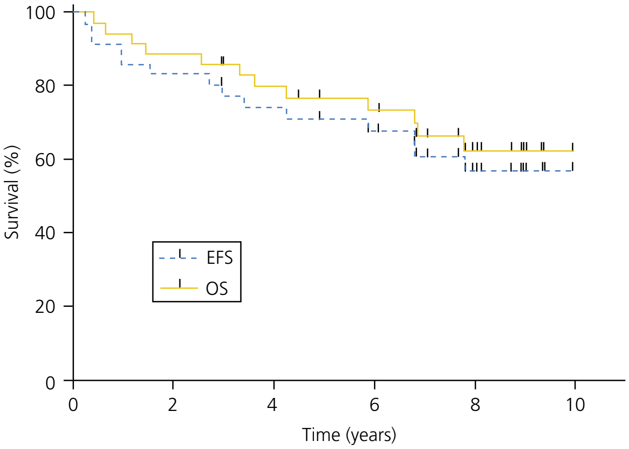

Figure 1.4 Kaplan–Meier estimates of event-free survival (EFS) and overall survival (OS) of 35 patients with recurrent or refractory B-cell lymphoma. These patients received autologous transplants and were given two courses of rituximab (4 weekly infusions, 6 weeks and 6 months following transplantation). Source: Weng et al. 2010 [167]. Reproduced with permission of the American Society of Clinical Oncology.

Conclusion

What has been accomplished in the field of allogeneic and autologous HCT during a half century of intense pre-clinical and clinical research? The answer to this question is: Long-term disease-free survival and cure in a substantial number of patients. Forty years ago, survival of our patients was measured in “days after transplantation” while now disease-free survival is expressed in years – or even – decades after HCT. In 2012, the 1,000,000th patient received a hematopoietic cell transplant (source: M.M. Horowitz, Center for International Blood and Marrow Transplant Research; see also Chapter 2 of this book). That means that 1,000,000 children, adolescents, and adults whose life expectancy was rather limited because of a serious disease were offered a chance for cure. This does not mean that all problems surrounding HCT have been solved during the past decades. Table 1.1 lists the problem areas and obstacles to success which patients and physician-investigators faced when transplantation became a treatment reality in the 1960s. A review of the list of problems in 2012 indicates that remarkable progress has been made in all areas:

1. It is now possible to offer HCT to older and medically infirm patients.
2. Through intense national and international efforts, the large number of volunteer donors and cord blood concentrates make it now possible to offer HCT to many patients who are lacking a suitably matched related donor.
3. Efforts to reduce extramedullary toxicities have been largely successful, especially through the introduction of reduced intensity regimens for allogeneic HCT and many transplant recipients never require admission to the hospital.
4. Graft failure is now a rare event because of (a) better tissue typing methods to identify the most suitably matched donor, and (b) the ability to mobilize and collect sufficient cells for autologous HCT.
5. Great strides have been made through preemptive therapy of cytomegalovirus infections. Potent new antibiotics and better antifungal agents have been introduced.
6. The greatest obstacle in allogeneic HCT remains graft-versus-host disease; however, potent immunosuppressive drugs have been developed and successfully added to our armamentarium.
7. Resistant malignant cells are difficult to eliminate and may lead to a relapse of the former disease after allogeneic or autologous HCT. On the other hand, harnessing the graft-versus-tumor effect is becoming increasingly possible and may be regularly attainable without GVHD in the near future.
8. Long-term observations have revealed the risk of new malignancies occurring after HCT. But we should also keep in mind that all treatments following the original diagnosis of cancer have a cumulative effect toward second malignancies, that is, it is not just the transplant effort which is the sole cause for new cancer.
9. Another painful lesson was learned from years and even decades of follow-up of HCT recipients who over time have developed heart, lung, and kidney diseases, as well as diabetes mellitus at a significantly higher rate than age-matched controls.
10. Many carefully performed studies have demonstrated that the quality of life of our patients is fortunately quite good. By far the majority of them consider the step to proceed to HCT as a very positive decision in their life.
11. Cost and charges for HCT are stunning as they are extremely high in most parts of the world. Leaders in hospital administrations should be urged constantly to use their best judgment pertaining to reasonable pricing of medical procedures.
12. Here is a final message from the authors of this chapter to all young readers, colleagues, and investigators in the field: Persistence in high-quality clinical research has allowed hematopoietic cell transplantation to become a procedure which can be widely offered with curative intent and curative potential. Transplantation of blood-forming cells will continue to evolve and develop. The future of HCT is really bright!

Table 1.1 Problem areas of allogeneic and autologous hematopoietic cell transplantation. Problems are listed in chronological order as they occur in the course of the respective transplant procedure

Allogeneic	Autologous
Age limit	Age limit
Availability of a suitable donor	Regimen-related toxicity
Regimen-related toxicity	Graft failure
Graft failure/rejection	Opportunistic infections
Opportunistic infections	Relapse
Graft-versus-host disease	New malignancies
Relapse	Long-term health problems
New malignancies	Quality of life issues
Long-term health problems	Cost/charges
Quality of life issues	
Cost/charges	

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CHAPTER 2

Uses and Growth of Hematopoietic Cell Transplantation

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Introduction

The first successful transplantations of allogeneic hematopoietic cells were performed in 1968 in three children with congenital immune deficiency diseases [1–4]. In each instance, hematopoietic cells were collected from the bone marrow of sibling donors who were genotypically identical or closely matched to the recipient for human leukocyte antigens (HLAs). Since then, more than 1,000,000 patients have received hematopoietic cell transplantations (HCTs) to treat life-threatening malignant and nonmalignant diseases. Current estimates of annual number of HCTs are 55,000–60,000, worldwide (Figure 2.1). Reasons for widespread use include proven and potential efficacy in many diseases, better understanding of the appropriate timing of transplantation and patient selection, greater availability of donors, greater ease of hematopoietic progenitor cell collection, and improved transplantation strategies and supportive care, leading to less transplantation-related morbidity and mortality and an increased ability to perform the procedure in older and sicker patients.

Changing indications for HCT

HCT has efficacy in many diseases (Table 2.1). In some, transplantation corrects congenital or acquired defects in blood cell production and/or immune function. In others, it restores hematopoiesis after high-dose (myeloablative) cytotoxic therapy for malignancy and/or provides potent anticancer adoptive immunotherapy. In the 1970s, more than half of the HCTs were performed for nonmalignant disorders: 40% were for aplastic anemia and 15% for immune deficiencies. Fewer than half were for cancers, and these were mostly for advanced acute leukemia. In the 1970s, Thomas and colleagues showed convincingly that some patients with refractory acute leukemia could achieve long-term leukemia-free survival with high-dose therapy and HLA-identical sibling marrow transplantation (see Chapter 1) [5,6]. Better outcome was subsequently demonstrated in patients transplanted in first or second remission [7–9]. Syngeneic (identical twin) and allogeneic HCTs were shown to produce cytogenetic remissions and long-term leukemia-free survival in chronic myeloid leukemia (CML) in the late 1970s and early 1980s [10–12].

Use of allogeneic HCT to treat leukemia increased dramatically in the 1980s. By 1985, about 75% of allogeneic transplantations were for leukemia, with approximately equal numbers for CML, acute myeloid leukemia (AML), and acute lymphoblastic leukemia (ALL); more than 90% were from HLA-identical sibling donors. Introduction of alternative targeted therapies for CML in the early 2000s led to decreased use of HCT, with current guidelines reserving HCT for patients who fail to respond to non-transplant therapy or who lose their response [13]. However, leukemia treatment still accounts for about 65% of allogeneic HCT procedures (Figure 2.2) (Center for International Blood and Marrow Transplant Research (CIBMTR) Statistical Center, unpublished data).

Experimental and clinical evidence for a dose–response effect of drugs used in lymphoma therapy led to trials of autologous HCT to allow dose intensification in non-Hodgkin lymphoma in the middle 1980s (see Chapter 1) [14–16]. Results were promising, and there was rapid acceptance of autologous transplantation as salvage therapy in persons failing conventional chemotherapy for lymphoma. This was followed by increasing use of autologous transplantation as consolidation of primary therapy in patients with high-risk disease. There is now more frequent use of allogeneic transplantation in lymphoma to harness immune-mediated graft-versus-lymphoma effects, especially in patients whose disease recurs after autologous transplantation, and in patients with follicular histology who are rarely cured by autologous transplantation [17]. Lymphoma accounted for 10% of allogeneic HCTs in 2011 (CIBMTR Statistical Center, unpublished data).

The rationale of dose intensification led to the application of high-dose therapy with autologous transplantation to many other hematologic and nonhematologic cancers over the past 10 years. One striking development was a dramatic increase in their use for breast cancer in the early 1990s. Breast cancer accounted for 16% of autologous transplants carried out in 1989–1990 and 40% in 1994–1995 [18]. However, results of randomized clinical trials in early and advanced breast cancer were disappointing (reviewed in [19–21]). In 1999, the use of HCT for breast cancer declined dramatically, and in 2011 breast cancer accounted for fewer than 5% of autologous transplants in North America. Treatment of solid tumors, primarily neuroblastoma and testicular cancer, still accounts for 5–10% of autologous transplants (Figure 2.2).

In 1996, results of a randomized trial comparing high-dose therapy and autologous HCT with conventional therapy for multiple myeloma were published; this study found a significant survival benefit with autologous transplantation [22]. Autologous HCT for myeloma increased dramatically after this report. Subsequent data suggested improved results with sequential autologous transplantations or autologous transplantation followed by allogeneic transplantation [23,24]. Multiple myeloma is now the single most

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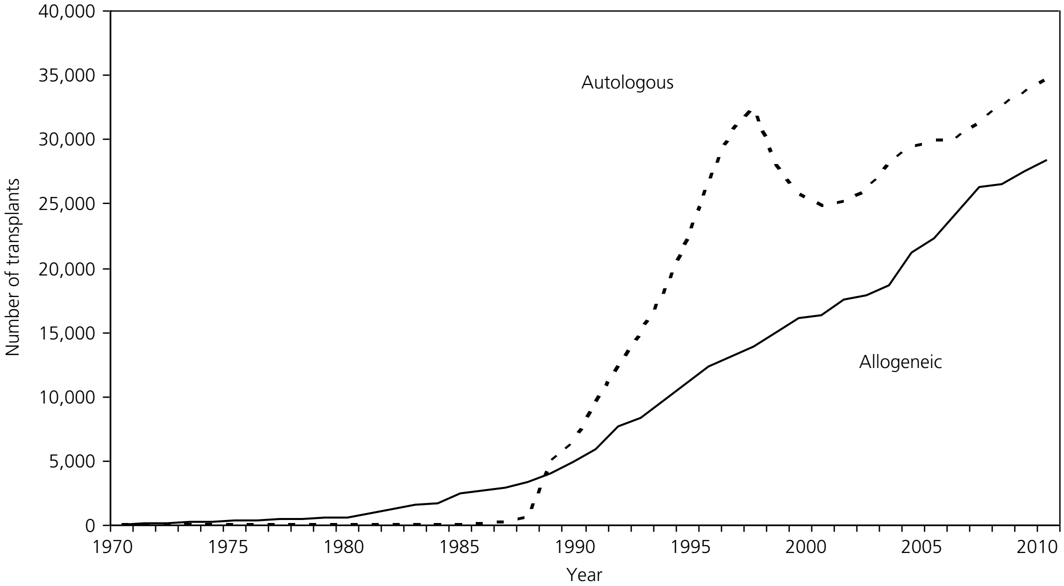

Figure 2.1 Numbers of allogeneic and autologous hematopoietic cell transplantations performed yearly worldwide. Data from the CIBMTR Statistical Center.

Table 2.1 Diseases in which autologous and/or allogeneic hematopoietic cell transplants may be used

Malignant	Nonmalignant
Leukemia/preleukemia	Severe aplastic anemia
Chronic myeloid leukemia	Paroxysmal nocturnal hemoglobinuria
Myeloproliferative syndromes (other than chronic myeloid leukemia)	Hemoglobinopathies
Acute myeloid leukemia	Thalassemia major
Acute lymphoblastic leukemia	Sickle cell disease
Juvenile chronic myeloid leukemia	Congenital disorders of hematopoiesis
Myelodysplastic syndromes	Fanconi anemia
Therapy-related myelodysplasia/leukemia	Diamond–Blackfan syndrome
Kostmann's agranulocytosis	Familial erythrophagocytic histiocytosis
Chronic lymphocytic leukemia	Dyskeratosiscongenita
Non-Hodgkin and Hodgkin lymphoma	Shwachman–Diamond syndrome
Multiple myeloma	Severe combined immune deficiency and related disorders
Solid tumors	Wiskott–Aldrich syndrome
Breast cancer	Inborn errors of metabolism
Neuroblastoma	Acquired autoimmune diseases
Sarcomas	
Ovarian cancer	
Small cell lung cancer	
Testicular cancer	

common indication for HCT, accounting for 50% of autologous transplants but fewer than 5% of allogeneic transplants (Figure 2.2). Although 30% of HCTs for myeloma in 2006 involved a planned autologous–autologous or autologous–allogeneic transplant approach, this strategy is currently controversial and only about 10% of HCTs for myeloma in 2011 involved a sequential approach (CIBMTR Statistical Center, unpublished data) [25]. The role of planned second transplants decreased to about 10% in 2011 and remains controversial [25].

The most common indications for allogeneic and autologous HCT in North America in 2011 are shown in Figure 2.2. About 75% of allogeneic HCTs are for leukemia or myelodysplasia: 37% for AML, 16% for ALL, 4% for CML, 14% for myelodysplastic or myeloproliferative syndromes, and about 5% for other leukemias. Eleven percent are for other cancers, including non-Hodgkin lymphoma (9%), multiple myeloma (<5%), Hodgkin disease (<5%), and other cancers (<5%). The remainder are for aplastic anemia (5%), immune deficiencies (<5%), and other diverse nonmalignant disorders. The most common indications for autologous transplants are multiple myeloma (50%), non-Hodgkin lymphoma (28%), Hodgkin disease (10%), leukemia (<5%), neuroblastoma (<5%), and other cancers (CIBMTR Statistical Center, unpublished data).

There is increasing interest in using HCT in several diseases where transplantation was not or was rarely used in the past, some with promising results in either anecdotal reports or phase II studies. These include sickle cell disease (see Chapter 73), inborn errors of metabolism (see Chapter 75), chronic lymphocytic leukemia (see Chapter 60), solid tumors such as ovarian, renal cell, and small cell lung cancer (see Chapter 63), and autoimmune diseases such as multiple sclerosis, systemic lupus erythematosus, systemic sclerosis, and severe rheumatoid arthritis (see Chapter 67). Taken together, these diseases currently account for fewer than 10% of HCTs, whether allogeneic or autologous, up from fewer than 5% five years ago. Several large trials of HCT for autoimmune disease are being conducted in Europe and in the United States. The prevalence of these diseases is high and, if trials confirm efficacy, the numbers of persons considered for HCT could increase dramatically. Hematopoietic cells are also ideal candidates as vehicles for gene therapy, and their use in this capacity is being explored in several settings (see Chapter 8). Finally, hematopoietic stem cells are now being used for the regeneration of non-hematopoietic tissues, including myocardium and nerves, in some phase I and II studies [26].

Changes in patient selection

In the 1970s, marrow transplants were often applied as desperate measures for desperate situations. Not infrequently, patients came to transplantation with longstanding, refractory cancers, with active infection, after receiving multiple transfusions, and with poor performance status.

The available drugs for prophylaxis of graft-versus-host disease (GVHD) were not optimal, nor were supportive care measures, especially antiviral and antifungal therapy. Not surprisingly, these procedures were associated with high risks for graft failure, GVHD,

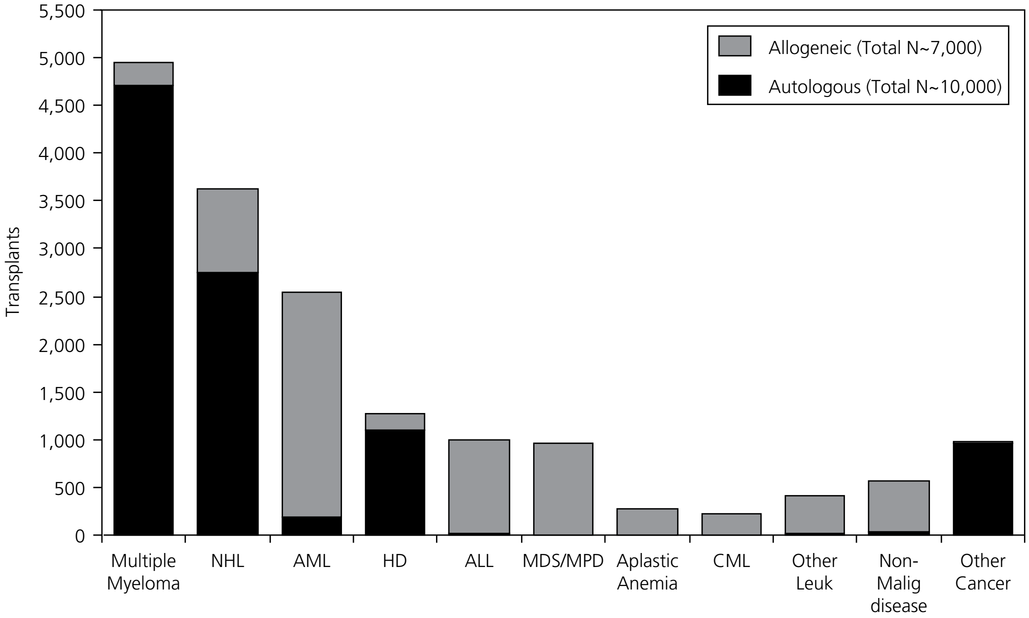

Figure 2.2 Indications for hematopoietic cell transplantation in North America, 2011. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDS/MPS, myeloproliferative disorder/myelodysplastic syndrome; NHL, non-Hodgkin lymphoma. Data from the CIBMTR Statistical Center.

infectious and non-infectious pneumonitis, non-pulmonary infections, and other complications, all leading to high transplant-related mortality. Much of the acceptance and growth of transplantation is attributable to a better understanding of factors leading to improved transplantation outcomes, especially selection of appropriate patients for transplantation at a point in their disease course when transplantation is most likely to be of benefit. Many studies in diverse diseases demonstrate that HCTs done earlier are associated with lower risks of both transplant-related mortality and disease recurrence.

Transplantation is now commonly applied as first- or second-line rather than "last chance" therapy. In the 1970s, only 20% of HLA-identical sibling HCTs for acute leukemia were carried out in first remission, while 60% were done for advanced disease (not in remission or in a third or subsequent remission). In contrast, in 2011, about 60% of HCTs for acute leukemia were done for patients in first remission, 20% in second remission, and only 20% for advanced disease (CIBMTR Statistical Center, unpublished data). Although delay beyond first remission may be appropriate for patients with standard-risk ALL or good-risk AML (who may have good outcomes with conventional therapy), most young patients with AML are probably best served by allogeneic transplantation carried out soon after diagnosis if a suitably matched donor, related or unrelated, is available. Such a strategy is not only associated with better outcome, but also decreases the chance that patients will develop an intervening complication (refractory relapse, life-threatening infection, or organ toxicity) that will preclude transplantation.

There has also been a trend for earlier use of autologous transplants. In 1989, the median interval between diagnosis and autologous transplantation for non-Hodgkin lymphoma was 23 months; in 2011, it was 14 months (CIBMTR Statistical Center, unpublished data). Similarly, in 1990, the median interval between diagnosis and autologous transplantation for multiple myeloma was 18 months; in 2011, it was 8 months. This change is attributable largely to the demonstration of efficacy of autologous transplantation as part of primary treatment or as first salvage therapy (see Chapters 56–59) for myeloma and lymphoma, leading to use earlier in the course of these diseases.

Earlier treatment accounts, in part, for the fact that patients in 2011 were less likely to have poor performance status at the time of transplantation than were patients in the 1970s. About 40% of patients transplanted in 1974–1979 had pretransplant Karnofsky scores of less than 80%, compared with 10% in 2011 (CIBMTR Statistical Center, unpublished data).

HCT is now used in much older patients than in the 1970s, when the median age of transplant recipients was 17 years and fewer than 5% were older than 40 years. In 2006, the median age of allograft recipients was 39 years, and of autograft recipients 56 years; in 2011, the corresponding figures were 47 and 56 years (CIBMTR Statistical Center, unpublished data). This is important since the diseases for which HCT is most frequently applied have their onset in older adulthood, often in the sixth decade. The ability to apply HCT in older patients makes it a useful treatment for many more patients. Autologous transplants have been successfully used in patients older than 70 years. Eighty-two percent of autologous transplant recipients in 2011 were over the age of 40 years, and 41% were older than 60 years. Allogeneic HCT is also being used in older patients, with the introduction of better GVHD prophylaxis, less toxic conditioning, and improved supportive care. Fifty-nine percent of allograft recipients in 2011 were older than 40 years, in contrast to only 28% in 1995. Forty-five percent of the allogeneic HCTs carried out in 2011 were in patients older than 50, and 20% were in patients older than 60 years. Several studies suggest that, among adults over the age of 30, increasing age has only modest effects on transplantation outcome, at least up to the age of 50 [27,28].

One recent approach to increase use of allografts in older adults is reduced-intensity or non-myeloablative conditioning. This strategy uses conditioning as immunosuppressive therapy to allow donor cell engraftment rather than as high-dose anticancer therapy. The approach relies on immune-mediated graft-versus-tumor effects for long-term disease control (see Chapter 21). First reported in the late 1990s, transplants using reduced-intensity conditioning now account for about 35% of allografts registered with the CIBMTR and 60% of allografts in patients over the age of 55 years. Data on long-term outcome or efficacy relative to conventional HCT are still lacking. A large randomized trial of high versus reduced intensity conditioning for patients receiving HLA-identical sibling or unrelated donor transplantation for AML or MDS is currently being conducted by the Blood and Marrow Transplant Clinical Trials Network.

Hematopoietic cell sources

In the 1970s and early 1980s, essentially all HCTs used cells collected from the marrow of closely HLA-matched related donors. The few transplantations done with HLA-mismatched related donors were associated with high risks of graft failure, GVHD, and poor outcome, except, in some series, when the disparity was limited to a single HLA antigen [29,30]. This limited application of HCT to the 25–30% of patients with an HLA-matched relative. Several developments dramatically increased the applicability of HCT to patients without HLA-identical relatives, including the use of autologous cells, collection of cells from blood rather than marrow, and use of unrelated donors.

Autologous transplantation

Although a few autologous transplantations were carried out before the 1970s, the approach of collecting cells from a patient before high-dose therapy with reinfusion afterwards did not generate much enthusiasm until the middle to late 1980s (see Chapter 1) [31]. The appeal of the approach rested in its applicability, since it did not require a donor, allowing dose intensification with hematopoietic cell support in many patients with chemotherapy-sensitive cancers. Autologous transplants were also associated with lower transplant-related mortality since GVHD did not occur and immune recovery was more rapid than after allografting. Although immune-mediated antitumor effects were also absent, and there was some concern about reinfusing cancer cells with the graft, early trials showed good results in persons with lymphomas failing other therapies. The technology diffused rapidly in the late 1980s, becoming the treatment of choice for those with relapsed lymphoma and increasingly used for other chemotherapy-sensitive but infrequently cured cancers, including acute leukemia, multiple myeloma, and selected solid tumors.

An important development allowing diffusion of the technology was the demonstration that hematopoietic cell grafts could be collected from peripheral blood in a limited number of leukaphereses after mobilization with chemotherapy or growth factors such as granulocyte- or granulocyte/macrophage-colony stimulating factor [32–34]. The resulting hematopoietic cell products contained large numbers of progenitor cells and led to faster hematopoietic recovery after transplantation. It also eliminated the need for a marrow harvest in an operating room. Whereas in 1989–1990, 85% of autologous transplants used cells collected from marrow, by 2000 fewer than 5% used marrow cells alone (CIBMTR Statistical Center, unpublished data). In contrast to allogeneic transplantations, many of which are still done in academic, tertiary care medical centers, autologous transplants were soon used in the community setting, partly because of the ease with which the hematopoietic cell product could be obtained, and partly because of the more rapid hematopoietic and immune recovery and lower frequency of transplant-related complications compared with allografting. This likely increased the numbers of patients offered HCT.

Allogeneic transplantation

There were anecdotal reports of successful HCTs using HLA-matched unrelated donors as early as 1973 [35–37], but the polymorphism of human HLA made the feasibility of finding such a donor for an individual patient low. In the middle 1980s, several national and international groups organized marrow donor registries with panels of HLA-typed volunteers agreeing to serve as donors for unrelated patients. There are about 20 million HLA-typed volunteer donors worldwide (see Chapter 37). Accessing these donors is facilitated by their listing in a compendium by Bone Marrow Donors Worldwide (www.bmdw.org), a collaborative effort of many national registries coordinated by the Europdonor group (www.europdonor.org), and by rapid computerized searching made available by several national and international registries. The largest donor registry is the US National Marrow Donor Program (NMDP; www.marrow.org), which has about 11 million donors on its file. The NMDP also has a web-based search application available for patients to perform a preliminary search (MatchView^{sm}) and has implemented an application, Haplogic^{TM}, to assist transplant physicians in estimating the probability of finding a fully matched unrelated donor for an individual patient.

Currently, Caucasian patients have a 70% or greater chance of finding a donor that is matched at high-resolution typing for HLA-A, B, C, and DRB1 through existing donor panels. Because of greater HLA polymorphism and fewer ethnically similar donors, patients in other groups have lower probabilities of finding an HLA-matched unrelated donor, but 50–70% will find a donor mismatched at only one of these loci, which may afford an acceptable if not optimal alternative [38].

The establishment of large donor panels dramatically increased the use of unrelated donor transplantation. While in 1985 fewer than 10% of allogeneic HCTs were from unrelated donors, in 2011 more than 40% were from unrelated donors. Unrelated donor transplantation accounted for much of the growth of HCT in the United States over the past decade (Figure 2.3). In some settings, survival after unrelated donor transplantation is similar to that after HLA-identical sibling transplantation, although the risks of graft failure and GVHD are higher (see Chapter 46). Unrelated donor HCT is associated with longer delays between diagnosis and transplantation, partly because of the time needed for the donor search and evaluation process. Delays are caused by reluctance on the part of some physicians to use what has historically been a more difficult transplantation approach early in disease. Recent data, however, suggest that HLA-matched unrelated donor transplantation should be considered whenever HLA-identical sibling transplantation is indicated [39].

As recently as 2002, most allogeneic HCTs used cells collected from marrow. For many years, there was reluctance to use cells collected from peripheral blood because of the large numbers of mature T lymphocytes, the cells that mediate GVHD, in blood-derived grafts. However, in 1995, three centers reported rapid hematopoietic recovery and acceptable acute GVHD after HLA-identical related peripheral blood allografts in a small number of patients [40–42]. A rapid increase in allogeneic peripheral blood HCTs followed. About 75% of related donor and 60% of unrelated donor HCTs now use cells obtained by leukapheresis.

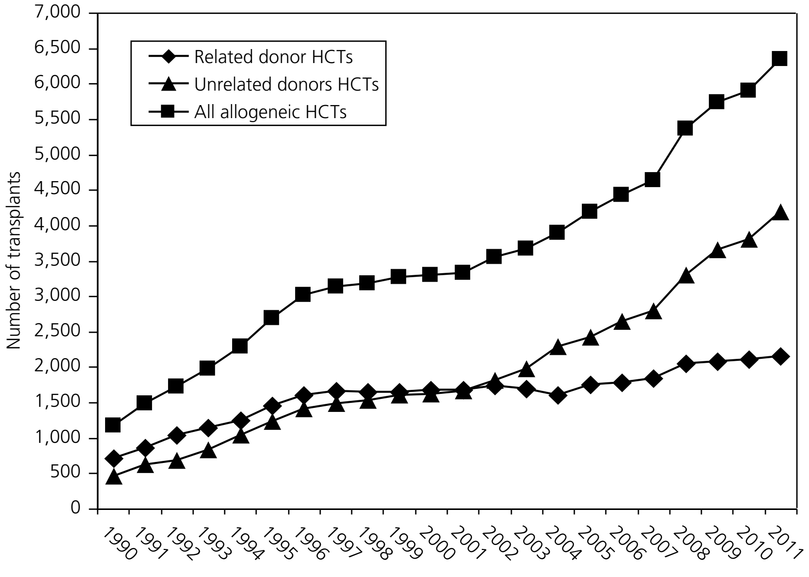

Figure 2.3 Annual numbers of allogeneic hematopoietic cell transplantations (HCTs) in the United States reported to the Center for International Blood and Marrow Transplant Research. Data from the CIBMTR Statistical Center.

Several randomized trials have compared the results of peripheral blood and marrow HCTs in the related donor setting [43,44]. Some, but not all, suggest an early survival advantage with peripheral blood HCT in patients with advanced disease. However, most studies also indicate an increased risk of chronic GVHD, associated in some studies with higher late mortality (see Chapter 42). Data about the relative efficacy of marrow and peripheral blood transplants in the unrelated donor setting are more limited. An observational study of unrelated donor transplant recipients conducted by the CIBMTR and the NMDP suggested that survival was similar, but GVHD more frequent with peripheral blood versus bone marrow grafts [45]. A single large randomized trial, conducted by the US Blood and Marrow Transplant Clinical Trial Network (BMT CTN), has addressed this issue in patients receiving myeloablative conditioning [46]. Among 550 patients randomized to receive blood or marrow grafts from unrelated donors, the probability of survival was virtually identical but graft failure was more common with marrow grafts while extensive chronic GVHD was more common with peripheral blood grafts. Of note, recipients of blood grafts had a 20% higher incidence of remaining on immunosuppression by 2 years after transplantation than marrow graft recipients. Whether this will change the current patterns of graft procurement remains to be seen. Data are still lacking regarding the optimal graft source when using reduced intensity conditioning.

Multiple experimental and clinical studies since the 1990s suggest that umbilical cord blood (UCB), which is rich in hematopoietic progenitors, may be a good source of allogeneic hematopoietic cells for transplantation (see Chapter 39). To be useful, there must be a large supply of cord blood units readily available. Several cord blood banks were established over the past two decades, with more than 500,000 units cryopreserved worldwide in 2012. The advantage of UCB transplantation is rapid availability of a graft without need for screening, typing and collecting cells from live donors, and the potential to target underrepresented ethnic groups for collection and storage. Most clinical data indicate that UCB transplantation has less potential for severe GVHD, even with a substantial degree of donor–recipient HLA mismatch, than adult donor transplants. UCB transplants now account for about 50% of the unrelated donor transplants carried out in children younger than 10 years old.

Hematopoietic recovery after UCB transplantation may be slow, particularly in adults and larger adolescents, in whom the cell dose (cells/kg of recipient weight) may be limiting. Consequently, UCB accounts for only 30% (increased from 20% in 2006) of the unrelated donor transplants done in children 10–19 years of age and only 15% (increased from 5% in 2006) of those in adults 20 years of age and older. However, a growing number of studies in adults have demonstrated the potential success of UCB transplantation [47–50]. Several approaches are being studied to increase the speed and certainty of engraftment. These include use of double UCB grafts, co-infusion of mesenchymal cells, co-infusion of T-cell-depleted haploidentical peripheral blood stem cells, injection of cord blood into the bone marrow directly, and cell expansion using a variety of techniques.

Use of relatives partially matched for HLAs is another way of offering HCTs to more people (see Chapter 45). In the United States, about 10% of allografts are from relatives sharing one HLA haplotype and mismatched for one or more antigens on the unshared haplotype. Results with more than one antigen disparity on the unshared haplotype were disappointing for a long time and there was little increase in the number of these transplants in the 1990s and early 2000s. Some studies suggest that the approach may be successful if T-cell-depleted grafts containing high doses of CD34 cells are administered and if donors are selected to maximize NK cell alloreactivity [51,52]. Another more recent approach involves use of unmanipulated cells with administration of high-dose cyclophosphamide 48–36 hours post-infusion to eliminate the most alloreactive cells [53,54]. The first approach is used more frequently in Europe and the second more frequently in the United States.

If alternative-donor HCTs, whether from unrelated volunteers, unrelated cord blood, or HLA-mismatched relatives, were to be applied in all or most indications currently considered appropriate for HLA-identical sibling transplantation, there would be about 13,000–16,000 allogeneic transplants yearly in the United States. This is far more than the estimated 6000–8000 now being done. It remains to be seen whether increasing awareness of the availability of unrelated donor marrow transplantation or the use of UCB or haplo identical transplants will increase these numbers. There are data to suggest that socio-economic and other, non-HLA issuesalso pose significant barriers to transplantation in suitable candidates [55,56].

Transplantation regimens and supportive care

Historically, with the exception of some transplantations for immune deficiencies, infusion of hematopoietic cells was preceded by intensive immunosuppressive and/or cytotoxic therapy. This treatment is aimed at eliminating malignant cells and, in the case of allografts, host immune cells that mediate rejection. In the 1970s and 1980s, the vast majority of HCTs for malignant disease (primarily leukemia) used a combination of high-dose cyclophosphamide and total body irradiation (TBI), with or without other drugs, for pretransplant conditioning. According to data reported to the CIBMTR, there has been a trend away from the high-dose radiation regimens used in the 1980s and early 1990s. About 35% of all allogeneic transplants for leukemia (60% of those in patients older than 60 years) are now done using reduced-intensity regimens. About 30% of allogeneic transplants for leukemia are done with high-dose busulfan, usually with cyclophosphamide with or without other drugs, and another 30% with high-dose TBI with cyclophosphamide or other high-dose chemotherapy. The remainder are carried out with a variety of high-dose chemotherapy regimens. High-dose therapy regimens for autologous transplants vary according to the underlying disease, but only a minority include TBI.

Another important change in allogeneic HCT strategy has occurred in the approach to post-transplant immune suppression. In the 1970s and early 1980s, methotrexate (MTX) was used. In the middle 1980s, the calcineurin inhibitor cyclosporine (CSP) was substituted for MTX in many centers. In the middle 1980s, a regimen combining MTX and CSP was introduced, which proved to be superior to either MTX or CSP alone for preventing GVHD [57,58]. Tacrolimus is now the more commonly used calcineurin inhibitor.The most common post-transplant immune suppressive regimen in 2011 was tacrolimus plus MTX, used for about 40% of allotransplants. Another 25% of allograft recipients received tacrolimus with mycophenolate mofetil or other drugs. About 35% received cyclosporine with MTX or other drugs. The risk of grade III–IV acute GVHD after HLA-identical sibling HCT decreased from more than 20% to less than 15% between the 1970s and the 1990s, and is less frequently fatal, accounting in part for the substantial decrease in early post-transplant mortality over the past three decades (CIBMTR Statistical Center, unpublished data) (Figure 2.4).

Also contributing to this decrease was better prevention of cytomegalovirus (CMV) disease through use of CMV-negative or filtered blood products for CMV-negative patients and prophylaxis, or early treatment, with ganciclovir in CMV-positive patients or CMV-negative patients receiving grafts from CMV-positive donors. This reduced the incidence of CMV pneumonia, a lethal complication of allogeneic transplantation, from about 10% to <2% (Figure 2.4). Noninfectious pneumonitis (idiopathic pneumonia syndrome) also decreased, possibly from decreased use of MTX and unfractionated radiation but also from better methods of identifying previously undetected micro-organisms. Use of peripheral blood hematopoietic cell grafts and growth factors has hastened hematopoietic recovery and decreased hospital stays, particularly after autologous grafts. Some of the latter are now carried out, at least partly, in the outpatient setting.

The net effect of these and other changes has been a decrease in transplant-related mortality in the first year after HLA-identical sibling HCT, according to CIBMTR data, from about 50% in 1970–1979 to about 15% in 2000–2009 (Figure 2.4). Transplant-related mortality after autologous transplants is even lower: about 5% after autologous transplants for leukemia, and <5% after autologous transplants for lymphoma and myeloma (CIBMTR Statistical Center, unpublished data). Causes of death after allogeneic and autologous transplantation are shown in Figure 2.5. Transplant-related toxicities predominate after allogeneic HCT; recurrent malignancy predominates after autologous HCT.

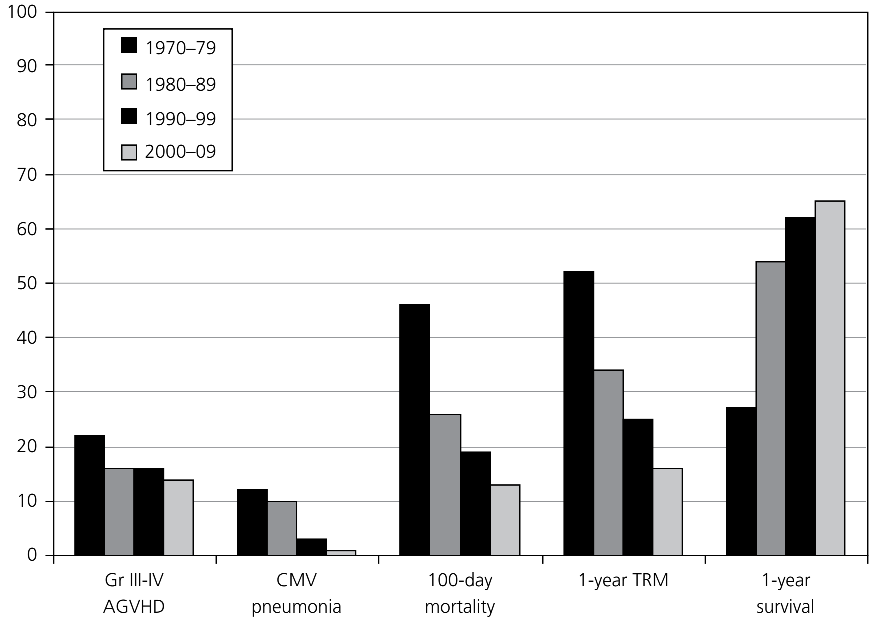

Figure 2.4 Incidence of grade III–IV acute graft-versus-host disease (AGVHD), cytomegalovirus (CMV) pneumonia, and 100-day overall mortality, 1-year transplant-related mortality (TRM), and 1-year survival after human leukocyte antigen-identical sibling hematopoietic cell transplantation (HCT) for leukemia among patients reported to the Center for International Blood and Marrow Transplant Research by year of HCT. Data from the CIBMTR Statistical Center.

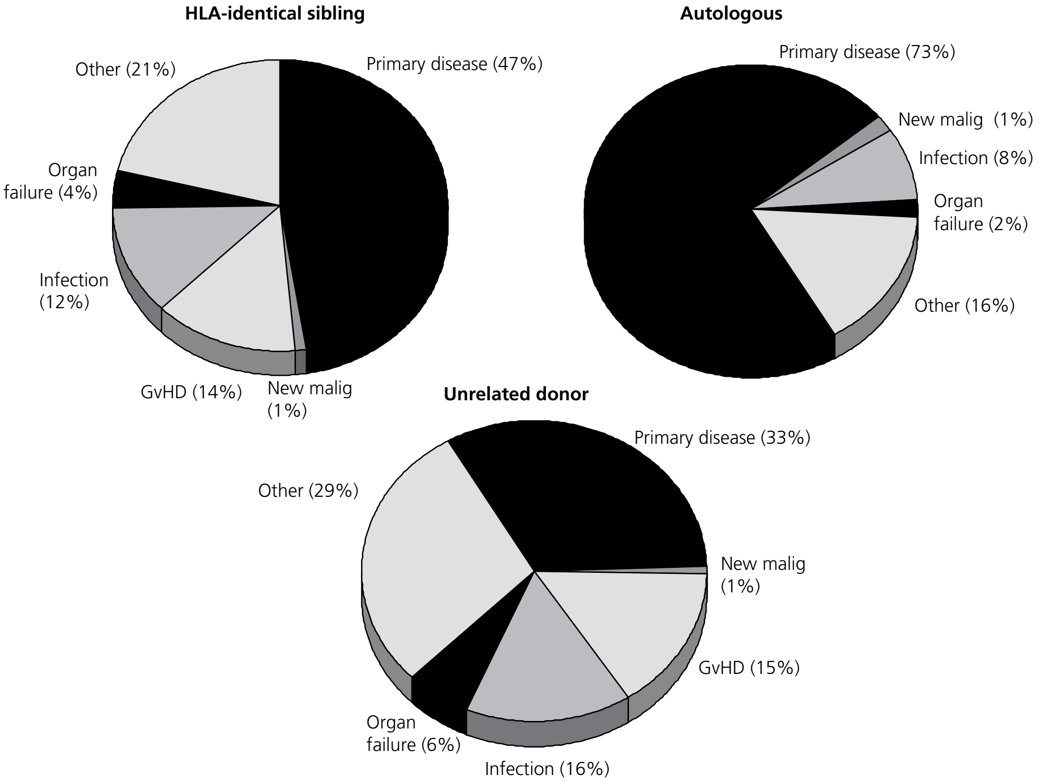

Figure 2.5 Causes of death after HLA-identical sibling, unrelated donor, and autologous hematopoietic stem cell transplantations done in 2008–2009. GVHD, graft-versus-host disease; IPn, interstitial pneumonitis.

Long-term survivors

The increasing use of HCT and better outcome of recipients means increasing numbers of long-term transplant survivors. There are now about 250,000 persons surviving 5 years or more after transplantation, and that number will grow rapidly. Most 5-year survivors are well, off all immune suppression, and leading normal lives. However, recent data suggest that the mortality rate for transplant recipients remains elevated compared to that of an age- and sex-matched general population for many years [59,60].

Transplant recipients remain at risk for late complications long after HCT (see Chapter 104) [59–61]. These include late infections, cataracts, abnormalities of growth and development, thyroid disorders, chronic lung disease, cardiovascular disease,and avascular necrosis. All of these are more frequent in patients with chronic GVHD. There is also an increased incidence of leukemias, myelodysplasias, and solid tumors in transplant recipients compared with the general population (see Chapter 105). Second cancers and other complications may not all be due to HCT per se but to the chemotherapy and/or radiation preceding the HCT. Regardless, lifelong surveillance is necessary, as is increased awareness of late complications among the many non-transplant physicians who will care for these patients. The CIBMTR and the European Group for Blood and Marrow Transplantation (EBMT) recently published guidelines for following transplant survivors [61].

Addressing barriers to use of HCT

In the first 2–3 decades of transplantation, availability of a suitable graft was a major barrier to use of HCT, particularly allogeneic HCT. Use of peripheral blood grafts for autologous transplantation, the dramatically increased availability of unrelated volunteer donors and cord blood units and improved outcomes of alternative donor transplantations now make this barrier surmountable for most patients. Yet, only about half of patients who might benefit from HCT undergo the procedure [55]. Socio-economic barriers are considerable. African Americans are significantly less likely to receive autologous, related donor or unrelated donor HCT than Caucasian patients with similar clinical characteristics [56].Lack of insurance, inadequate insurance and high out-of-pocket costs pose significant barriers to patients in the United States(reviewed in [62]). Healthcare infrastructure may also pose barriers. A recent study of allogeneic HCT activity and transplant center capacity from 2005 to 2009 in the United States showed that the number of HCTs increased 30% but HCT bed capacity increased only 17% [63]. A recent NMDP initiative to assess healthcare system capacity resulted in a series of recommendations to address barriers to transplantation, including promoting public policies that ensure adequate financial coverage for all aspects of the transplantation process [64].

Assessing and improving results of HCT

The field of transplantation has been well served by international collaboration to assess outcomes through international outcomes registries such as the CIBMTR (www.cibmtr.org) and the EBMT (www.ebmt.org). Hundreds of transplant centers have contributed clinical data on hundreds of thousands of patients, allowing observational studies to be carried out addressing many important issues in HCT (see Chapter 28). The past 10 years have seen an increasing number of multi-center prospective clinical trials related to HCT and, importantly, the establishment of networks dedicated to addressing issues in HCT. The EBMT has also established a clinical trials office to facilitate multi-center, multi-national trials. In the United States, the BMT CTN (www.bmtctn.net), a large multi-center network sponsored by the US National Heart Lung and Blood Institute and the National Cancer Institute, has accrued more than 5000 patients to trials since 2003 [65]. These initiatives have significantly increased the infrastructure available to translate our rapidly advancing knowledge in cell biology, immunology, and genetics into treatment strategies that will improve outcomes for our patients.

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SECTION 2 Scientific Basis or Hematopoietic Cell Transplantation
SECTION 2A Hematopoiesis and Stem Biology Transplantation

CHAPTER 3

Generation of Definitive Engraftable Hematopoietic Stem Cells from Human Pluripotent Stem Cells

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Introduction

In 1998, Thomson et al. described the first derivation of human embryonic stem cells (hESCs) from the inner cell mass of human blastocysts [1]. hESCs are characterized by their unique self-renewal and pluripotent capacity: they can proliferate almost indefinitely in vitro and have the capacity to differentiate into every cell type of the body. This landmark finding generated great expectations: the possibility of limitless supplies of engraftable tissues of all types, including hematopoietic tissues. Moreover, these cells represent a powerful tool to study embryonic development and lineage specification. They can also provide an unlimited resource of specific cell types for drug screening and toxicity testing.

Stem cells are divided into three categories based on their differentiation potential: totipotent, pluripotent, and multipotent (Table 3.1). Fertilized eggs and each cell of the embryo up to the four-cell stage are totipotent. In a suitable environment, they can form an entire organism including all embryonic, extra-embryonic and adult cell types. In contrast, pluripotent cells are not able to independently form a placenta, or to organize the formation of a proper primitive streak. ESCs are pluripotent cells. They can self-renew and differentiate into any cell type of the body. Somatic stem cells that are found in the tissues of adult body or in the cord blood are multipotent. They have a restricted differentiation potential.

Pluripotent cells are transiently present during embryonic development, from the late morula-stage embryo to the primitive ectoderm of the preimplantation blastocyst. True pluripotent cells are generally not found in adult organisms though some reports indicate that they may be derived from testis. While ESCs are considered the counterpart of the pluripotent cells from the embryo, it has been argued that ESCs are culture “artifacts” that do not have an equivalent in the body. Of note, some differences between pluripotent cells in vivo and those cultured have been shown, including gene expression, proliferation rate, cytokine dependence, fidelity of imprinting, and epigenetic changes [2].

In 2006,Yamanaka’s group in Japan demonstrated that somatic cells could be reprogrammed into pluripotent cells that resembled embryonic stem cells [3]. The authors introduced four genes (*Oct4*, *Sox2*, *KLF4* and *c-Myc*) into embryonic or adult fibroblasts and selected the cells that reactivated ESC-specific markers. These cells, called iPS for “induced pluripotent stem,” could self-renew and differentiate into all three germ layers. Their pluripotency was confirmed by the generation of chimeric mice and germline transmission after blastocyst injection [4,5]. Furthermore, the therapeutic potential of these iPS cells was demonstrated in a humanized mouse model for sickle cell anemia [6]. In this report, mutant donor fibroblasts were reprogrammed into iPS and the genetic defect was repaired by homologous recombination. Then the repaired iPS cells were differentiated into hematopoietic progenitors and these cells were transplanted into the donor mice (Figure 3.1). All hematologic and systemic parameters of sickle cell anemia significantly improved after transplantation, providing proof of principle for using iPS in therapeutic settings.

In 2007, two separate teams demonstrated the reprogramming of human fibroblasts using two slightly different sets of four genes [7,8]. This remarkable finding opens the door to generate patient- and disease-specific pluripotent cells (Figure 3.2). In the past 8 years, a large number of publications reported the derivation of iPS lines from different human cell types: fibroblasts [9], adipocytes [10], $CD34^+$ cells [11], endothelial cells [12], or keratinocytes [13], and the usage of several different technologies to introduce the four transcription factors. Although most of the “early” iPS lines were generated using an integrative retroviral or lentiviral system, the most recent lines have been derived using non-integrative systems such as the Sendai virus [14], episomal vectors [15,16], RNA transfection [17], and protein transduction [18]. These iPS lines are completely transgene-free and therefore represent a safer source of cells for transplantation. Thus, the reprogramming of adult somatic cells to ESC-like cells by overexpression of the four “Yamanaka” factors is a very attractive approach to generate pluripotent cells with the same genomic material as the donor. Therefore, these cells could

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provide a unique source of unlimited histo-compatible cells for transplantation.

Several groups have demonstrated the capacity of hESCs and iPS cells to differentiate into blood cells [19–23]. Pluripotent stem cells (PSCs) could offer a new source of hematopoietic cells for transplantation. Since these cells would be genetically identical to the person from whom they were derived, they would be particularly appealing as cell therapy. However, the process of blood differentiation from PSCs remains inefficient, the risk of teratoma formation from residual undifferentiated PSCs cannot be dismissed and there is evidence that reprogrammed cells are genetically unstable. Therefore, a long list of issues remains: (1) how can we efficiently derive HSCs or mature blood cells from PSCs?; (2) how can we isolate HSCs or mature blood cells from the heterogenous population of differentiated hPSCs?; (3) are HSCs derived from PSCs capable of hematopoietic repopulation in vivo?; (4) can we generate a sufficient number of mature blood cells for transplantation?; and (5) can we generate immunologically compatible cells?

In this chapter, we present the progress that has been made and the obstacles along the path to the practical use of hPSC-derived HSCs or mature blood cells in clinical transplantation.

Table 3.1 Sources of stem cells and their differentiation potential

	Potential	Sources	Examples
Totipotent cells	All embryonic, extra-embryonic, and adult cells	Early embryo before the four-cell stage	Zygote Each cell from the two- or four-cell stage embryo
Pluripotent cells	All embryonic and adult cells Some extra-embryonic	Early embryo: morula and blastocyst stage Epiblast	Embryonic stem cells Induced pluripotent stem cells
Multipotent cells	Restricted to specific lineages	Umbilical cord Fetus Adult body	Hematopoietic stem cells Mesenchymal stem cells Neuronal stem cells Skin stem cells

Generation of HSCs from ESCs

What have we learned from murine ESCs?

Mouse ESCs (mESCs) possess a robust capacity for hematopoietic specification in vitro. Factors involved in the steps of differentiation from ESC to HSC have been described (Figure 3.3). Two main procedures have been used to differentiate ESCs into blood: embryoid body formation and co-culture with stromal cell lines. Embryoid body formation is routinely used to induce the differentiation of ESCs. Embryoid bodies (EB) can be generated by culturing ESCs in

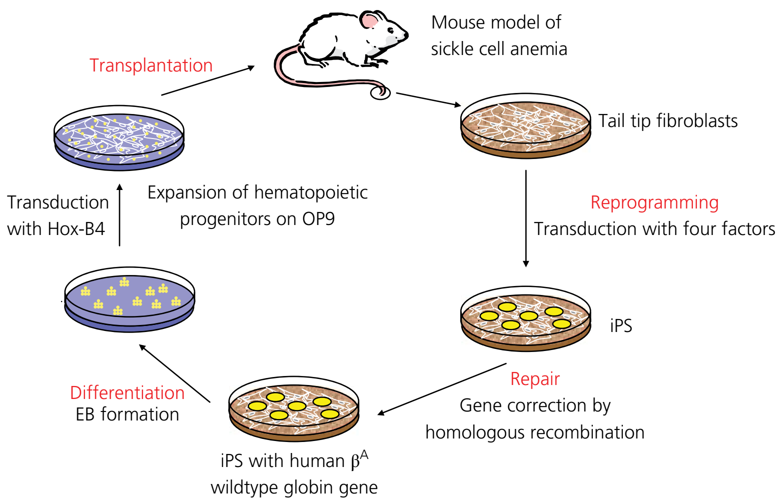
Mouse model of sickle cell anemia
Transplantation
Tail tip fibroblasts
Transduction with Hox-B4
Expansion of hematopoietic progenitors on OP9
Reprogramming
Transduction with four factors
iPS
Differentiation
EB formation
Repair
Gene correction by homologous recombination
iPS with human β^A wildtype globin gene

Figure 3.1 Scheme for sickle cell anemia correction in mice combining reprogramming, gene transfer and cell therapy. EB, embryoid body; iPS, induced pluripotent stem.

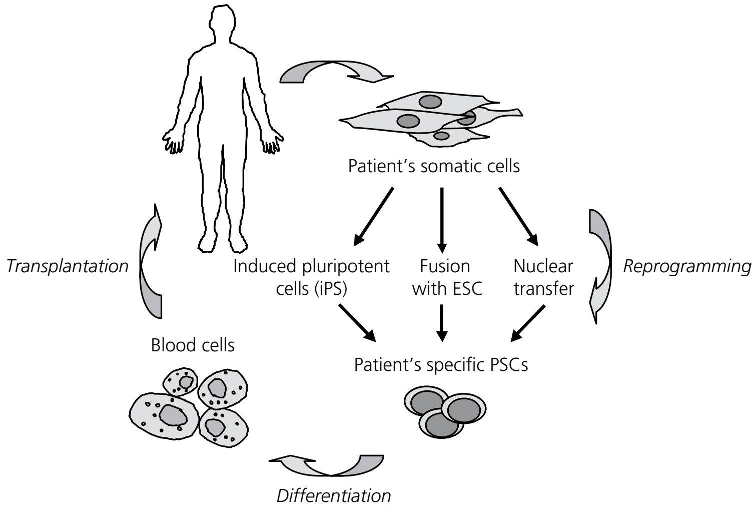
Patient's somatic cells
Transplantation
Induced pluripotent cells (iPS)
Fusion with ESC
Nuclear transfer
Reprogramming
Blood cells
Patient's specific PSCs
Differentiation

Figure 3.2 Generation of patient-specific human pluripotent cells.

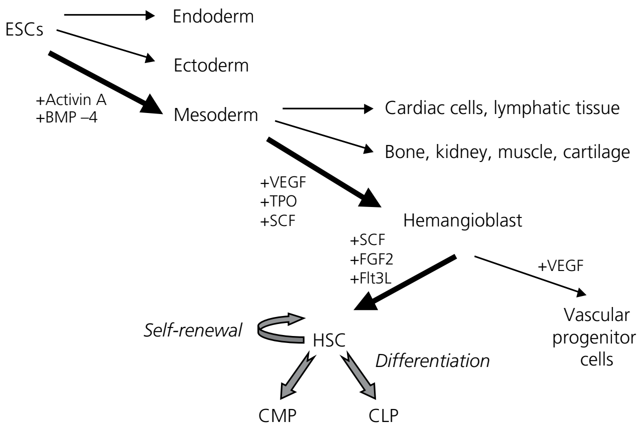

Figure 3.3 Cascade of differentiation from embryonic stem cells (ESCs) to blood cells. CLP, common lymphoid progenitor; CMP, common myeloid progenitor. For other abbreviations, see text.

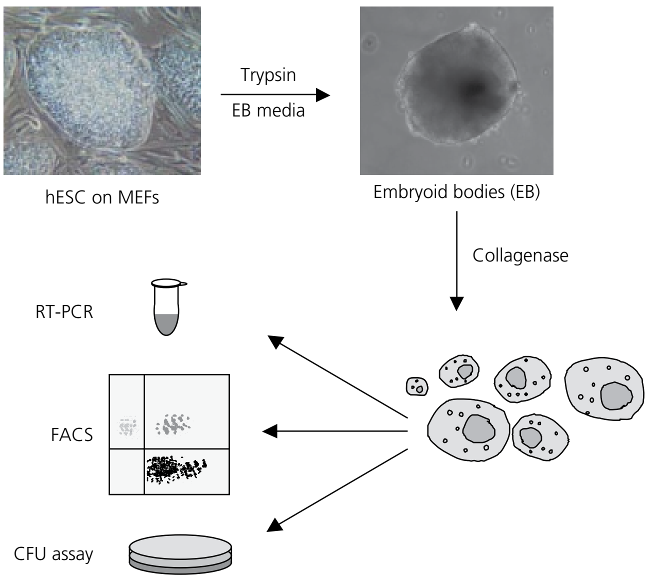

Figure 3.4 Differentiation of human embryonic stem cells (hESCs) by embryoid body (EB) formation and subsequent analysis of blood progenitor cells appearance via reverse transcriptase polymerase chain reaction (RT-PCR), fluorescence activated cell sorter (FACS), or colony-forming unit (CFU) assay. MEF, mouse embryonic fibroblast.

hanging drops for several days, by culturing the cells in 1% methylcellulose or by plating ESCs in low-attachment dishes, allowing the cells to form aggregates. In the absence of factors that maintain pluripotency, these cells will spontaneously differentiate into all three germ layers.

Gordon Keller first showed that the sequence of events occurring in vitro, from primitive ectoderm to mesoderm to hematopoietic cells, parallels the different steps of the onset of hematopoiesis in vivo by observing the expression of several specific markers [24]. The emergence of hematopoietic progenitors can be detected by the colony-forming unit (CFU) assay. During mESC differentiation, CFU numbers increased from day 3 to day 6, reaching 1% of the total population of EB cells at day 6. At this stage, the majority of precursors was primitive erythroid cells – small nucleated cells expressing embryonic globin. At day 10, the population of primitive erythroid cells decreased, while the populations of macrophages (CFU-M), adult erythroid cells (CFU-E), neutrophils/macrophages (CFU-GM), and mixed precursors (CFU-GEMM) increased. Mast cells and lymphoid lineages were detected at a later stage (day 12–14), but to a lesser extent, suggesting that this in vitro differentiation system is not optimal for the generation of lymphoid lineages.

Hematopoietic development occurred even in the absence of supplemental hematopoietic growth factors. In order to provide a supportive microenvironment, ESC differentiation was often performed directly on stromal cells such as OP9, a stromal cell line derived from newborn op/op mouse calvaria (that does not produce macrophage colony-stimulating factor (M-CSF)), S17 and MS-5 mouse bone marrow stromal cell lines, or AM20–1B4, a stromal cell line derived from the aorta–gonad–mesonephros (AGM) region. Both protocols, EB formation and culture on stromal cell lines, permitted the derivation of primitive and definitive hematopoietic cells with a large predominance of primitive blood cells.

Derivation of HSCs from hPSCs

Several laboratories have derived hematopoietic precursors from hESCs or iPSCs using the two approaches mentioned earlier: embryoid body formation (Figure 3.4) and co-culture with stromal cells.

The first evidence for blood cell derivation from hESCs was described by Kaufman et al. [19]. They co-cultured undifferentiated hESCs on irradiated S17 or C166 (a yolk sac endothelial cell line) for 17 days and showed the emergence of a small population (1–2%) of $CD34^+CD38^-$ cells, consistent with the phenotype of early hematopoietic precursors. This co-culture gave rise to CFUs with a peak at day 17–18 and colonies from multiple hematopoietic lineages. Other groups have co-cultured hESCs on the stromal cell line OP9, showing a faster and greater efficiency for the derivation of $CD34^+$ cells reaching up to 20% after 7 days. Subsequent co-culture of isolated $CD34^+$cells on the MS-5 stromal cell line induced the production of macrophages, mature granulocytes, natural killer (NK) cells, and B lymphocytes [20–22]. The same approach was used to test blood differentiation from several iPS lines and gave very similar results, albeit with greater disparities between lines [25]. Additionally, stromal cells derived from the mouse AGM region or fetal liver, were shown to enhance hematopoietic differentiation from hESC cell lines [26] and favor the derivation of HSCs with long-term repopulation activity.

Despite the relative effectiveness of this co-culture system, reproducibility may be a problem since stromal cells are very sensitive to culture conditions (including density, serum lot, and passage number). Stromal-free approaches, typically using an embryoid body system, have also been explored to test the potential of hESCs and hiPSCs [27] to differentiate into hematopoietic cells. The influence of bone marrow protein-4 (BMP-4) and cytokines on the hematopoietic differentiation within EBs has been assessed and the successful generation of $CD45^+$cells has been shown when combinations of cytokines (stem cell factor (SCF), FMS-like tyrosine kinase-3 ligand [Flt3L], interleukin-3, interleukin-6, granulocyte CSF, and BMP-4) were used [23]. Later, vascular endothelial growth factor-A_{165} (VEGF-A_{165}) was shown to enhance the selective induction of erythropoietic development [28]. These results indicate that hematopoietic commitment can be induced by exogenous factors, an important step to improve the derivation of blood cells from hPSCs. In an effort to eliminate high variability in the differentiation systems, a blood differentiation protocol in serum-free medium was developed [29]. Moreover, Elefanty's group improved the efficiency and reproducibility of the experiment by controlling the size of the EBs [30]. These systems are all highly empirical and remain fairly primitive. It is anticipated that better understanding of the molecular events involved may permit a more targeted and robust system to generate functional HSCs.

Derivation of hemangioblasts from hESCs

It is established in the murine system that both hematopoietic and endothelial cells develop from a common transient precursor, the hemangioblast. Initial evidence came from an in vitro mouse ESC differentiation system [31]. Within 3 days of differentiation into EBs, a population of cells with hemangioblastic properties could be detected. When cultured in methylcellulose, hemangioblasts could form blast colonies that had both endothelial and hematopoietic potential. The blast-colony-forming cells expressed genes, such as *Scl*, *Flk1*, and *CD34*, which are common to both hematopoietic and endothelial cells. These blast colonies contained endothelial progenitors as well as primitive and definitive hematopoietic cells. Importantly, clonal analysis demonstrated that a single cell could result in the development of both blood vessel and blood cells. Since this seminal report, the existence of the hemangioblast has been demonstrated in both embryonic and adult murine tissues [32,33].

Demonstration of a comparable human cell has been more complicated. However, a number of reports now support that hemangioblasts can be generated from human pluripotent stem cells [34–36]. In particular, a method in which human ESCs were first differentiated into EBs (by BMP-4, VEGF, SCF, thrombopoietin, and Flt3L) and, after 3.5 days, dissociated and plated in semisolid medium, has been shown to improve several aspects of hemangioblast generation. Specifically, the system has several advantages: (1) it is serum free (and therefore more reproducible than protocols using serum due to variations between lots); (2) it allows the generation of a high number of blast cells (20–50 million blast cells can be generated in a week from 1.2 million undifferentiated hESCs); (3) these blast cells can be cryopreserved; and (4) they can differentiate into endothelial cell and multiple hematopoietic lineages. This protocol represents a significant advance toward the derivation of a high number of hematopoietic cells from hESCs for clinical use. Interestingly, when applied to hiPS lines, this protocol showed a markedly decreased efficiency of hemangioblast production, mostly due to senescence and apoptosis [37]. At the same time, another report demonstrated the limited neuronal differentiation potential of iPS lines compared to hESC lines, regardless of the reprogramming method used. These results challenged the idea that iPS cells could provide an unlimited source of cells for transplantation [38]. Although these reports are of concern, they are limited and contrast with many studies that have shown similar yield and proliferation for derivatives of hESC and hiPSC.

Derivation of blood-specific lineages from hPSCs

The development of culture conditions that induce specific lineages is of great interest for both studying the events resulting in lineage differentiation and generating cells that might be useful in adoptive transfer for therapy. Erythroid cells can be used in transfusion to treat patients with anemia or patients who had massive blood loss due to trauma. Platelet transfusions are often prescribed for patients undergoing chemotherapy, bone marrow transplant, or other organ transplant. It is also given to patients with life-threatening thrombocytopenia. Many pre-clinical studies have shown the potential for using T cells, dendritic cells, or NK cells in immunotherapies to treat cancer. Human pluripotent stem cells could provide a renewable cell source for these cell therapies. With the rapid progress in the generation of safer iPS lines, it is now conceivable to obtain patient-specific mature blood cells suited for transplantation.

T cells

In 2006, a system enabling the derivation of T cells from hESCs was established [39]. This two-step protocol, combining preliminary differentiation on OP9 followed by secondary culture into human thymic tissue in immunodeficient mice, showed that hESCs could yield mature T cells when placed in a supportive environment. Resultant thymocytes were able to respond to T-cell receptor-mediated signals. This protocol was later improved by replacing the OP9 co-culture with EB formation in the first step of the differentiation [40]. Although these findings could have therapeutic implications, for instance for patients with X-linked severe combined immunodeficiency disease or infected with human immunodeficiency virus, the in vivo secondary culture is cumbersome and the number of T cells produced is limited. Another system was developed to efficiently induce T-cell differentiation from mouse ESCs [41], where, after an initial differentiation on OP9, the cells were transferred to OP9 expressing the Notch receptor ligand Delta-like 1 (OP9-DL1). The co-culture on OP9-DL1 was also shown to enhance T-cell differentiation of human hematopoietic progenitor cells from bone marrow or umbilical cord blood (42,43). This in vitro approach has since been tested on hESCs with limited success. Two groups were unable to derive T cells from several human ESC lines [44,45] whereas one report demonstrated successful generation of T cells from one hESC line (H1) using this protocol [46]. After 10–12 days on OP9, the authors noted the formation of endothelium-lined cell clumps, reminiscent of blood island structures, which they called hematopoietic zones (HZs). They contain $CD34^{hi}CD43^{lo}$ cells that can be picked and differentiated further on OP9-DL1 cells. After 4–6 weeks in culture on OP9-DL1, 15–50% of the human cells were $CD3^+$. These cells were shown to be functional and polyclonal T cells.

Although, there is evidence that mature T cells can be derived from human pluripotent stem cells using a completely in vitro culture system, it is clear that more efficient, more robust, and more consistent in vitro protocols need to be developed. Additionally, iPS lines have been generated from T cells by several groups [14,47]. This opens the door for the generation of autologous iPS lines with antigen-specific T-cell receptors (TCRs) that could be used in adoptive T-cell therapy to treat cancer. Although this strategy seems promising, the time, cost, and low efficiency of T-cell differentiation remain major obstacles.

B cells

Differentiating pluripotent stem cells into B lymphocytes has proven to be extremely difficult. In 2005, Vodyanik et al. differentiated hESCs with a two-step protocol and detected a low percentage (around 2%) of $CD19^+CD45^+$ B-cell precursors. The hESCs were initially differentiated on OP9 stroma followed by the isolation of the $CD34^+$ cells by MACS and their culture on MS-5 stroma for 21 days [20]. Using a similar protocol, pre-B cells were generated from human iPS cells [48]. These cells were positive for CD19 and CD10 but negative for CD5 and IgM, consistent with a pre-B phenotype.

NK cells

Currently, a large number of clinical trials are under way to test the safety and efficacy of NK-cell based immunotherapy to treat cancer. Evaluating different sources of NK cells for cell therapy is important to obtain large numbers of cells.

hESCs have been shown to differentiate into mature functional NK cells [23,49,50]. CD34$^+$/CD45$^+$ cells were obtained by differentiating hESCs on the stromal cell line S17 and these cells were further directed to differentiate into NK cells by co-culture on the murine fetal liver AFT024 cell line. A highly enriched homogenous population of NK cells was obtained within 4–5 weeks. These cells displayed a normal phenotypic profile with expression of CD56, killer immunoglobulin-like receptor, and CD94/NKG2, markers of mature NK cells. More importantly, these cells exhibited cytolytic activity against human tumor cells by direct cell-mediated cytotoxicity and antigen-dependent cellular cytotoxicity. The same group reported the generation of NK cells from iPS lines and demonstrated their anti-HIV activity [51]. Thus, these cells are not only a valuable means to study NK development, but could also provide a novel source for cellular immune therapy. However, one major limitation of this protocol is the use of a two co-culture system. The development of new, feeder-free differentiation culture is essential to move toward PSC-NK cell therapy.

Macrophages

Using a related strategy, macrophages were derived from hESCs [52]. After 14–17 days of initial differentiation on the S17 stromal cell line, the hESC-derived CD34$^+$ cells were grown in semisolid methylcellulose medium to obtain myeloid colonies. They were then cultured for an additional 2 weeks in liquid culture with cytokines (granulocyte–macrophage CSF and macrophage colony-stimulating factor). The resulting cells expressed CD14, human leukocyte antigen-DR (HLA-DR), CD4, CCR5, and CXCR4, and exhibited normal functions, as measured by phagocytosis, upregulation of B7.1 and cytokine secretion in response to lipopolysaccharide stimulation. The cells were also able to be genetically modified by lentiviral transduction, suggesting potential opportunities for their use as protein-secreting immune cells. Improved protocols for the derivation of macrophages from hESC and hIPS lines have been described [53,54], offering higher efficiency and a feeder-free system.

Dendritic cells

Dendritic cells (DCs) are potent antigen-presenting cells (APCs) with an ability to elicit primary T-cell responses. They can be used to stimulate the immune response against tumor-associated or viral antigens or, in contrast, to diminish immune response in autoimmune diseases or after transplantation. Immature DCs can be isolated from peripheral blood or derived from bone marrow CD34$^+$cells [55,56]; however, hPSCs could potentially provide an unlimited number of DCs. A three-step approach was used to generate DCs from hESCs [57]. These cells expressed similar markers to HSC-derived DCs, such as CD1a, CD9, CD68, and CD86. Moreover, these cells were functional, as shown by their ability to induce the proliferation of adult and cord blood T cells and their capacity to present antigens through the major histocompatibility complex (MHC) class I pathway. Using the same three-step approach, functional DCs were generated from additional hESC and hiPS lines [53,58]. The iPS-DCs were shown to be functional through their capacity to induce the proliferation of cytomegalovirus specific autologous T cells after being loaded with cytomegalovirus peptide. This three-step protocol was further improved by replacing the OP9 co-culture with a clinically compatible feeder-free system [59,60].

One strong advantage in using pluripotent stem cells as a source of APCs is their potential to be genetically modified. One group reported the derivation of genetically manipulated DCs from hESCs [61]. They used a plasmid to overexpress the human mutated CD74 in hESCs and selected clones with high transgene expression at the pre-DC stage. They further show that these DCs could activate specific T cells without adding peptides. Thus, with the rapid development of genome modification technologies like Zinc-finger, TALEN, and CRISPR, the generation of genetically engineered hPSC DCs could offer a promising tool for antigen-specific immune therapy.

Erythroid cells

PSCs, as self-replenishing units for the production of large numbers of cells, have long been considered as a mode of generating red blood cells ex vivo. One consideration, however, is that the red blood cells produced may represent those of early embryonic development. The primitive erythroid cells found in the yolk sac during human development have a characteristic morphology: they are nucleated and express mostly embryonic globin(ε, ζ, and α). In the fetal liver, macrocytic erythrocytes are enucleated and express fetal globin ($\alpha2\gamma2$), while mature, marrow-derived erythrocytes are characterized by adult globin ($\alpha2\beta2$).

Many reports have shown the generation of erythrocytes from hESCs and hiPSCs. A review from Chang, et al. summarizes the work published in the past 10 years [62]. In brief, red blood cells could be produced from any PSC, but the efficiency and maturity of the cells varied depending on the protocol used and the lines. The highest efficiency (200,000 erythroid cells per hESC) was obtained after co-culture on OP9 and MS5 [63]. However, only a small percentage of these erythroid cells were enucleated and they only expressed embryonic globin. In contrast, other groups were able to derive erythroids with a higher percentage of enucleated cells, up to 66% using the EB method [64,65], but the efficiency was lower and the erythroid cells remained of embryonic origin as shown by the lack of β globin expression. In conclusion, regardless of the differentiation method, it remains a challenge to produce fully mature erythroid cells from pluripotent stem cells. Interestingly, this is a problem for any cell type generated from PSCs and emphasizes the importance of the microenvironment for the full maturation of PSC-derived cells. One way to overcome this problem is to induce terminal differentiation in vivo. A recent paper from Kobari et al. showed that erythroblasts could achieve full maturation (nucleus expulsion and expression of adult Hb) a few days after injection into NOD/SCID mice [66]. Although, this is a proof of principle that mature erythroid cells can be obtained from PSCs, this method is not suitable for the generation of red blood cells for clinical use.

Megakaryocytes/platelets

Developments in differentiation protocols and the fact that platelets are anucleate (thereby eliminating the concern about genetic stability of pluripotent cells) have rapidly made this an area where pluripotent cell products are moving to the clinic. In 2008, Takayama et al. established a protocol to generate functional platelets from human embryonic stem cells. Human ESCs were first differentiated by co-culturing on irradiated C3H10T1/2 or OP9 cells in the presence of VEGF. After 2 weeks in this condition, hESCs formed sac-like structures containing round, hematopoietic-like cells. In the presence of TPO, SCF and heparin, these cells further differentiated into megakaryocytes expressing CD41a, CD42a, and CD42b that could release functional platelets [67]. Using a different protocol, Lu et al. were able to generate platelets from hESC with higher efficiency and showed their functionality in vivo [68]. Because platelets are enucleated and irradiation can be used to eliminate possible contaminating nucleated cells, they are the most promising pluripotent stem cell-blood derivative candidate for clinical trials. One of the major obstacles to the use of PSCs derived platelets for transplantation is the number of platelets needed. The standard dose is between 3×10^{11} to 6×10^{11} platelets. However, one clinical trial using hiPSC-derived platelets is in preparation in Japan, led by Dr. Koji Eto with phase I/II scheduled in few years.

Isolation and/or expansion of hPSC-derived HSCs

It is clear that hPSCs can be differentiated into blood cells at various stages of commitment. Although the protocols cited earlier allowed for blood cell generation, the cells were not homogeneous, and the isolation of the cells of interest would be necessary before transplantation. Isolation would address the major concern that some undifferentiated PSCs could remain in the population of cells being transplanted. Since undifferentiated PSCs have the potential to form teratomas (benign tumor-containing cells from the three germ layers) when injected into SCID mice, it is crucial to eliminate any residual undifferentiated PSCs prior to transplantation. A negative selection could be performed using well-known surface markers specifically expressed in hPSCs, such as TRA-1-60, stage-specific embryonic antigen-3 (SSEA-3) or SSEA-4. Alternatively, it has been proposed to generate a human PSC cell line that expresses the herpes simplex virus thymidine kinase gene [69]. Since this gene confers sensitivity to the drug ganciclovir, specific elimination of transplanted hESC-derived cells could be accomplished by use of ganciclovir in the case of tumor development.

Another issue is the presence of cells from other lineages in the heterogeneous population of differentiated cells. Thus, an attractive approach is to positively isolate HSCs or specific blood lineages before transplantation. A limitation is the lack of defined surface markers for the prospective isolation of long-term HSCs. Mouse long-term repopulating HSCs are well characterized, and a panel of surface markers is commonly used to isolate these cells, such as Lin^-, Kit^+, Sca^+, and $\mathrm{Thy1}^{\mathrm{lo}}$ [70]. In 2005, a family of cell surface receptors, the signaling lymphocyte activation molecule family (SLAM), that distinguished long-term HSCs (LT-HSCs) from multipotent progenitors (MPPs), was identified in mice [71]. While CD150 was present exclusively at the surface of LT-HSCs, CD48 was specifically expressed in MPPs. The combination of these two SLAM markers (CD150/CD48) was proven to be very efficient for the isolation of LT-HSCs. Both LT-HSCs and MPPs can give rise to all blood lineages, but only LT-HSCs can maintain lifelong production of blood cells. Thus, the development of this simple and direct method to isolate LT-HSCs was a major step forward to improve transplantation efficiency. However, the expression pattern of CD150 and CD48 is different in humans [72] and their utility to isolate non-human primate LT-HSC has been questioned [73]. Other methods, using CD49f have been shown to be more promising [74].

This marker, like many used to isolate cells, has not been validated in cells derived from pluripotent cells. Whether markers identified on cells derived from the intact organism can be used to isolate the appropriate cell derived from cultured pluripotent cells is not clear. For example, several markers known to be expressed on hematopoietic progenitors, such as CD90, CD133, and CD117, are also expressed in undifferentiated hESCs, limiting the use of these markers for HSC isolation. It is therefore not known if the HSC-enriched $\mathrm{CD34}^+/\mathrm{CD90}^+/\mathrm{CD38}^-$pattern applies to cells derived from human ESCs [75].

Another hurdle for the generation of HSCs from PSCs is the lack of culture conditions allowing the expansion of these cells. During hematopoietic differentiation in vitro, the number of cells with hematopoietic activity (based on the colony-forming cell (CFC) assay) reaches a peak, usually around 2–3 weeks into the differentiation but then rapidly decreases as the cells further differentiate. Additionally, the timing of the peak is different for each line and each protocol used. Unless new culture systems are developed to expand HSCs in vitro, it will be technically challenging to isolate a sufficient number of PSC-derived HSCs for transplantation.

Several strategies have been proposed to enhance HSC ex vivo expansion and a few reports showed improvement in HSC culture systems in the past few years. For instance, using a chemical screen, Boitano and colleagues identified a purine derivative called SR1 that supported a 50-fold increase in cord blood $\mathrm{CD34}^+$ cells [76]. Recently, Zandstra's group reported an 11-fold increase of HSC by inhibiting feedback signaling from differentiated blood cells [77]. Another approach would be to mimic the bone marrow niche using a 3D culture system. A German group has done preliminary work comparing the ability of different 3D scaffolds to support CB $\mathrm{CD34}^+$ expansion [78]. The development of a culture system to sustain HSC growth is currently an active area of investigation.

Are PSC-derived hematopoietic progenitors capable of engraftment and hematopoietic repopulation?

Transplantation of HSCs derived from mPSCs

Although the derivation of hematopoietic progenitor cells from mPSCs has been well documented in the last 25 years, only a few groups have reported a robust long-term repopulation from HSCs derived from mPSCs. In the first reports, the authors overexpressed the transcriptional regulators Hoxb4 and Cdx4 [79,80] in HSCs to increase their engraftment into lethally irradiated mice. In brief, Hoxb4 expression was induced in embryoid bodies at day 4 by addition of tetracycline, followed by dissociation at day 6 and replating on OP9. Only the primitive hematopoietic cells expressing Hoxb4 or the combination Hoxb4/Cdx4 were able to expand in this culture condition. After an additional 2 weeks of co-culture, all of the cells were transplanted into irradiated mice. Engraftment and multilineage repopulation was demonstrated, albeit with low lymphoid potential. Later, similar results were obtained with the overexpression of a LIM-homeobox transcription factor: Lhx2 [81]. This system was more favorable to B-cell differentiation but inhibitory for T-cell differentiation. Although these studies demonstrated the potential for HSCs derived from PSCs to be fully functional, this overexpression system would not be safe for therapeutic applications due to the risk of malignant transformation. Thus, the generation of functional and engraftable HSCs from PSCs remains extremely challenging.

The lack of engraftment of PSC-derived HSCs is thought to be due to the primitive nature of the cells generated. In mammals, hematopoietic development follows a complex process with two successive waves of primitive and definitive hematopoiesis occurring in distinct anatomical sites [82]. Primitive hematopoiesis arises in the blood islands of the yolk sac by day 18 of gestation in humans. It is transient and allows for the production of nucleated erythroblasts, as well as macrophages and megakaryocytes. These primitive red blood cells are critical for the survival of the early embryo, which develops in a highly hypoxic environment. The second wave of hematopoiesis is located in the AGM region in the embryo proper. It is thought that definitive HSCs migrate from the AGM to the fetal liver, where they expand extensively before reaching their final niche in the bone marrow during the second trimester. These definitive HSCs are mostly quiescent but can give rise to all blood cell lineages. In the 1970s, it was thought that yolk sac HSCs colonized the fetal liver, and later the adult bone marrow, giving rise to definitive HSCs. Studies in the 1990s, however, demonstrated that the first definitive HSCs originated from the AGM, independent of the yolk sac blood cells [83,84].

For a decade, this theory of two independent waves of hematopoietic onset, one from the yolk sac that supplies the embryo and one from the AGM that provides definitive adult HSCs, was accepted by many scientists in the field. However, this theory was then challenged by an in vivo cell-tracing approach to follow the fate of yolk sac hematopoietic cells during mouse development [85]. Using time–genetic labeling of yolk sac hematopoietic cells, it was demonstrated that yolk sac-derived cells can indeed migrate into sites of definitive hematopoiesis and contribute to blood cell production in adults. Work from another group further demonstrated that definitive HSCs could be derived from yolk sac if the cells were co-cultured with an AGM stromal cell line [86]. This observation implied that the AGM provided the specific microenvironment allowing the maturation of the yolk sac primitive HSCs into mature definitive HSCs. It may therefore be possible to instruct primitive cells derived from PSCs to provide engraftable HSCs.

Transplantation of HSCs derived from human PSCs

The repopulating potential of hPSC-derived HSCs has been reported to be quite poor [87]. However, when hESC-derived HSCs were injected directly into the marrow cavity, rather than the bloodstream, of irradiated immunodeficient mice, human blood cells were found at the site. There was even evidence that similar cells could be secondarily transplanted [88]. However, in all cases the level of engraftment was very low (<1%) and inferior to that of somatic sources of HSCs. The engraftment was slightly increased when the hESC were differentiated in a co-culture system using primary cells or cell lines derived from the AGM or fetal liver [89]. In this study, human blood cells were detected in the injected and non-injected femur, as well as the peripheral blood of all surviving mice. These hESC-derived HSCs were also capable of secondary transplantation. However, the level of blood reconstitution in the peripheral blood remained very low. Overexpression of Hoxb4 in HSCs derived from hPSCs was also tested, but unlike in the mouse system, the engraftment potential was not increased [90]. The enforced expression of Cdx4 or Lhx2 has not been tried yet. In conclusion, little progress has been made over the past decade to enhance the capacity of HSC-derived PSCs to engraft.

Can we generate immunologically compatible HSCs?

Another major concern for the use of hPSC-derived blood cells in transplantation settings is their potential for being rejected by the recipient in the same manner that any conventional transplanted organ is rejected. One approach to circumvent this problem is to use traditional immune regimens to prevent PSC derivatives rejection. Pearl et al. demonstrated that a short treatment with a combination of three co-stimulatory receptor blocking agents could enhance engraftment of hESC or hiPSC and, more importantly, their derivatives [91]. However, this procedure may not be adequate for highly immunogenic blood cells.

Several additional strategies have been proposed to limit the likelihood of tissue rejection if hPSC-derived hematopoietic cells ever become available for transplantation: (1) the creation of a global bank of hPSCs; (2) the use of the potential immune privileged status of hPSC derivatives; (3) the generation of tailor-made hPSCs by reprogramming patients' somatic cells (induced pluripotent stem (iPS)); and (4) the direct reprogramming of patients' fibroblasts into blood cells. In the following paragraphs, we review these different approaches.

Creation of a global hPSC bank

Attempts to define the HLA complexity of an hPSC bank needed to cover a reasonable segment of the population have been performed. One study analyzed the blood group and HLA types of 10,000 UK cadaveric organ donors for compatibility with 6577 patients registered on the UK kidney transplant waiting list, and used it as a simulation model for the potential requirement for hESC-derived cell transplantation [92]. This study was based on a six-antigen match. The authors reported that as few as 150 hESC cell lines would provide a full match for a minority (less than 20%) of patients, but a beneficial match (meaning one HLA-A or one HLA-B mismatch only) for 38% of the recipients and an HLA-DR match for 84%. They suggested that only 10 cell lines homozygous for common HLA types would provide a complete match for 38% of the patients and a beneficial match for 67%.

One caveat of this study is the underrepresentation of particular ethnic groups. Another study carried out on the Japanese population showed similar results [93], estimating that a bank of human ESC cell lines derived from 170 randomly selected embryos would provide 80% of patients with, at worst, a single mismatch at one HLA locus. We have to keep in mind that this data was obtained from a relatively homogeneous population. A much larger number of cell lines will be required to offer a good match to ethnic minorities. The ethical aspects associated with the creation of an hPSC bank are complex and have been well articulated elsewhere [94].

Immune privileged?

Several studies have suggested that hESCs and their differentiated derivatives are immune privileged and may circumvent rejection [95,96]. Supporting this claim, hESCs have been shown to express low levels of MHC class I proteins and to lack expression of MHC class II antigens [97]. However, MHC class I expression increased during differentiation and could be rapidly induced by interferon treatment. Since NK cells target low-expressing MHC class I cells through a process called "missing self," the expression of NK receptors on the surface of hESCs was checked [97]. hESCs did not express the NK receptor and thus were not subjected to NK-mediated lysis. This finding suggested that engrafted hESCs could escape the innate immune response.

In another study, the same group further studied the immune response against hESCs and derivatives in vivo [96]. Although teratomas could develop into NOD/SCID mice after 1 month, no tumors were found in NK-cell-deficient, B-cell-deficient, or immune-competent mice, implying that xenorejection of hESCs was T-cell dependent. In order to mimic the clinically relevant transplantation settings, they used a humanized (Trimera) mouse model [98] to evaluate allorejection of hESCs. Both hESCs and their derivatives could escape rejection and form teratomas when injected into the mice reconstituted with human peripheral blood monocytes. In contrast, transplanted human skin fibroblasts and B lymphocytes induced a donor leukocyte response and were completely eliminated.

The alloantigen-specific immune response against hESCs was also examined in vivo [95]. hESCs were injected intramuscularly into immunocompetent mice. Unlike the human MBA-1 control cells (megakaryocytic cell line), hESCs did not induce an inflammatory response in these mice. Moreover, hESCs and their derivatives had a limited ability to induce T-cell-mediated immune responses. In fact, their data suggested a direct inhibitory effect of hESCs on T-cell proliferation in response to allogeneic DCs. These immune-privileged properties of hESCs are reminiscent of the tolerance of maternal lymphocytes for embryonic cells. However, these properties were challenged in another study [99]. The authors demonstrated that hESCs induced a similar response to human fibroblasts on naïve and immunized T cells, both directly and in the presence of syngeneic DCs. More data needs to be collected to confirm and understand the partial immune privilege revealed in these reports. The hESC derivatives used in these studies were the result of random differentiation through teratoma formation. No MHC class II expression was detected in this population of cells, which suggests a poor efficiency for hematopoietic differentiation or the presence of immature blood cells only. Blood cells are particularly immunogenic, so further work needs to be performed on the immune response of this specific lineage.

Pluripotent stem cells tailor-made by reprogramming patients' somatic cells (iPS)

One strategy to avoid graft rejection is to generate cell lines with the genomic material of the patient. In this case, the match would be perfect and the risk of rejection minimal. Several methods have been attempted to generate patient-specific pluripotent stem cells such as somatic cell nuclear transfer, somatic–embryonic cell fusion or the generation of parthenogenetic ESCs. However, these methods were unsuccessful for human cells or had strong limitations.

It is clear now that the method of choice to generate patient-specific pluripotent stem cells is somatic reprogramming. This method was described for the first time by Shinya Yamanaka in 2006, an achievement that earned him the Nobel Prize of Medicine in 2012. This relatively simple protocol allows somatic cells to be reprogrammed by overexpressing four genes (*Oct4*, *Sox2*, *Klf4*, and *c-Myc*). The iPS lines generated through this protocol show great similarity to hESCs: they can expand indefinitely and can be differentiated into any lineage, in theory, offering an unlimited supply of blood cells. With the recent advances in the generation of integration- and transgene-free iPS lines, iPS-derived cells could be an ideal source for hematopoietic cell transplantation.

In principle, the risk of immune rejection in an autologous setting, where the patient's own iPS cells are used as a source of cells for transplantation, should be minimal. However, one report, published in *Nature*, showed an unexpected immune response following iPS cell transplant in genetically matched mice [100]. The team generated iPS lines from mouse skin cells and transplanted them into mice that were genetically identical to the donors. Surprisingly, most of these iPS lines could not form teratomas since they quickly triggered an immune rejection. Although this study serves as a cautionary note for the use of PSCs in clinical settings, additional work must be done to evaluate the immune rejection of iPS derivatives instead of undifferentiated iPS as described in this paper. Moreover, iPS lines generated using different reprogramming technologies should be assessed to find out whether the reprogramming methods could influence the immunogenicity of the resulting iPS lines.

Nevertheless, producing new iPS lines for each patient would be an extremely expensive, time-consuming and complex process. A proposed alternative is to create banks of hiPS lines with HLA genotypes that would match a majority of the potential recipients. Unlike hESC banks, iPS banks could select the donors based on their HLA haplotype and therefore be less random. Four reports described different models to define the number of iPS lines needed to offer a match for some specific populations. Two studies focused on the Japanese population. Okita et al. derived iPS lines from two individuals that could, in theory, match ~20% of the Japanese population [101]. Another group calculated the number of iPS lines appropriate to cover most of the Japanese population. They estimated that a cell bank of 50 iPS lines would offer a matched line to 90.7% of the population [102]. A similar study showed that a bank with 150 iPS lines would be sufficient to match 93% of the UK population [103]. This report used the HLA database established by the Bone Marrow Donors Worldwide (BMDW) to evaluate the feasibility of selecting donors homozygous for each HLA haplotype. Other models were established to evaluate the size of the banks to match potential recipients from different countries. This is summarized in a review from Zimmermann [104]. Overall, the feasibility of such banks has been demonstrated and the derivation of GMP-grade iPS lines has already been accomplished.

Direct reprogramming of patients' fibroblasts into blood cells

An interesting new approach for obtaining blood cells was published in 2010 [105]. It originated from an observation that many colonies with round cells, resembling blood cells, were generated during the reprogramming of human dermal fibroblasts into pluripotent stem cells. These cells were $CD45^+$ and lacked the pluripotent stem cell marker TRA-1-60. Interestingly, the authors showed that most of these $CD45^+$ cells expressed only one of the four "Yamanaka" factors: Oct4. Based on this observation, they tested whether overexpression of Oct4 was sufficient to directly reprogram fibroblasts into blood cells. Using a lentivirus system to introduce Oct4 into fibroblasts, they were able to get 24–38% of $CD45^+$ cells 21 days after transduction. They demonstrated that these cells were multipotent hematopoietic progenitors capable of differentiating into myeloid, erythroid, and megakaryocytic lineages in vitro and in vivo. Unlike the erythroid cells generated from PSCs, the erythroid cells derived with this direct reprogramming system express adult β-globin protein. Although this work has not yet been reproduced by other groups, many reports on direct conversion of fibroblasts into neurons [106], motor neurons [107], cardiomyocytes [108], and endothelial cells [109] have been published within the last few months.

In conclusion, this approach offers a faster and cheaper way to generate hematopoietic cells. Nevertheless, the limited proliferation of the fibroblasts and of the multipotent hematopoietic progenitors is a major restriction to the use of this technology to obtain the large number of cells needed for transplantation.

Conclusion

The ability to use pluripotent cells derived either from embryos (hESCs) or reprogrammed somatic cells (iPSCs) as a source of HSCs for transplantation remains a distant possibility. More experiments need to be done to understand how these cells normally mature to acquire the characteristics of adult HSCs, and how these steps in ontogeny may be controlled and accelerated in laboratory settings. It is worth recalling that approximately 20 years separated the first derivation of mESCs [110,111] and definitive proof of long-term multilineage engraftment of HSCs derived from mESCs [112].

Research on the derivation of definitive engraftable HSCs from human pluripotent stem cells is still in its infancy. In contrast, striking progress has been accomplished in the derivation of mature blood cells. In particular, new protocols were developed to generate mature functional erythrocytes and platelets on a large scale. Since these blood cells are enucleated, their transfusion presents a lower risk for the patient. Therefore, the transplantation of red blood cells and platelets from immunologically compatible PSCs represents the most practical strategy for PSC-derived blood cell-based therapy.

Since the previous edition of *Thomas' Hematopoietic Cell Transplantation*, three clinical trials using derivatives of embryonic stem cells have been allowed to proceed by the U.S. Food and Drug Administration (FDA). The transplantation of hESC-derived oligodendrocytes in spinal cord injury was begun but closed prior to completion due to financial considerations. Two additional clinical trials are still active testing the safety of using hESC-derived retinal epithelial cells in Stargardt macular dystrophy and age-related macular degeneration. Therefore, regulatory guidelines governing pluripotent cell products are being developed and clinical testing has begun. These critical first steps will greatly influence whether and how hematopoietic cells from pluripotent cells will be introduced into medicine.

We cannot know yet whether pluripotent cells will provide a clinically useful source for hematopoietic cell transplants in humans. However, these cells have tremendous potential as both a research tool and a possible therapeutic agent, a potential that can only be defined by further vigorous investigation.

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CHAPTER 4

Hematopoietic Stem Cells, Regenerative Medicine, and Leukemogenesis

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Introduction

Stem cells are single cells that are capable of both self-renewal and differentiation [1–3]. In essence, self-renewal means that the cells can divide without undergoing discernible differentiation, and that they possess properties that allow large numbers of self-renewing cell divisions to occur in a regulated fashion over the life span of a host. Hematopoietic stem cells (HSCs) are therefore blood-forming cells that, at the single cell level, can duplicate by self-renewal as well as produce all types of differentiated blood cells [4]. HSCs were the first prospectively isolated stem cells that give rise to organs or tissues [5,6], and their properties stand as a model for other tissue and organ stem cells. Based on their definition, stem cells must make their first appearance at the beginning of organ and adult tissue development, because at this stage there are no residual cell types that were first seen in the embryonic stages that precede fetal development.

Failure of hematopoietic cell transplanters and journals to use appropriate terms to describe the cells that are transplanted

Many investigators misuse the term stem cells when they transfer other populations of cells. In this volume, and hopefully in the journals that serve this field, hematopoietic cell transplantation (HCT) means that the inoculum contains hematopoietic cells. Bone marrow (BM), mobilized peripheral blood (MPB), and umbilical cord blood (UCB) are common sources of cells for HCT. Collections of cells from these sources can be enriched for hematopoietic stem and progenitor cells that express CD34 – that is, $CD34^+$ hematopoietic cells – for use in HCT. Transplantation of candidate HSCs in humans has only occurred rarely [7], and different labs use different markers to isolate these candidates. It would seem logical for investigators to specify the phenotypic markers expressed on or absent from the claimed HSCs – for example, $CD34^+38^{lo}CD90^+Lin^-$ HSCs, or $ALDH^+$ HSCs, or dye exclusion cells in the side population, called SP cells, or the more restrictive lowest 10% of these, called SP tip cells. Hematopoietic progenitors may be named either by their function, if enriched or purified, for example, common lymphoid progenitors (CLPs), or by their marker phenotype, for example, $CD34^+38^+CD123^+CD45RA^-Lin^-$ common myeloid progenitors (CMPs).

History of the HSC

Following the demonstration that just lethal doses of whole body irradiation caused death by hematopoietic failure, and that hematopoietic failure could be averted either by shielding hematopoietic organs or by transplantation of unirradiated bone marrow [8–10], the critical study that set the stage for the concept of HSC demonstrated that injected bone marrow reconstituted the hematopoietic system rather than provided radiation repair factors [11–13]. In our view, the seminal set of studies in the HSC field began with the observation by Till and McCulloch that transplantation of limiting numbers of bone marrow cells into lethally irradiated mice gave rise to colonies in the spleen that contained all elements of the myeloerythroid (but not lymphoid) lineages and that the number of colonies was proportional to the number of bone marrow cells injected [14].

Two experiments followed these observations that opened the field to stem cell biology and changed the thinking of the hematology community. First, pre-irradiation of the marrow donor at doses that would allow the survival of some cells that had random chromosome translocations or inversions resulted in the finding that each spleen colony was the product of a clonogenic precursor [2]. Second, at least some of these colonies contained cells that also contained spleen colony-forming cells, as well as cells that could be radioprotective [2,15,16]. Later, it was shown that the daughter cells of some of these spleen colony-forming cells included lymphocytes [17]. These experiments indicated that there probably exist, in the bone marrow, single cells capable of self-renewal and full differentiation to hematopoietic fates. These important experiments were carried out mainly in the 1960s, before the discovery of monoclonal antibodies [18] or the development of high-speed multiparameter fluorescence-activated cell sorters [19]. The eventual isolation of HSCs required not only these innovations, but also the development of in vitro and in vivo assays for the identification and quantification of clonogenic precursors of T-cell [20], B-cell [5], and myeloerythroid lineages [21–24]. Many important enrichments of these precursors were accomplished using cell sorting with monoclonal antibodies and lectins [25]. However, prospective isolation of HSCs was not accomplished until a simultaneous analysis of all lineages was coupled with quantitative reconstitution of hematopoiesis in irradiated mice by limiting numbers of candidate HSCs [5,26–30].

We have isolated both mouse and human HSCs, and their surface phenotypes are shown in Figure 4.1 [29,31,32]. They share the properties of expressing Thy-1 ($Thy1.1^+$ or $CD90^+$) and lacking the expression of a collection of mature blood cell lineage markers (Lin^-) and both express c-kit. In addition, mouse HSCs express the Sca-1 (stem cell

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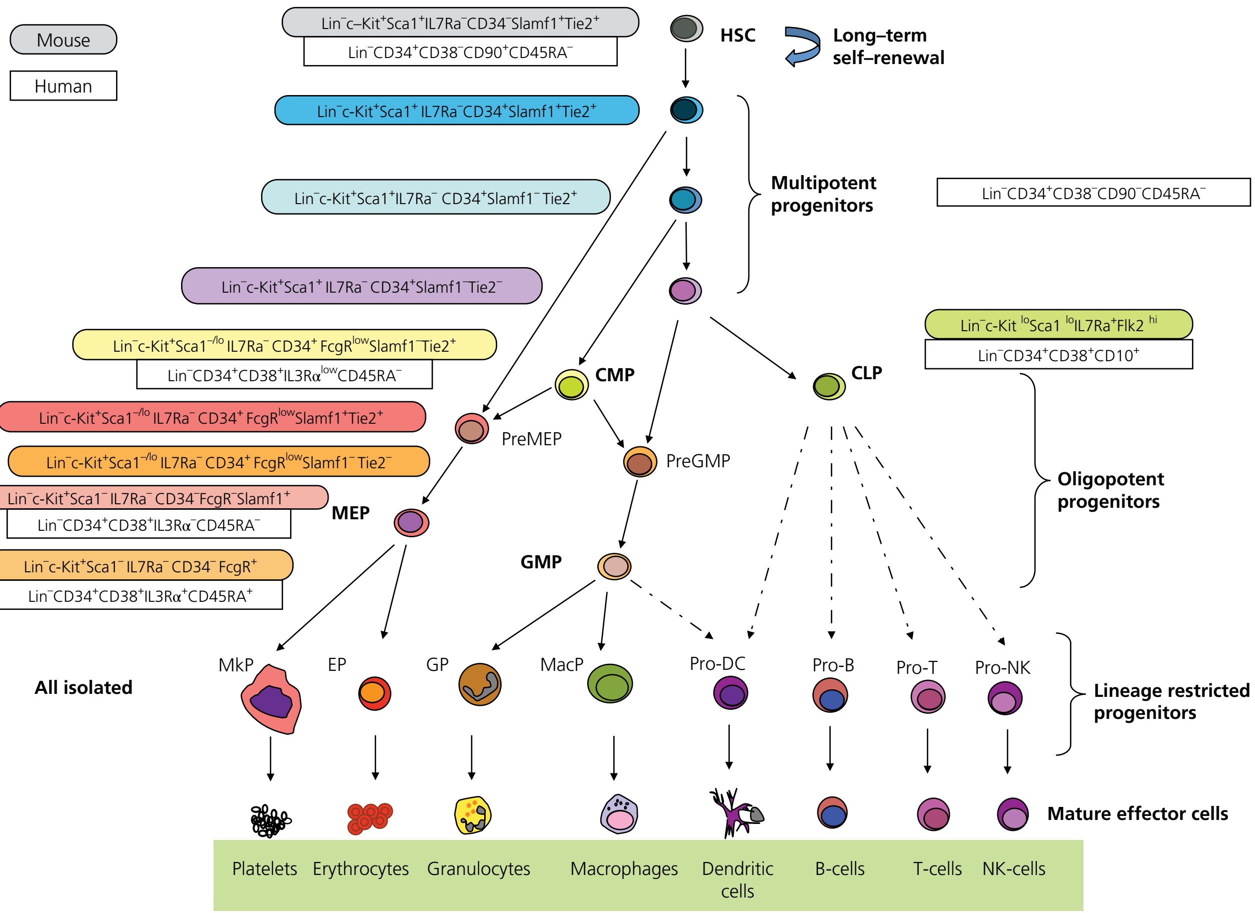

Mouse
Human
Lin$^-$c–Kit$^+$Sca1$^+$IL7Ra$^-$CD34$^-$Slamf1$^+$Tie2$^+$
Lin$^-$CD34$^+$CD38$^-$CD90$^+$CD45RA$^-$
HSC
Long–term self–renewal
Lin$^-$c-Kit$^+$Sca1$^+$ IL7Ra$^-$CD34$^+$Slamf1$^+$Tie2$^+$
Lin$^-$c-Kit$^+$Sca1$^+$IL7Ra$^-$ CD34$^+$Slamf1$^-$ Tie2$^+$
Multipotent progenitors
Lin$^-$CD34$^+$CD38$^-$CD90$^-$CD45RA$^-$
Lin$^-$c-Kit$^+$Sca1$^+$ IL7Ra$^-$ CD34$^+$Slamf1$^-$Tie2$^-$
Lin$^-$c-Kit$^+$Sca1$^{-/lo}$ IL7Ra$^-$ CD34$^+$ FcgR^{low}Slamf1$^-$Tie2$^+$
Lin$^-$CD34$^+$CD38$^+$IL3Rα^{low}CD45RA$^-$
CMP
Lin$^-$c-Kit ^{lo}Sca1 ^{lo}IL7Ra$^+$Flk2 ^{hi}
Lin$^-$CD34$^+$CD38$^+$CD10$^+$
CLP
Lin$^-$c-Kit$^+$Sca1$^{-/lo}$ IL7Ra$^-$ CD34$^+$ FcgR^{low}Slamf1$^+$Tie2$^+$
PreMEP
Lin$^-$c-Kit$^+$Sca1$^{-/lo}$ IL7Ra$^-$ CD34$^+$ FcgR^{low}Slamf1$^-$ Tie2$^-$
PreGMP
Oligopotent progenitors
Lin$^-$c-Kit$^+$Sca1$^-$ IL7Ra$^-$ CD34$^-$FcgR$^-$Slamf1$^+$
Lin$^-$CD34$^+$CD38$^+$IL3Rα^-CD45RA$^-$
MEP
Lin$^-$c-Kit$^+$Sca1$^-$ IL7Ra$^-$ CD34$^-$ FcgR$^+$
Lin$^-$CD34$^+$CD38$^+$IL3Rα^+CD45RA$^+$
GMP
All isolated
MkP
EP
GP
MacP
Pro-DC
Pro-B
Pro-T
Pro-NK
Lineage restricted progenitors
Mature effector cells
Platelets
Erythrocytes
Granulocytes
Macrophages
Dendritic cells
B-cells
T-cells
NK-cells

Figure 4.1 Hematopoietic lineage tree, showing cells at successive stages of differentiation with distinctive marker profiles (immunophenotypes) in mouse and human. The hematopoietic stem cell (HSC) has long-term reconstituting, self-renewing capacity; the multipotent progenitors have limited or no self-renewal leading to transient but multilineage reconstitution. CMP, common myeloid progenitor; CLP, common lymphoid progenitor; MEP, megakaryocyte/erythroid progenitor; GMP, granulocyte/macrophage progenitor; MkP, megakaryocyte progenitor; EP, erythroid progenitor; GP, granulocyte progenitor; MacP, macrophage progenitor; Pro-DC, dendritic cell progenitor; Pro-B, B lymphocyte progenitor; Pro-T, T lymphocyte progenitor; Pro-NK, natural killer cell progenitor. (For a color version of this figure, please see the color plate section.)

antigen 1) marker [29,30,33,34] and do not express surface Flk2/Flt3 [31,32], whereas their downstream multipotent progeny express Flk2 [32]. Long-term HSCs (LT-HSCs) were also found to express CD150 (SlamF1). We isolated human HSCs of the phenotype CD34$^+$ [35] Thy-1$^+$Lin$^-$ [36] that were enriched for T, B, and myeloid cell potential in clonogenic assays, and were efficient at restoration of the respective human hematopoietic lineages in SCID-hu mice (i.e., mice with severe combined immunodeficiency bearing renal subcapsular grafts of human fetal liver and thymus) [36]. Human LT-HSCs are, in addition, CD38$^-$ to CD38^{lo} [36–40]. We further characterized LT-HSCs to be contained within the Lin$^-$CD34$^+$CD38$^-$CD90$^+$CD45RA$^-$ fraction of cord blood. Transplantation of as few as 10 of these cells resulted in long-term repopulation in immunocompromised mice. In addition, we found the Lin$^-$CD34$^+$CD38$^-$CD90$^-$CD45RA$^-$ population to contain candidate human multipotent progenitors (MPPs), cells with limited self-renewal potential capable of transient multilineage blood reconstitution [41].

In mice, the Sca-1$^-$, Lin$^+$, c-kit$^-$, Thy-1$^-$, or Thy-1^{hi} subsets do not contain any long-term multilineage hematopoietic potential [34]. In humans, CD34$^-$, Lin$^+$, or Thy-1 cells also do not contain in vitro or in vivo long-term multilineage hematopoietic precursor potential However, some research groups have reported at least some HSCs may be CD34$^-$ [42–44], or Thy1$^-$ [45]; and $\alpha6^+$ [46], as in mice [47]. Only CD34$^+$ or CD34$^+$90$^+$ human HSCs have been successfully transplanted in humans.

In mice, transplantation of limiting dilution or single LT-HSCs results reproducibly in a high frequency of hosts (5–40%) showing long-term multilineage reconstitution, with self-renewal of HSCs [27,28,48–50]. The bone marrow of allogeneic human HCT transplants contains self-renewed HSCs that also engraft upon serial transplantation in SCID-hu mice [36].

Properties of mouse HSCs and other MPPs

Transplantation of downstream short-term (ST)-HSCs or MPPs results in robust but transient multilineage reconstitution after which residual host HSCs recover [28,29,32]. However, no matter how many ST-HSCs or MPPs are transplanted, at no time is there any measurable de-differentiation from the short-term cells into the long-term pool [30].

In the normal steady state, 2–8% of LT-HSCs randomly enter cell cycle each day, whereas higher percentages of ST-HSCs and MPPs are in cell cycle at any time [51–53]. Interestingly, when steady-state bone marrow LT-HSCs are isolated according to their position in the cell cycle, those cells with 2N DNA content efficiently provide radioprotection and long-term multilineage reconstitution, whereas the dividing subsets (>2N DNA content) are less efficient at reconstitution and similarly less efficient at homing directly to the bone marrow [51,52,54]. Within the 2N DNA pool of LT-HSCs, the G_0 cells engraft more readily than those in G_1 [52]. Following transplantation, a much higher fraction of LT-HSCs remain in cell cycle in the radioprotected host, and this period can extend for at least 4–5 months after transplantation [30,50,51,55]. Given the poor transplantability of cycling HSCs, it is conceivable that protocols that test cells for "stemness" by serial transplantation could be confounded by these lingering effects of mobilization and radiation on cell cycle status and reconstitution ability [50,51,55].

Although in the steady state most HSCs are quiescent, and only a small fraction of LT-HSCs is in cell cycle, this ratio can be dramatically altered by a number of circumstances. For example, upon transplantation into irradiated hosts, probably every HSC that lands in a hematopoietic microenvironment undergoes, at least initially, symmetrical self-renewing cell divisions to expand the HSC pool. In addition, the techniques used to mobilize HSCs into the bloodstream usually operate by first causing all HSCs to enter the cell cycle non-randomly [54,56,57]. As shown in Figure 4.2, treatment of a mouse with cyclophosphamide (CY) (which should kill no more than 4–8% of LT-HSCs, but should have a dramatic effect on both multipotent progenitors and oligolineage progenitors (see later) because they are largely in cell cycle) followed by granulocyte colony-stimulating factor (G-CSF), results in every HSC entering cell cycle, leading to a 12- to 15-fold increase in the steady-state number of LT-HSCs [56].

HSCs must go through many self-renewing cell divisions over the life of an animal, raising the question of whether they are susceptible to a natural limit on their number of cell divisions [58], and whether they have a special mechanism for maintaining telomeres, to avoid programmed cell senescence resulting from critical telomere shortening [59,60] (see also Chapter 6). Both mouse and human HSCs have telomerase activity but telomere length declines in the progeny of HSCs that have undergone a large number of cell divisions [61]. Therefore, the relatively high telomerase activity in HSCs [62] is not sufficient to completely prevent telomere shortening. In the absence of functional telomerase reverse transcriptase (TERT) the progeny of dividing HSCs lose telomeres much more rapidly [61,63].

Transgenic mouse strains that overexpress the TERT component at high levels in HSCs do not reduce the length of their telomeres [64]. Nevertheless, these HSCs still lose transplantation activity after the fourth or fifth serial HSC transplantation [63]. This *loss* of transplantability in HSCs that retain telomeres points to other phenomena limiting the transplantation of these cells, including perhaps the persistent entry into cell cycle, programmed cell life spans independent of telomere length, or random factors associated with transplantation and/or cell division [61,65].

Programmed cell death or senescence of HSCs need not occur only as a result of extensive numbers of HSC cell divisions. There are several genetic loci that regulate, in mice, both the frequency of HSCs in bone marrow and the frequency of HSCs that are in cell cycle [66–68]. One genetic program that likely regulates HSC numbers is that leading to programmed cell death inhibitable by the antiapoptotic proteins MCL1 and BCL2 (and by extension, other antiapoptotic genes such as BCL-x). In mice that have an enforced high level of expression of hBCL2 in HSC, the steady-state levels of HSCs are increased four- to five fold, and the competitive repopulation activity of these HSCs is higher than that of CD45 congenic HSCs transferred into the same irradiated host [69,70].

There are several genes whose expression is required to initiate: hematopoiesis alone, hematopoiesis and angiogenesis, or angiogenesis alone; these include *AML-1/RUNX-1* [71], *SCL-TAL* [72], *FLK1* [73]. At least some of these genes are expressed intrinsically in HSCs [74–79].

Genetic pathways for the self-renewal of HSCs

Self-renewal is the single distinguishing characteristic between LT-HSCs and the rest of the multipotent and oligopotent progenitors in the hematopoietic system (see Chapter 6). For self-renewal to occur for life and for the renewed cells to contribute to hematopoiesis, HSC proliferation must be possible in the absence of differentiation, and life span limitations such as telomere shortening, programmed cell death, and programmed cell removal must be held in check. Several of the genes already found to be involved in HSC functions prohibit differentiation. For example, *BMI1* in the gene expression inhibitor complex PRC1/2 affects H3K27 trimethylation [66,80–82] and inhibition of TET2 and its regulated DNA demethylation pathways [83,84]. In addition, HSCs express higher levels of telomerase and its associated telomere length retention elements,

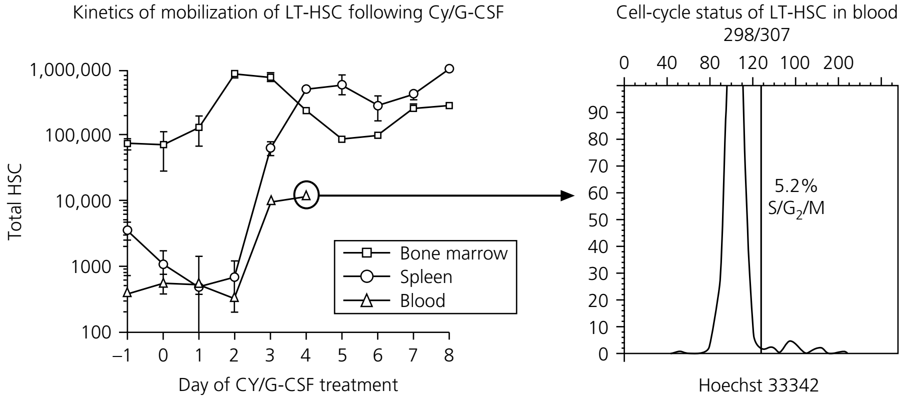


Figure 4.2 The total number of long-term hematopoietic stem cells (LT-HSCs) in the bone marrow, spleen, and blood of mice on successive days of CY/G-CSF treatment. Total bone marrow hematopoietic stem cells (HSCs) were calculated by assuming that the femurs and tibias (less the epiphyses) contained 15% of all bone marrow in the mouse. Total HSC level in the blood was calculated by assuming that the total blood volume was 1.8 mL. Cell-cycle status of LT-HSCs in the blood was determined by Hoechst DNA staining. Source: Wright et al. 2001 [54]. Reproduced with permission of the American Society of Hematology.

and also express antiapoptotic genes such as *MCL1* [85], and perhaps *BCL2* [86]. While LT-HSCs can be expanded in a variety of situations in vivo, the attempt to expand rigorously purified mouse or human HSCs in vitro with conventional cytokines such as steel factor (SLF), thrombopoietin (TPO), interleukin 11 (IL-11), IL-6, IL-3, and Flt3L, alone or in combination in serum-containing medium has never resulted in more than a minor expansion in HSCs by phenotype, and little or no expansion by function [87–93]. Many investigators were led astray by the expansion of cells expressing CD34, but a broad variety of non-HSCs are also CD34$^+$, and these cells were the result of proliferation of HSCs and progenitors coupled with cell differentiation. The introduction of the antiapoptotic protein BCL2 into HSCs in mice allowed HSCs to survive serum-free culture conditions, and so the response of LT-HSCs to factors in the absence of differentiating factors present in serum, such as monocyte/macrophage colony-stimulating factor (M-CSF), G-CSF, granulocyte–monocyte colony-stimulating factor (GM-CSF) and others, could be studied [69,70]. Any combination of these cytokines and others when added to these BCL2-containing HSCs led to a massive burst of proliferation and progenitor expansion by most HSCs, but little or no detectable HSC expansion [69,70]. HSCs responding to IL-3 give large bursts of proliferation with concomitant differentiation along the myeloid and mast cell lineages, without self-renewal of HSCs. One might have hoped that the HSC response to SLF alone would have been self-renewing divisions, as mutations in the *SLF* gene or its receptor, c-kit, lead to fetal or early neonatal morbidity, usually because of profound anemia [94–99] (reviewed in [100]). SLF is a survival cytokine for resting and active HSCs [70], and antibody blockade of its signaling through c-kit can deplete HSCs [101]. However, the response of single highly purified Bcl2-expressing LT-HSCs to SLF in serum-free medium was proliferation without detectable self-renewal, but with differentiation through both common lymphoid and common myeloid progenitor pathways [70]. Stimulation with TPO led to mild proliferation, and dedicated differentiation along the megakaryocytic lineage. Interestingly, analysis of mRNA from HSCs and highly purified myeloid progenitors revealed that HSCs might have the highest expression of the TPO receptor c-mpl, of any of these cell populations [74,102–104, https://gexc.stanford.edu/model/3/gene/Mpl]. TPO is produced by osteoblasts in the bone marrow and blocking antibody to it induces release of HSCs from the niche and their increased proliferation [105]. Together, these data suggest that TPO is important for HSC proliferation and self-renewal after transplantation but also promotes quiescence by promoting retention in the niche, with increased integrin expression and prevention of cycling and subsequent HSC exhaustion.

Some early experiments indicated that other factors may be involved in HSC expansion. For example, activation of the Notch-1 receptor mimicked by retroviral transduction with the intracellular activated form of Notch-1 occasionally led to the generation of mouse cell lines with many of the characteristics of HSCs, although this response was neither general nor robust [106,107]. Provision of *HoxB4* as a retroviral insert in semipurified mouse hematopoietic progenitors led to vigorous proliferation and at least some retention of hematopoietic multilineage and long-term reconstituting activity in vitro and in vivo [108–110]. However, there has yet to be a rigorous demonstration that this outcome was caused by expansion of HSCs alone, rather than by provision of other signals that enable their engraftment [109–111].

The Wnt/Fzd/Dsh/GSK-3β/β-catenin pathway is also implicated in HSC self-renewal (reviewed in [112,113]). Wnt, a highly hydrophobic protein binds to surface frizzled (Fzd), a 7TM protein, in combination with a low-density lipoprotein-associated receptor, usually low-density lipoprotein receptor-related protein (LRP) 5. Binding of Wnt to Fzd leads to activation of disheveled (Dsh). The action of Dsh allows β-catenin to dissociate from the complex that includes APC, axin, and GSK-3β; this prevents GSK-3β phosphorylation of β-catenin, which, therefore, can translocate to the nucleus, where it becomes available for binding to Lef/TCF DNA binding transcription factors to activate transcription [112,113].

The addition of partially or fully purified Wnt3A to highly purified LT-HSCs in serum-free medium results in clonal proliferation of HSCs. In vitro, these HSCs go through a significant expansion and up to 50% of the expanded progeny are cells of the LT-HSC phenotype [114,115]. Transfection of non-degradable β-catenin lacking the *N*-terminal phosphorylation sites were reported to drive LT-HSC into massive and prolonged expanding cell divisions, with expansion of LT-HSCs both by phenotype and by function upon transplantation [114]. Inhibitors of Wnt signaling even blocked SLF and other cytokine-mediated proliferation of LT-HSCs [114]. When LT-HSCs are transfected with a reporter of LEF/TCF + β-catenin activity in the nucleus and then transplanted into lethally irradiated hosts, LT-HSCs reading out the reporter are retained in vivo long term, whereas myeloid progenitors derived from these LT-HSCs do not express the reporter [114]. Taken together, these results indicated that the Wnt/Fzd/β-catenin pathway is likely important in self-renewing cell division of LT-HSCs. When activated by Wnt3A, LT-HSCs upregulate expression of both *Notch-1* and *HoxB4*, genes also implicated in the self-renewal of HSCs. However, the regulated deletion of the β-catenin gene in transplanted bone marrow failed to affect the engraftment of hematopoiesis or the retransplantation capacity of this marrow [116]. Contradictory results were recently reported with a different gene promoter encoding the Cre recombinase required for β-catenin deletion [117].

The *BMI1* gene is a member of the polycomb family, which has a role in silencing gene expression by site-specific deacetylation of histones associated with those genes, as well as trimethylation of H3K27 [80]. *BMI1* is expressed at high levels in mouse and human LT-HSCs, and mice with an artificially mutated *BMI1* gene have a profound defect in the transplantation and self-renewal of LT-HSCs [78,80]. The two transcripts of the p16 locus, P16INK4A and P19ARF, are overexpressed selectively in the hematopoietic tissues in the *BMI1*-mutant mice, thus indicating a role of *BMI1* in down-regulation of genes whose expression could lead to differentiation and/or is contrary to self-renewal of HSCs [118]. These anti-self-renewing effects could include decreased viability, enforced differentiation, or direct regulation or inhibition of genes involved in the self-renewing pathways such as those of Wnt/Fzd/β-catenin and Notch [106,107,112,114]. *BMI1* mutants show no differences in expression of *HoxB4* but an increase of *HoxA9* [80], genes that also have some role in HSC functions [111,119].

JunB negatively regulates mouse LT-HSCs, and in the absence of JunB the HSC pool expands. In the expanded pool, p16ink4a and p19arf are expressed at lower levels than wild-type HSC, and bcl2 and bclxl are expressed at higher levels in JunB knock-out HSCs than in wild-type HSCs [120]. A number of genes have recently been reported to play a role in HSC self-renewal, including *JunB*, *Sox17*, *Cited2*, *Mcl1*, *Tel/Etv6*, *Gfi1*, *Pten*, and *Stat5*. Chapter 6 covers self-renewal in more detail, but, taken together, these results indicate that the control of self-renewal is a complex, multipathway process, much of which is probably controlled by the HSC niche.

Migration of HSCs

Hematopoietic capacity is known to be present in the adult mouse at the highest levels in bone marrow, at about one-tenth of that level in the spleen, and at 1/100 of the bone marrow level or less in blood [121]. In the early experiments that showed this, the ability of marrow or tissue transplants to retain erythropoiesis could not be assigned directly to the action of HSCs. There was no clear explanation as to why hematopoietic activity might be present in organs other than the bone marrow, and certainly not in the blood. It was only when clinical hematopoietic cell transplantation groups described the phenomenon of hematopoietic activity increasing in the blood of cytotoxic drug-treated individuals that the possibility of HSC mobilization into the blood was considered [122,123].

Empirical protocols using cyclophosphamide plus cytokines such as G-CSF, GM-CSF, SLF alone or in various combinations with G-CSF, IL-1, IL-3, led to efficient mechanisms to mobilize hematopoietic cells into the bloodstream [124–128]. In these early experiments, this activity was measured in terms of *in vitro* tests for hematopoietic colony-forming cells (CFCs), but CFC assays alone are not specific for HSC versus oligolineage progenitor activities [36,129]. However, there could be no doubt that HSCs were involved when MPB transplants were used in hosts conditioned with high-dose chemotherapy, resulting in both early and sustained hematopoiesis. When cyclophosphamide and G-CSF are used in the mouse model – wherein one can analyze directly the daily changes in marrow, spleen, and blood – it is clear that nearly every HSC and MPP enters cell cycle rapidly [56]. These reach a peak in the marrow, at which time the cells appear rapidly in blood and spleen (Figure 4.2). At all times the HSCs appearing in the blood have 2N amount of DNA, indicating that they are not proliferating in the blood; in fact, the fold increase in their numbers would have ruled out a blood-only expansion of the HSC pool [56]. Bromodeoxyuridine (BrdU) incorporation into their chromosomal DNA during the expansion phase of HSCs in the "mobilized" bone marrow results in the labeling of virtually every HSC and, interestingly, just 1 or 2 days later, the 2N HSCs found in the blood are all labeled with BrdU [56]. Thus, mobilization appears to be a consequence of marrow HSC proliferation and accumulation of emigrant HSCs in extramedullary sites such as spleen, liver, and blood. HSCs have low levels of receptors for G-CSF [102,103,130], yet G-CSF triggers mobilization of highly purified HSCs [131,132]. In the early phases of mobilization, there is a rise in cells of the myelomonocytic series, and it has been shown that the elaboration locally by myeloid cells of matrix metalloproteinases such as MMP9 can serve to cleave cell–cell interaction molecules known to be active between HSCs and marrow stroma [133]. Similarly, elaboration of the chemokine IL-8 occurs and infusion of IL-8 alone can cause a significant and rapid mobilization of HSCs [134,135]. The actual nature of molecules involved in HSC release is not clear, although it is known that HSCs express integrin $\alpha_4\beta_1$, which allows them to attach to hematopoietic stromal cell VCAM-1 [136–140]. Also, HSCs express the c-kit receptor, which allows binding to at least cell-surface SLF on stroma. Although HSCs have a number of other members of the adhesion and integrin family, the role of each of these in the following remains unclear: cell proliferation, detachment from stroma, local emigration into sinusoids, movement through the bloodstream, margination on distant blood vessels, transendothelial migration at these vessel sites, and localization to microniches that support LT-HSCs. In the adult mouse, homing of infused HSCs appears to involve both cell surface integrin $\alpha_4\beta_1$ and cell surface CXCR4, a receptor for the chemokine SDF-1 [141–143]. Of all known chemokines, SDF-1 is the only one to which adult mouse HSCs migrate in vitro, and this is chemotactic movement [143]. However released, co-infusion of labeled HSCs and labeled red blood cells (RBCs) from mobilized blood into the blood of same-stage mobilized animals results in the rapid emigration of HSCs out of the blood and these HSCs do not reappear in the bloodstream for the next several hours (Figure 4.3). These HSCs non-randomly home to hematopoietic sites in mobilized animals: bone marrow, spleen, and liver – all tissues with sinusoids lined by macrophages. These observations fit with previous studies on the rapid egress of infused lymphocytes out of the bloodstream into tissues via recognition of particular vascular addressins for which they have cognate homing receptors [144,145]; and the rapid egress from

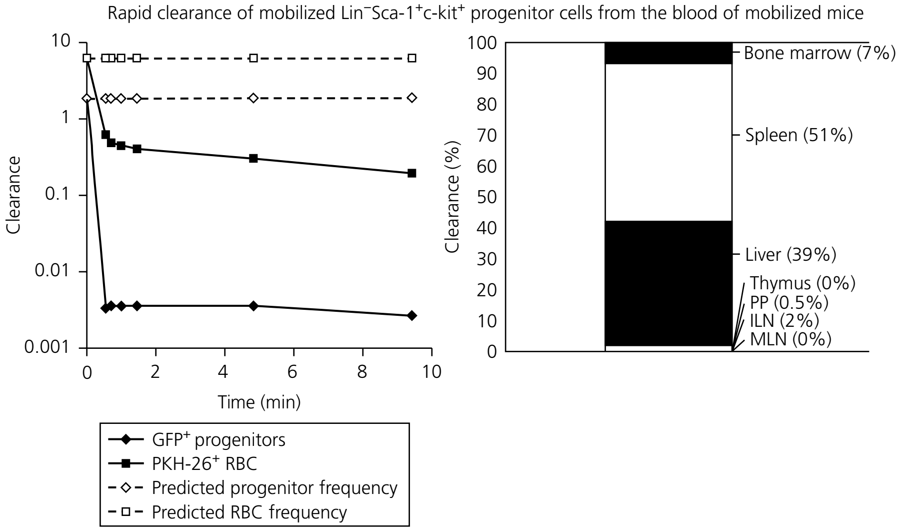

Rapid clearance of mobilized Lin$^-$Sca-1$^+$c-kit$^+$ progenitor cells from the blood of mobilized mice

Figure 4.3 Rapid clearance of mobilized hematopoietic progenitor cells from the bloodstream. Clearance from the blood of eGFP$^+$ progenitors and PKH-26$^+$ red blood cells (RBCs), and predicted progenitor and RBC frequencies, respectively. In vivo homing to different organs of mobilized and consecutively transplanted hematopoietic progenitor and stem cells 3 hours after injection. Source: Wright et al. 2001 [54]. Reproduced with permission of the American Society of Hematology.

the bloodstream of other nucleated cells such as monocytes [146]. There are similar HSC, MPP, and myeloid progenitor fluxes in normal mice. Maintenance of the approximate 10–100 HSCs found in the blood of mice, given a residence time in the blood of 5 min or less, would require fluxes of well over tens of thousands of HSCs and MPPs per day. Data suggest that HSCs and progenitors circulate through the blood and reside in the tissues for up to 36 hours before returning to the blood via the lymphatics [147]. It is not yet clear whether these cells are passing through the blood in one pass or whether they form a specialized pool of HSCs and MPPs that have a higher probability of reentering the blood than cells resident in deep marrow niches. If HSCs found in the blood are also derived from dividing HSCs, nearly one out of every two daughter cells from a self-renewing HSC division would have to enter the bloodstream.

Consistent with this emigration rate, infusion of purified LT-HSCs into untreated immunodeficient mice (to avoid immune rejection of cells with antigenic markers) led to a dose–response engraftment of marrow sites with 0 to 250 HSCs, while up to 5000 infused HSCs engrafted no more marrow sites than did 50 infused HSCs. This plateau was about 0.5% of marrow sites. However, 50 cells on day 1 and another 50 cells on day 2 each engrafted about 0.5% of the marrow sites [148]. Thus, about 0.5% of the marrow niches for HSCs appear to be available at any moment, and these appear to be occupied by the next interval. Pre-clearing of HSCs with a cytoreductive anti-c-kit antibody alone in immunodeficient mice leads to up to 80% engraftment with purified HSCs given in large numbers at the point that the mAb reaches its lowest concentration in the blood [101].

The migration of HSC into hematopoietic tissues involves a step of crossing macrophage-lined sinusoids. During mobilization, HSCs and progenitors upregulate the expression of the integrin-associated protein CD47 [149]. One function of CD47 is as a ligand for the SIRPα macrophage receptor [150]. In the absence of CD47, macrophage-bound cells are phagocytosed, whereas cells expressing high levels of CD47 signal the macrophage through SIRPα to block phagocytosis. We have found that leukemias transfected with mouse CD47 at levels approximating those found on mobilized HSCs are protected from phagocytosis in vitro and engraft in vivo, whereas the same leukemias lacking these levels of CD47 are phagocytosed in vitro and fail to engraft in vivo [149]. Further, HSCs from CD47-knockout mice fail to engraft in wild-type congenic mice [149]. These experiments provide evidence that, during migration, itinerant HSCs express high levels of CD47 to gain entry to hematopoietic sites.

Hematopoietic stem cell and progenitor niches

Interpreting the fate of recently self-renewed and resting HSCs calls for a deeper examination of hematopoietic niches in the marrow. Niches that commit recently entering HSCs to the myeloid or lymphoid pathways need not be adjacent to the HSC niche from which the parent, uncommitted HSCs came if the daughter cells are constantly migrating. In addition, a large flux of recirculating HSCs likely provides for the continuous replacement of empty HSC niches with functional HSCs. In the absence of such a pool of migrating HSCs, the death of an HSC could mean the permanent loss of that functional niche. This finding has relevance when one considers claims of plasticity of stem cells in one or more tissues [48]. If tens of thousands of HSCs are fluxing through tissues every day, finding HSC activity in those tissues might result only from these itinerant HSCs, rather than transdifferentiation of local tissue-specific stem cells into hematopoietic fates [48].

The understanding of the anatomical sites for HSC niches followed hypothetical analyses of the need for such niches to control HSC numbers and to account for their differentiation to progenitors. Schofield proposed that such niches would be most likely placed next to bone [151], and several later studies showed that a subset of osteoblast-lineage cells likely were part of the niche [152,153]. Much earlier, Dexter et al. developed bone marrow stromal cultures that supported myeloerythropoiesis [22,154], and Whitlock and Witte developed stromal cultures that supported B lymphopoiesis [155]. Whitlock et al. cloned single stromal cells from these cultures, and developed several stromal lines, some of which supported only myeloerythropoiesis, and others, myeloid production followed by B lymphoid production [156]. For both of these types of cultures, only HSCs could initiate long-term cultures [155,156] that included B and myeloid outcomes from single cells. The stromal cell clones that allowed single HSCs to produce multiple lineages expressed several surface markers, including 6C3/Bp1, a transmembrane protein with an ectodomain peptidase that is expressed on early B lineage progenitors [157] and is induced by IL-7 on these stromal and B lineage cells [158]. The prospective isolation of mouse and human HSCs used one such clone, AC6.2.1, in the readouts of multilineage precursors. Both Dexter and Whitlock-Witte cultures gave accurate readouts of mouse and human HSCs and hematopoietic progenitors and the adhesion molecules, for example integrin $\alpha_4\beta_1$ with VCAM-1 [23,136,137,158], that these stem and progenitor cells use to bind to the stroma. The lines that support myelopoiesis often have hematopoietic cultures that overgrow with macrophages, and a line from the CSF-1 osteopetrotic mouse, OP9, supports myelopoietic cultures over the long term [159]. The modern approaches to the cellular components in the hematopoietic marrow niche now include at least three cell lineages, the osteoblast subset [160], a nestin$^+$ stromal cell innervated by SNS peripheral nervous system neurons [161], and blood vessel cells [162]. Programmed knockdown of genes in the endothelial lineages leads to diminution of HSC levels.

Dissociated cells from early mouse fetal bone, taken prior to the seeding of hematopoiesis at embryonic-day (E)15, transplanted in matrigel ectopically under the kidney capsule form new bones, with cartilage components and complete intraosseus bone marrow, with blood vessels, blood lineage cells, and HSCs from the host [163]. Prospective isolation of a clonal precursor that self-renews and forms bone, cartilage, and at least four distinct hematopoietic supporting stromal cells has been accomplished [163,164]. The stroma include 6C3$^+$ cells with similar profiles to the AC6.2.1 population that was clonally isolated from Whitlock-Witte cultures 26 years ago [164]. These newly isolated cells support HSCs to conduct full hematopoiesis and self-renew in vitro. They also contain mRNA encoding Wnt4 and CXCL12, while a separate subset expresses cell surface CD90 and includes mRNA expressing SLF. Intravenously infused pure HSCs can be found in cavities bounded by the 6C3$^+$ and the CD90$^+$ stroma, neither of which are on the surface of osteoblasts or endothelial cells [164]. Taken together, these studies indicate the existence of a very complex mouse niche with multiple stromal cell types and segregated expression of HSC-supporting cytokines.

Ontogeny of HSCs

It is tacitly assumed that HSCs derive from the embryonic mesoderm. In mammals, the first site of definable blood cells appears to be the yolk sac blood islands, in mice at about 7.5 days post-coitum (dpc) [165,166]. At 7–8 dpc, mouse yolk sacs contain cells capable of responding in vitro to produce hematopoietic CFCs [165,166]. The yolk sac connects to the developing embryo/fetus [167] at about 8.5 dpc via the development of the vitelline vessels, which feed into the embryo proper. Just after that 8–8.5 dpc interval, hematopoietic cells appear in and around the dorsal aorta of the developing fetus (reviewed in [168]). Nearly 40 years ago, we showed that transplantation of 8–9-dpc yolk sac blood island cells into the yolk sac cavity of 8-dpc allogeneic hosts results in mice that have lifelong presence of donor-derived day 10 CFU-S in marrow as well as thymocytes [169]. At about 10–11 dpc, hematopoiesis begins in the fetal liver in the mouse, and continues there throughout fetal life.

Where do the first HSCs develop [170]? At 3.5 dpc, the blastocyst implants into the uterus, and its inner cell mass appears to be a population of pluripotent cells without defined commitments [167]. Between 3.5 and 5.5 dpc, gastrulation occurs with the formation of the three germ layers [167]. It has been a mystery whether the developing post-gastrulation mesoderm cells emigrate separately to yolk sac and embryo to give rise to independent origins of hematopoiesis; or whether only one of these sites is the initial site of commitment to hematopoiesis, and the other sites that appear later result from migration [168,169,171,172]. The idea of separate origins for extra-embryonic (yolk sac) hematopoiesis and intra-embryonic hematopoiesis was supported by the fact that yolk sac erythropoiesis produced embryonic and fetal hemoglobins, while lifelong hematopoiesis produced mainly adult hemoglobin [171,173]. These were called, respectively, primitive and definitive hematopoiesis [173]. Until 2007, no experiment directly assessed whether these two kinds of hematopoiesis have distinct or common origins at the cellular level. To address this question Nishikawa's group used an inducible Runx1/aml1 reporter system to label extra-embryonic HSCs [174]. When embryos were induced at 7.5 dpc, the time that HSCs first appear in the yolk sac blood islands [169,175], labeled hematopoietic cells were observed in the fetus and adult. As circulation was not established at this time-point, these data suggest that the labeled HSCs that persist and give rise to ongoing hematopoiesis must have derived from yolk-sac HSCs that migrated into the embryo once circulation was established [174]. Because the placenta and the allantois at 9 dpc have some Runx1$^+$ cells, they are other possible sites of HSC generation in these experiments if the tamoxifen inducer has a half-life in the embryo greater than a day.

Hematopoiesis and angiogenesis are closely associated in both the developing blood islands and the developing dorsal aorta, and there is good evidence that mutant mice lacking the cell surface receptors for angiogenic peptides fail to develop either hematopoietic or angiogenic cells [73,176]. While these experiments suggest that a bipotent precursor called the hemangioblast exists that can give rise to both angiogenic stem cells and HSCs, only a small number of hemangioblast cells capable of producing both outcomes in vitro can be prospectively isolated from the pre-yolk sac embryo [177]. Rather than there being a hemangioblast precursor, it is possible that mutant mice that cannot make blood vessels properly, cannot make blood because primitive stem cells travel via the blood, or that blood vessels make factors important for hematopoiesis. Nevertheless, there are several independent studies that show the existence of hemogenic endothelial cells in mice [178] and zebrafish [179]. Whether the progeny of cells that appear to bud from hemogenic endothelium is the HSC or some other cell type is still unclear.

A set of our experiments supports the hypothesis that the majority of embryonic blood cells found in yolk sac blood islands do not share a clonal progenitor in the yolk sac, and therefore many derive from pre-hematopoietic mesodermal cells in the yolk sac [180]. Tetrachimeric mice were generated by injection of four distinctly colored single cells into developing blastocysts. Analysis of the color combinations observed in the yolk sac of the resulting embryos revealed endothelial and hematopoietic cells of different colors in all individual blood islands, indicating that they developed from more than one cell (Figure 4.4). If both hematopoietic and endothelial cells derived from a common hemangioblast progenitor, each blood island would be monochromatic in these mice. However, single-color blood islands were not observed. Instead the majority of blood islands contained blood and endothelial cells of multiple but not necessarily the same colors, indicating that multiple progenitors of each type came together to form each blood island [180]. About 15–20% of the blood islands examined could have derived from hemangioblasts; when we lineage-marked cells with the Flk1.cre/floxed Lacz$^-$GFP constructs in the same ES cells, about 20% of blood cells were GFP$^+$, indicating an activated Flk1 locus, but 80% were Lacz$^+$GFP$^-$. These data indicate that about 20% of early blood cells derive from a possible hemangioblast progenitor, and about 80% derive from a hematopoietic progenitor that did not go through a hemangioblast pathway. Similar conclusions were drawn by Shalaby et al. [73]. However, Choi et al. report that all yolk sac cells are derived from Flk1$^+$ precursors [181], a finding different from ours.

There is an interesting point coming out of embryonic stem cell biology that may be relevant to these issues. ES cells are derived from the inner cell mass of blastocysts, and likely represent pluripotent stem cells in the mouse at approximately 3.5 dpc. They are maintained as pluripotent cells by the addition of LIF (leukemia inhibitory factor) to their cultures, at least in mouse ES lines [182]. Removal of LIF from these pluripotent ES lines results in the onset of hematopoiesis and, during this development, 5 to 8 days after LIF was withdrawn, cells capable of in vitro hematopoiesis emerge. These cells also express some markers (AA4.1$^+$c-kit$^+$CD34$^+$Lin$^-$CD41$^+$CD45$^+$) shared with HSCs, but at this point are Sca1$^-$ [183]. Yolk sac blood island cells at 9 dpc are also AA4.1$^+$c-kit$^+$CD34$^+$Lin$^-$cD41$^+$CD45$^+$ [169,175]. Thus, these two systems appear in parallel to produce embryonic HSCs. However, the ES derived "HSCs" express adult hemoglobin, and can produce myeloerythroid and lymphoid cells. Despite this, ES-derived HSCs did not engraft in irradiated newborn or adult hosts [183], indicating the requirement for further development of these cells to give rise to transplantable HSCs. HSCs are in far greater numbers at all times in the yolk sac blood islands than in the embryo proper (e.g., within the dorsal aorta) [183,184]. While the numbers of HSCs in the yolk sac at all times could support the number of HSCs found in 11.5-dpc fetal liver, the number in the embryo proper in the entire dorsal aorta could only supply a fraction of those in the 11.5-dpc fetal liver. These findings indicate that yolk sac blood island cells represent a site of both embryonic and definitive hematopoiesis.

The major site of the next (fetal) stage of hematopoiesis, after the yolk sac blood islands, appears to be the fetal liver [166]. The numbers of HSCs in the fetal liver increase logarithmically from 11–15 dpc. After 15 dpc, fetal liver HSC numbers level off, but hematopoiesis continues to expand in the liver [33,185,186]. At 16 dpc, hematopoiesis appears in the fetal spleen in mice, and at 18 dpc, in the fetal bone marrow.

The initiation of fetal liver hematopoiesis is blocked in mice with mutations in integrin $\alpha_4\beta_1$ (VLA-4) or integrin $\alpha_5\beta_1$ (VLA-5) [187,188]. Because integrins are involved in cell migration, immigration of

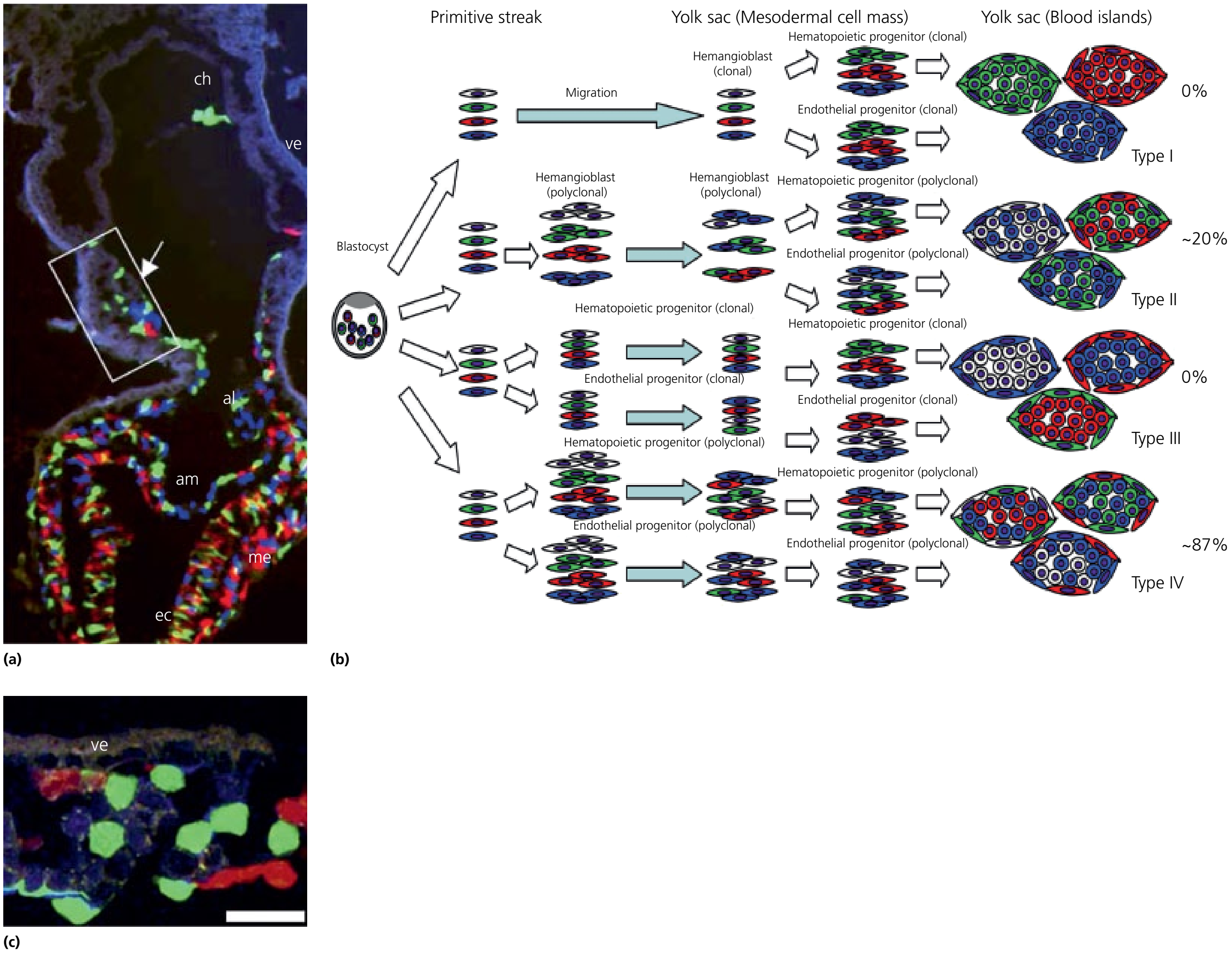

ch
ve
al
am
me
ec
(a)
Primitive streak
Yolk sac (Mesodermal cell mass)
Yolk sac (Blood islands)
Blastocyst
Migration
Hemangioblast (clonal)
Hematopoietic progenitor (clonal)
Endothelial progenitor (clonal)
0%
Type I
Hemangioblast (polyclonal)
Hemangioblast (polyclonal)
Hematopoietic progenitor (polyclonal)
Endothelial progenitor (polyclonal)
~20%
Type II
Hematopoietic progenitor (clonal)
Endothelial progenitor (clonal)
Hematopoietic progenitor (clonal)
Endothelial progenitor (clonal)
0%
Type III
Hematopoietic progenitor (polyclonal)
Endothelial progenitor (polyclonal)
Hematopoietic progenitor (polyclonal)
Endothelial progenitor (polyclonal)
~87%
Type IV
(b)
ve
(c)

Figure 4.4 Chimeric analysis of yolk sac blood islands with fluorescent ES clones. Tetrachimeric mice were generated by injection of four distinctly colored single cells into developing blastocysts. The resulting embryos were then analyzed for color combinations observed in the YS and revealed endothelial and hematopoietic cells of different colors in all individual blood islands, indicating that they developed from more than one cell. (a) A chimeric embryo at the early neural plate stage (EB). A white rectangle indicates an immature blood island. The arrow indicates the direction of tissue extension; ec, embryonic ectoderm; me, intra-embryonic mesoderm; ve, visceral endoderm; am, amnion; al, allantois; ch, chorion. (b) Experimental scheme indicating the possible derivations of blood islands and the % of each type observed. (c) A type IV chimeric blood island with EGFP, ECFP, and mRFP1 endothelial cells and ECFP, EGFP, and non-fluorescent hematopoietic cells. Source: Adapted from Ueno et al. 2006 [180]. Reproduced with permission of Elsevier.

these cells into or beyond the fetal liver may require steps that include expression of $\alpha_4\beta_1$ or induce the expression of integrin $\alpha_5\beta_1$ at this stage of the liver. Integrin $\alpha_4\beta_1$ is used by trafficking HSCs throughout life to enter sites of hematopoiesis [140], and so the endowment of expression of integrin $\alpha_4\beta_1$ permits fetal liver HSCs to be assayed by transplantation in fetuses or adults. In contrast, neither yolk sac blood island HSCs nor ES-derived HSCs in their native state can be transplanted into adult irradiated mice. The transition of yolk sac HSCs and ES-derived HSCs from that embryonic stage to the fetal stage reportedly can be facilitated by enforced expression by *HoxB4*, or by *HoxB4* and *Cdx4* in these cells [108]. Therefore, there are important clues to how embryonic HSCs, presumably capable of primitive hematopoiesis, can develop into fetal HSCs capable of definitive hematopoiesis.

During the various stages of fetal HSC functions, it initially seemed possible that waves of HSCs emerged from tissues such as the fetal liver at specific time points to be accepted by and to engraft in secondary sites such as fetal spleen, fetal bone marrow, and lymphoid sites such as the thymus [170]. However, HSCs are found constitutively in the blood throughout fetal life, and it must be the preparedness of the developing organs such as the spleen, marrow, and thymus that results in the movement of hematopoiesis to those sites [189].

A detailed examination of HSCs in the developing fetal liver with respect to T-cell developmental potentials has revealed that changes are occurring at the level of HSCs which show that HSCs develop in a quantal but dynamic process [190]. Developing T cells gain their specificity and their function in a coordinated series of molecular events, including selection of which class of T-cell receptor genes are rearranged and selected, and to which distant sites the T cells will migrate. Within the thymus, during fetal development, the first T cells to emerge use products of the T-cell receptor Vγ3 and T-cell receptor δ gene families, followed by the T-cell receptor Vγ4, then Vγ2 and Vγ5 and T-cell receptor $\alpha\beta$ gene families. In mice the T-cell receptor V genes at the γ locus that must rearrange to be expressed are, extending from the most proximal site of potential rearrangements, Vγ3, Vγ4, Vγ2, and then Vγ5. The first T cells to emerge in the thymus are Vγ3 cells, which colonize the skin only during fetal life [191]. While Vγ3 T-cell development is shutting down in the thymus, Vγ4 T-cell development arises, and these T cells will migrate during fetal life to the presumptive female genital tract epithelia and the tongue epithelium of both sexes [190,191]. The development of Vγ4 T cells decreases at about day 16 or 17 of fetal life, to be overtaken by the almost simultaneous onset of: Vγ2 T cells, which will inhabit lymph nodes and secondary lymphoid sites such as the spleen; Vγ5 T cells, which will inhabit the entire gastrointestinal tract; and αβ T cells, which are largely restricted to lymphoid tissues.

The kinetics of T-cell development can be studied with the use of fetal thymic organ cultures (FTOCs), although these are not physiologic cultures. When single clonogenic 12-dpc fetal liver HSCs are added to FTOCs, those thymuses produce Vγ3, then Vγ2, then Vγ5, and TCRαβ T cells [190]. When 14-dpc fetal liver HSCs are placed at clonal levels into the same stage FTOC, Vγ3 T cells do not emerge, but the rest of the repertoire is developed. Finally, 12-dpc fetal liver HSCs added directly into the adult thymus cannot produce the fetal program of Vγ3 T cells, but can produce the mature program of Vγ2, Vγ5, and TCRαβ cells [190].

Taken together, these studies indicate that, probably with each cell division, the fetal liver HSCs are changing (usually reducing) their developmental fates, which would only be read out when their progeny entered the fetal thymus, and the change in these fates and the readout of these fates is dependent upon factors within HSCs and within the thymus microenvironment. After birth, and at least in young adult mice, the numbers of LT-HSCs, ST-HSCs, and MPPs are regulated at a relatively constant level.

The aging of HSCs: clonal selection versus regulated epigenetic change

HSCs in the mouse go through cell autonomous changes in numbers and developmental potentials during aging. We first reported that there is a gradual loss of cells of the MPP phenotype and an increase in both the number of LT-HSCs and the fraction of LT-HSCs that are in the cell cycle [65], most dramatically seen in the marrow of geriatric mice. With a refinement of markers for LT-HSCs versus all other multipotent progenitors, we found that the number of LT-HSCs increased as reported, but that they retained a low level of cell-cycle activity; the increase was found primarily in a MPP fraction [77]. In old mice, myelopoiesis is retained or even enhanced, while lymphopoiesis in both T- and B-cell lineages falls. These fates are cell intrinsic; co-transplantation of 2-month-old and 2-year-old LT-HSCs into irradiated young mice led to increased output of LT-HSCs from the old versus young stem cells, and less output of CLPs and T and B cells from the old LT-HSCs than from the young LT-HSCs [77]. These same trends were seen in the small number of aged hosts transplanted with young and old HSCs. The cell intrinsic biologic properties of old versus young HSCs were mirrored by a microarray analysis of these highly purified cells: young HSCs had both myeloid and lymphoid transcripts, but old HSCs overexpressed myeloid and underexpressed lymphoid transcripts [77]. Of the top 32 myeloid transcripts overexpressed in old HSCs, 13 have been identified as proto-oncogene or translocation partners of myelogenous leukemia proto-oncogenes in humans. As myelogenous leukemias increase in incidence with age, the initiating events may occur in HSCs, and if they do, genomic events such as translocation and inversion may occur preferentially at highly transcribed loci.

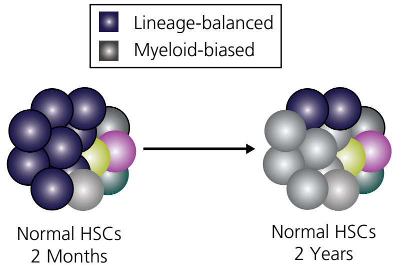


Figure 4.5 At birth, most of the hematopoietic stem cell (HSC) clones in mice are *lineage-balanced*, that is, equally capable of generating various lineages. However, with age, clones biased towards generating myeloid lineages (*myeloid-biased*) predominate, due to either clonal selection or epigenetic changes in the originally lineage-balanced clones. (For a color version of this figure, please see the color plate section.)

In aged mice with predominantly myeloid-biased HSCs the majority of HSCs are CD150^{hi}, while in young mice the majority are CD150^{lo-med}, with rare CD150^{hi} cells. CD150^{hi} HSCs from young or old mice are myeloid biased, while CD150^{lo-med} cells from young or old mice are balanced or lymphoid biased at the single cell level [192]. This opens the possibility, shown in Figure 4.5, that mice are born with various clones of HSCs with preset hematopoietic potentials, and that during aging, the myeloid-biased subset outcompetes the balanced or lymphoid-biased clones. Since the same distribution of myeloid-biased versus balanced HSCs is found in human bone marrow – balanced in youth but with a dominance of myeloid-biased cells in the elderly [193] – it appears that this is an evolutionarily conserved phenomenon. We speculate that before trains, planes, and cars, most species encountered the microbes in their microgeography by puberty, giving rise to long-lived memory T- and B-cell clones against them. Therefore the demand for naïve immunocompetent T and B cells is diminished, and the need for the continual generation of CLPs that give rise to them also decreases as the myeloid-biased HSC clones become dominant in the aged.

Does hematopoiesis only derive from HSCs and do HSCs give rise only to blood?

Several years ago, there were reports that bone marrow hematopoietic cells could give rise to brain cells, that brain stem cell populations can give rise to blood, that fat cells can give rise to neurons and mesenchymal cells, that muscle stem cells can give rise to hematopoietic and myogenic outcomes, and a host of other types of transdifferentiations (reviewed in [194]). What was lacking in most of these experiments was the demonstration that the transplanted population was in fact a stem cell purified to homogeneity that could give rise at the clonal level to two different tissue types, and that the tissue types that had been generated were robustly characterized as functional mature cells of that tissue without cell fusion.

Attempts to repeat the experiments showing transdifferentiation of CNS stem cells to hematopoietic tissues have thus far failed. A reexamination of the cells in muscle that give rise to blood show them to be CD45^{+} committed HSCs, separable from muscle progenitors that, on their own, give rise to muscle [195,196]. In some circumstances, regenerating muscle following an injury can incorporate cells whose markers are expressed in the regenerated muscle tissue, and the most common transplantable source of these cells is bone marrow [197,198]. While some claims have been made that these are derived from HSCs, in fact single purified HSCs, even over long intervals, cannot contribute to muscle or any other tissue [48]. In another example, hepatocytes bearing markers from injected purified HSCs regenerated, following multiple rounds of selection through liver toxicity [199]. However, recent evidence [200,201] indicates that these regenerated hepatocytes are mainly a result of rare cell fusion events between myelomonoctic progeny of the hematopoietic stem cells and some precursor of liver cells. In studies of neural tissue, the marrow cells or HSCs or circulating precursor cells that enter undamaged or kainite-damaged brains have limited fates as microglia and $CD45^+$ cells, as well as rare binucleate Purkinje cells derived by cell fusion [202]. Following the studies arguing for transdifferentiation, reports again were published supporting the possibility of pluripotency in non-germline cells in postnatal mammals. These reports claimed that true pluripotent cells exist in adult bone marrow and blood, and are $CD45^-$ "very small" (3–5 μ) embryonic-like stem cells [203,204]. We found no such pluripotent cells in mouse blood or marrow [205], and others have been unable to find them in human hematopoietic tissues [206].

The many claims that transdifferentiation could occur reflect a problem in stem cell biology as a young field. Most new investigators to the field did not appreciate the importance of purifying the populations to homogeneity, populations of cells of apparent phenotypic and functional homogeneity, and did not mark cells retrovirally or with other genetic markers to follow the fate of clonogenic precursors.

The transition from discovery to accepted scientific fact

It is important to note that for a reported discovery to become accepted as scientific fact, the following several criteria need to be met:

1. The initial discovery must be published in fully peer-reviewed journals.
2. The experiment as published must be repeatable in many independent laboratories.
3. The phenomenon described should be so robust that other experimental methods must reveal it.
4. In the case of transplantation, the tissue regeneration must be therapeutic in quantity and quality.

Based on these criteria, we do not believe there is sufficient evidence for the claims that tissue-specific stem cells in a postnatal organism can transdifferentiate and, upon transplantation, generate another tissue. In addition, bone marrow is not the same as HSCs, but is a population of mature or maturing cells, and certainly includes at least two or three stem and progenitor populations: HSCs, mesenchymal stem (more accurately, stromal) cells (MSCs), bone and cartilage lineage cells, and endothelial cells. Thus, it is inappropriate for such initial claims of transdifferentiation to be considered true enough to be the basis of clinical trials or care protocols or public funding and policy decisions.

Lineage committed hematopoietic progenitor cells

Considerations for the definition/isolation of hematopoietic progenitors

The developmental process from HSCs to mature cells must involve intermediates that have lost stem cell potentials but are not yet terminally differentiated. An important question and active area of research regards the sequence in which lineage commitment occurs. Several findings suggested the existence of oligopotent progenitors. First, the lack of both T and B lymphocytes in patients with severe combined immunodeficiency syndromes (SCID) was taken as evidence for the existence of oligopotent, lymphoid-committed progenitor cells or lymphoid stem cells. However, the loss of lymphocytes caused by adenosine deaminase (ADA) deficiency [207,208] or by mutations of the common cytokine receptor γ-chain (γ_c) [209,210] does not necessarily imply a common progenitor. Rather, several lymphoid cell types or their progenitors may be most susceptible to the induced alterations [211] or may be dependent on the same signal transduction mechanisms. This same caution needs to be applied to the interpretation of hematopoietic phenotypes of genetically altered mice, as mutants of Ikaros [212] or Notch-1 [213] that could either be essential factors for common or multiple different progenitors [214].

Second, leukemia cells can be viewed as cells that are arrested at early developmental stages but have gained self-renewal capacity. The occurrence of leukemias that either co-express antigens normally associated with myeloid or lymphoid cell types (mixed lineage leukemias) or that harbor two leukemia populations of clonal origin (bilineal leukemias) could be taken as evidence for the existence of bipotent B-myeloid or T-myeloid progenitors [215–220] (or reviewed in [221–223]). However, leukemia clones could be derived from HSCs, from MPPs, or from restricted progenitors that, because of their altered gene expression profile, display cell surface gene products that are normally not present at these developmental stages.

Third, the fact that single cells in bone marrow give rise to colonies that contain all myeloid but no lymphoid progeny in vitro (colony-forming unit – granulocyte/erythrocyte/macrophage/megakaryocyte, CFU-GEMM) or in vivo (mixed CFU-S) suggests the existence of oligopotent myeloid progenitors. However, cells that give a myeloid readout could also be multipotent but did not find the conditions to read out all possible progeny [23,224,225].

In light of these problems, several prerequisites need to be met to define restricted progenitors in a developmental hierarchy. First, to prospectively identify candidate populations, they need to be isolated to the highest possible homogeneity/purity. Second, if a population shows oligopotent differentiation activity, it must be demonstrated that at least some single cells within this population give an oligopotent readout, that is, that this population is not a mixture of different monopotent progenitors. Third, it needs to be shown that single oligopotent progenitors are not HSCs or MPPs. These prerequisites are difficult to achieve. At any given time point, progenitor cells may undergo intrinsic "stochastic" commitment events, may not find suitable microenvironments to read out all their possibilities, or may even not read out at all [226,227]. However, by applying the above criteria, we and others were able to identify oligopotent developmental intermediates in mice and humans. These data strongly support the hypothesis that multipotent hematopoietic progenitors first lose their self-renewal ability and consecutively commit to either the lymphoid or myeloid developmental pathway. Although not formally proven, based on frequency, cycling status, and in vitro and in vivo expansion potential of bone marrow lymphoid and myeloid committed progenitors, it seems possible that all hematopoiesis develops through either a common lymphoid or common myeloid developmental stage.

Common lymphoid progenitor cells and lymphoid development

Lymphoid cell development from HSCs is dependent on externally provided differentiation, growth, and survival factors. Of those, IL-7 may be most important. Its cognate high-affinity receptor is a complex composed of the IL-7Rα chain [228] and the γ$_c$ chain [210,229]. Neutralizing antibodies to IL-7 or genetic ablation of either IL-7 or IL-7Rα inhibit both T- and B-cell development in vivo [230,231]. Targeted deletion of the γ$_c$ gene leads to the loss of T and B cells and the additional loss of natural killer (NK) cells [232–234], presumably because of the impaired formation not only of the IL-7R, but also the IL-2R and IL-15R that may be non-redundant cytokines for NK cell development [235–237]. Furthermore, mice that lack Jak3, a signal transduction molecule associated with γ$_c$ [238,239], display a similar phenotype as the γ$_c$-deficient mice [240–242]. Also, patients with genetic defects in Jak3 show similar defects in lymphoid development as patients with γ$_c$ gene disruptions (X-SCID) [243,244]. Based on these data, IL-7 is recognized as a non-redundant cytokine for both T- and B-cell development that could act as a proliferation factor, a survival factor, and/or could initiate lineage-specific developmental programs. IL-7R is expressed on both developing T and B cells, and in mature T cells [245]. In genetically modified mice that are either IL-7Rα$^{-/-}$, γ$_c^{-/-}$, or IL-7$^{-/-}$, T-cell progenitors express low levels of Bcl-2, an antiapoptotic protein [246]. However, incubation of T cells from IL-7$^{-/-}$ mice with recombinant IL-7 results in the upregulation of Bcl-2 [247], and enforced expression of Bcl-2 in either IL-7Rα$^{-/-}$ or γc$^{-/-}$ mice leads to significant rescue of αβ T-cell development [247,249]. Therefore, a critical role of IL-7 in developing and mature αβ T cells is the promotion of survival via expression of Bcl-2 or other antiapoptotic proteins, such as, possibly, Bcl-Xl [250]. However, enforced expression of Bcl-2 was not sufficient to rescue B-cell development and γδ T-cell development [69,248,249]. Here, IL-7R-mediated signals are necessary for the rearrangement of immunoglobulin heavy chain V segments via *Pax*-5 gene activation [251,252] and for V-J recombination of γδ TCR genes via Stat5 [253,254]. Therefore, IL-7R signaling can either lead to the transmission of "trophic" survival signals in the T-cell lineage or "mechanistic" differentiation and proliferation signals in the B and γδ T-cell lineage, respectively. Thus, it was reasonable to search for oligopotent common lymphoid progenitors (CLPs) within the Lin^{-}IL-7Rα^{+} fraction in adult mouse bone marrow. We identified a population of Lin^{-}IL-7Rα^{+}Thy-1^{-}Sca-1^{lo}c-kit^{lo} cells that also express γc, indicating that this population possesses functional IL-7R (Figure 4.1) [248]. In contrast, both LT-HSC and ST-HSC populations are IL-7Rα^{-}. These CLPs possess rapid and potent T, B, and NK cell-restricted differentiation activity in reconstitution assays. In both in vitro and in vivo assays, Lin^{-}IL-7Rα^{+}Thy-1^{-}Sca-1^{lo}c-kit^{lo} cells completely lack myeloid differentiation activity [248]. Also no day 8 and day 12 CFU-S activity could be detected [129,248]. Injection of 1000 CLP could generate $0.6 - 1.1 \times 10^7$ CD3^{+} spleen T cells by 4–6 weeks, and 1.4×10^7 B220^{+} spleen B cells by 2 weeks in vivo. CLP-derived T- and B-cell generation peaks 7–10 days earlier than similar numbers of T and B cells derived from the same number of HSCs [248]. However, in contrast to HSC-derived cells, numbers of CLP-derived T and B cells begin to decline after 4–6 weeks, indicating that this population has no or limited self-renewal activity. Using a two-step assay we demonstrated that CLPs contain clonogenic progenitors for both T and B cells. Single cells were cultured in methylcellulose in the presence of SLF, IL-7, and Flt3 ligand to expand cell numbers, and a portion of the day 3 colonies were picked up and injected directly into the thymus. Those injected into the thymus differentiated into all stages of T-cell development and, in some cases, differentiated into both T and B lineage cells. The further cultured cells formed B-lineage colonies composed of pro-B and pre-B cells [248]. Therefore, the defined CLPs match the criteria for the definition of oligopotent progenitor cells. We have subsequently shown that the purity of CLP is further refined based on its expression of Flk2, whereas the Flk2^{-} fraction of the previously defined CLP contains B-committed cells [255]. Accordingly, CLPs exist downstream of Flk2^{+} ST-HSCs and MPPs in normal hematopoiesis [31,32,256].

Based on their frequency in bone marrow, cycling status, and in vivo T-, NK-, and B-cell generation potential, CLPs theoretically could be a developmental intermediate for most if not all mature lymphoid cells. The commitment of CLP to either the T, B, or dendritic cell lineage may be determined simply by the microenvironment and signals that CLPs encounter. However, whether CLPs are an indispensable stage of T- and B-cell development remains unclear.

Coffman et al. identified the B220 marker that is present on all B-lineage cells [257] and early B-cell progenitors were found to be B220^{+}, CD43^{+}, CD24^{-}BP1/6C3^{-} [157,158]. Hardy and colleagues further subdivided B-cell progenitors into fractions: prepro-B cells (fraction A0, AA4.1^{+}CD4^{lo}B220^{-}HSA^{-}; A1, AA4.1^{+}CD4^{+}B220^{+}HSA^{-}; A2, AA4.1^{+}CD4^{-}B220^{+}HSA^{-}); and pro-B cells (fraction B, B220^{+} CD43^{+}HSA^{+}6C3/BP-1^{-}; C, B220^{+}CD43^{+}HSA^{+}6C3/BP-1^{+}) [258–260] (also reviewed in [261]). The majority of fraction A1 cells are c-kit^{-}, lack myeloid potential, but possess minor T-cell potential that can be detected after intrathymic injection. Pro-B cells are B-lineage committed, rearrange D_H-J_H genes (fraction B), and undergo V-DJ recombination (fraction C). In view of their T- and B-cell readout capacity, the A0 and A1 fractions could contain CLPs, but because different markers were used for their isolation, a direct comparison of data from these populations is not possible. However, a majority of A0 and A1 fraction cells do not express IL-7Rα, but A2 cells do. B-lineage development has subsequently been studied in detail by a variety of groups using different combinations of markers that prevented direct comparison of the data [258,260,262–268]. Subsequently, we performed analyses to clarify these data by incorporating the majority of these described markers and others in our experiments. These studies allowed us to identify a committed B-lineage progenitor downstream of the CLP with the surface phenotype of B220^{+}CD43^{+}AA4.1^{+}Flk2^{+}CD27^{+}IL7Ra^{+}c-kit^{lo}CD24^{-}CD19^{-}CD11c^{-} Ly6c^{-}CD4^{-} [269]. We later found a population of cells in the CLP gates that expresses Ly6D, and these appear to be the earliest committed B-lineage cells in the mouse identified to date [269].

The earliest thymic progenitors (DN1, double negative 1 cells) are CD4^{lo}CD8^{-}CD44^{+}CD25^{-}c-kit^{+} cells [270–273]. Most of these early thymic progenitors are T-cell committed (pro-T cells); however, some cells within this population are capable of differentiating into B, NK, and dendritic cells (DCs), as well as, at a low frequency, myeloid cells [273], but their clonal origin has not been established [272–274]. Whether the earliest thymic progenitor population contains a small number of recently homed CLPs that, within this microenvironment, preferentially differentiate into T cells, or whether CLPs commit to the T-cell lineage while still in bone marrow and successively home to the thymus [275] has been controversial. By reductive isolation of cells with a single marker at a time, we have found that the thymic progenitors in the bone marrow are all of the CLP phenotype [276].

Allman et al. [277] suggest that thymopoiesis is maintained through early thymic progenitors (ETPs) that may develop from bone marrow progenitors more closely related to HSCs and not via a CLP-dependent pathway. This is based on findings that some cells (ETPs) within the DN1 fraction: do not express IL-7Rα at high levels; have some myeloid potential; give rise to T and B cells with kinetics that resemble those of ST-HSCs [32] more closely than that of CLPs; and were present at near-normal frequencies in Ikaros$^{-/-}$

mice while CLPs were not detectable by phenotype. While these data add important information on possible alternative T-cell developmental pathways, they do not assess directly how many ETPs or mature T cells are CLP-derived or whether most thymopoiesis is independent of CLPs. Both MPPs and CLPs injected intravenously give rise to thymocytes, but the MPPs do so with delayed kinetics reflective of their homing to the BM and giving rise to CLPs that then go on to seed the thymus directly. Additionally, thymic myeloid cells were observed to derive from transplanted MPPs and not CLPs, suggesting that these cells are the progeny of myeloid progenitors that also enter the thymus. Further, when Flk2$^+$ CLPs were plated in conditions capable of supporting differentiation into multiple lymphoid and myeloid lineages, single CLPs gave rise to T, B, NK, and DC lineages [276]. Approximately 50% of single CLP clones gave rise to both B- and T-lineage cells in this assay and none gave rise to any macrophages or neutrophils [278]. Taken together, these data confirm the role of CLPs as potent progenitors of both B and T lymphocytes in vivo and in vitro. Also of note, CLPs co-transplanted with HSCs in autologous or allogeneic transplantation models can rescue mice from otherwise lethal cytomegalovirus (mCMV) infections [279], providing additional evidence for the robust *and* functional T and/or NK cell reconstitution capacity of CLPs in vivo.

In humans, evidence for a T/NK bipotential precursor population in the fetal thymus [280] and a terminal deoxynucleotidyl transferase (TdT) positive candidate lymphoid precursor population in CD34$^+$ bone marrow cells has been reported [281]. However, clonal analysis was not carried out. A subpopulation of the CD34$^+$TdT$^+$ cells express the neutral endopeptidase CD10 [282]. Later it was shown that fetal and adult bone marrow Lin$^-$CD34$^+$Thy-1$^-$CD38$^+$CD10$^+$ cells contain clonal progenitors of B, NK, and dendritic cells [283]. As a population, these cells give rise to T cells in the SCID-hu thymus assay but could not generate myeloid progeny. Another report stated that cord blood CD34$^+$CD38$^-$CD7$^+$ cells contain clonal B, NK, and DC precursors; however, T-cell readout was not evaluated [258]. Therefore, both studies suggest but do not formally demonstrate the existence of CLPs in humans. IL-7 signaling may not be essential for normal B-cell development in humans, because B cells can be generated without IL-7 in vitro [284], and disruption of the IL-7R in vivo causes T-cell but not, consistently, B-cell deficiencies [285,286] (reviewed in [282]). Human early lymphoid progenitors, in analogy to mouse CLPs, express IL-7Rα. Indeed, Lin$^-$CD34$^+$CD38$^+$CD10$^+$ cells can be subdivided into a CD10$^+$IL-7Rα$^-$ and a CD10^{lo}IL-7Rα$^+$ fraction, with the latter being highly enriched in clonal B-cell progenitors [287]. It has also been shown that pro-B cells proliferate and differentiate in culture with IL-7 and BM stromal cells, whereas pre-B cells are no longer responsive to IL-7 [288]. It is important to determine whether CD10^{lo}IL-7Rα$^+$ cells or CD10$^+$IL-7Rα$^-$ cells contain both T- and B-cell progenitors and whether thymus seeding cells share some of these phenotypes (for a review of human early thymocyte development see [289]).

Common myeloid progenitor cells and myeloid development

The identification of the CLP in the IL-7Rα-expressing fraction of mouse bone marrow that holds no myeloid differentiation activity [248] suggested that complementary progenitors common to all myeloid cells may exist in the IL-7Rα$^-$ cell fraction. In addition, we had noted that the Sca-1$^-$ subset of Thy-1^{lo}Lin$^-$ cells contained myeloerythroid progenitors [30]. Therefore, to exclude HSCs and CLPs, we searched for myeloid progenitors within the Lin$^-$IL-7Rα$^-$Sca-1$^-$c-kit$^+$ bone marrow fraction. We identified three myeloid progenitor populations [102]: common myeloid progenitor cells (CMPs, FcgR^{lo}CD34$^+$; now known to be Sca-1^{lo}, not Sca-1$^-$ [290]), and their lineal descendants; megakaryocyte/erythrocyte progenitor cells (MEPs, FcgR^{lo}CD34$^-$); and granulocyte/macrophage progenitor cells (GMPs, FcgR^{hi}CD34$^+$).

These three myeloid progenitor subsets likely represent the major pathways for myeloid cell differentiation because they contain the vast majority of myeloid progenitor activity in steady-state bone marrow. CMPs give rise to all myeloid colonies in vitro including CFU-Mix; GMP give rise to CFU-G and CFU-M as well as CFU-GM; and the MEPs give rise to BFU-E (burst-forming unit, erythroid), CFU-Meg, and CFU-MegE, each with high cloning efficiency. We demonstrated that CMPs as a population differentiate in vitro into cells with MEP and GMP phenotypes and functions [102]. Furthermore, the majority of single CMPs generate both GM- and MegE-related progeny. Upon in vivo transfer, the three cell populations give short-term but not long-term readout, corresponding to their in vitro activities, and indicating that they have only limited if any self-renewal activity [129].

MEPs provide radioprotective cells for lethally irradiated mice housed under infection control conditions [129]. Lethally irradiated mice injected with MEPs, CMPs, ST-HSCs, or MPPs show transient donor-derived hematopoiesis sufficient to sustain survival while reconstitution with residual host HSC occurs [29,69,129]. In addition, the majority of day 8 CFU-S activity resides within the MEP population, but is absent from the GMP population [129], supporting previous findings that day 8–9 CFU-Ss are largely erythroid [30,291]. Our data also confirm previous findings that the majority of day 12 CFU-S activity resides not within lineage-committed progenitors but within the more primitive HSC populations, with some activity in CMPs and MEPs [29,30,292]. Neither B- nor T-cell differentiation activity was detectable in either MEPs or GMPs. CMPs could not generate T cells; however, a small number of B-cell progeny were detectable in vitro and in vivo [102]. We also isolated a pure population of megakaryocyte progenitors (MKP) in mice. These Lin$^-$c-kit$^+$Sca-1$^-$CD34$^+$CD9$^+$CD41$^+$ cells are highly efficient at producing megakaryocyte CFC in vitro, micro-megakaryocyte loci in vivo, and donor-derived platelet production in vivo, while they are devoid of erythrogenic activity [293].

The subsequent identification of additional markers has enabled higher resolution delineation of myeloid developmental pathways. First, we and others found that the CMP population, like the CLP, was heterogeneous for Flk2 expression [255,294] and the Flk2$^+$ fraction contained the CMP activity and could give rise to GMPs and MEPs and all myeloerythroid progeny including DCs. Subsequently, the addition of CD150 (Slamf1), CD105 (Enderlin), and CD41 to the analysis revealed further heterogeneity within the myeloid progenitor populations [295]. These markers revealed that the previously defined CMP could be further subdivided into CD150$^+$ and CD150$^-$ fractions, and the MEP can be divided into three subpopulations based on endoglin and CD150 expression. Detailed analyses with these markers led to the identification of isolatable preGM, preMegE, and preCFU-E populations that give appropriate lineage restricted readouts in vitro and in vivo [295]. The current lineage trees from mouse and human HSC are shown in Figure 4.1.

These data are consistent with another study that used a Gata1-GFP reporter to identify a population that refines the CMP phenotype or precedes it in the developmental scheme [290]. Specifically, this population is Lin$^-$Kit$^+$Sca^{int}Flk2$^-$ Gata1^{lo} and gives rise in vivo to all myeloid lineages, but has very low lymphoid readout. Using a PU.1 reporter, this study also identified a GLMP that could give rise to lymphocytic and myeloid lineage cells but not platelets. These results are similar to those from Adolfsson et al. which indicated that MPPs that express the highest levels of Flk2 have lost platelet-forming ability [296]. This is also consistent with data suggesting that PU.1 represses MegE development [297].

Alternative developmental pathways

There are several recent in vivo studies as well as in vitro data that suggest that myeloid versus lymphoid differentiation may not always be the first lineage determination made during hematopoietic development and either bipotent B-cell/myeloid, or T-cell/myeloid progenitors, or both may exist. In support of a B/macrophage progenitor, it was demonstrated that several B-cell lines can be modified to produce cells with features of macrophages by in vitro manipulations [298–300]. Importantly, transfection of v-raf into B-cell lines established from Eμ-*myc* transgenic mice could convert them into macrophages that maintained the identical rearrangement of an IgH gene [299]. This observation suggests that latent developmental potentials that are not accessible under normal conditions may be regained through genetic alteration. Indeed, in the first experimental reprogramming of an in vivo cell, we have shown that CLPs, which under normal conditions never generate myeloid readout, produced granulocytes and macrophages after they were genetically engineered to express the human IL-2 receptor β chain (hIL-2Rβ) or the granulocyte–macrophage colony-stimulating factor receptor (GM-CSFR) and were stimulated with the cognate ligands [301]. This was blocked by enforced expression of Pax-5 [302].

Also in vitro, single FL cells could give rise to both B/myeloid and T/myeloid progeny [224,303,304]. In these experiments using a FTOC system in the presence of 60% O_2 for single cell readout, Kawamoto et al. [304] detected B/T/myeloid, B/myeloid, T/myeloid, but never T/B bipotent progenitors from FL cells populations [304]. However, in these high O_2 concentration cultures, only ~5% of single $Lin^-Sca\text{-}1^+c\text{-}kit^+$ HSCs gave rise to multilineage (T/B/myeloid) outcomes, suggesting that this system is not reproducible enough to demonstrate all differentiation capacities of HSCs. Also, differentiation into T/myeloid or B/myeloid lineages might simply occur because of the "random" commitment of HSCs in these culture conditions. Alternatively, T/B committed bipotent progenitors might not be contained in the $Lin^-Sca\text{-}1^+c\text{-}kit^+$ population but only in the $Lin^-Sca\text{-}1^{lo}c\text{-}kit^{lo}$ CLP fraction [248,305]. Bipotent B/macrophage progenitors were also described at a very low frequency (~0.02%) in adult hematopoiesis in vitro [306], supporting the hypothesis that a B/macrophage developmental line might be conserved beyond fetal hematopoiesis.

As described earlier, additional data also suggest that HSCs and progenitors circulate through the blood and reside in the tissues before returning to the blood via the lymphatics [147]. Further, these cells were shown to differentiate in the tissue directly into dendritic, myeloid, and $B220^+$ cells. This result together with results showing that HSCs respond to a reduced hematocrit [307], in vitro data providing evidence for direct differentiation to megakaryocyte development [70], and studies suggesting oligo and bipotent progenitors, suggest that HSCs percolate through the tissues as a means of directly assessing specific hematopoietic and immune demands. It is reasonable to hypothesize that these cells reflect the responsiveness of the hematopoietic system and indicate that steady-state hematopoiesis, when demand for each lineage is constant, is distinct from hematopoiesis during infection or blood loss, when there is a specific need for an increase in certain lineages. Being able to shut down production of lineages not in demand would also be a means of conserving resources such that white blood cells are not generated at the expense of red blood cells (RBCs) during anemia and, conversely, RBCs and platelets are not generated at the expense of innate or specific immune cells when these are in demand.

Human myeloid progenitors

As in the case of CLPs, multiple studies suggested the existence of human oligopotent myeloid progenitors [308–312]. We identified human bone marrow and cord blood cell populations that are counterparts of the mouse CMPs, GMPs, and MEPs [287]. They are negative for multiple mature lineage markers (including early lymphoid markers that might define human CLPs as CD7, CD10, or IL-7Rα) and all are $CD34^+CD38^+$. They are distinguished by the expression of CD45RA, an isoform of the CD45 cell-surface tyrosine phosphatase that can negatively regulate at least some classes of cytokine receptor signaling [313], and IL-3Rα, a receptor that, upon activation, supports proliferation and differentiation of primitive progenitors [70,314,315]. $CD45RA^-IL\text{-}3R\alpha^{lo}$ (CMPs), $CD45RA^+IL\text{-}3R\alpha^{lo}$ (GMPs), and $CD45RA^-IL\text{-}3R\alpha^-$ (MEPs) show high myeloid cloning efficacy but low long-term (stromal hematopoietic) culture-initiating cell (LTC-IC) capacity, indicating that they do not self-renew. Although FcγR II-III (CD16/CD32) expression distinguishes mouse CMPs and GMPs, it was not detectable on any of the human myeloid progenitor populations; and all human clonal myeloid progenitors express CD34 while mouse MEPs are $CD34^-$ [102]. Interestingly, in mouse myeloid progenitors, IL-3Rα as well as CD45RA expression were both negative to low in MEPs, intermediate in CMPs, and positive in GMPs, without providing sufficient discrimination to clearly set a cut-off level between the different cellular fractions. As with the mouse myeloid progenitors, CMPs give rise to MEPs and GMPs in vitro, and a significant proportion of CMPs have clonal granulocyte/macrophage and megakaryocyte/erythrocyte readout potential. We did not test T-cell differentiation ability; however, no in vitro B or NK cell readout could be detected. However, some B cells might develop from large numbers of transplanted CMPs. As in the case of mouse CMPs, it is important to clarify whether the CMP population contains oligopotent B/myeloid progenitors, or monopotent B-cell progenitors that share CMP phenotypes but do not read out in our in vitro assay, or whether in vivo B-cell readout resulted from small numbers of contaminating B-cell progenitors in the large number of transplanted cells (see later).

Lineage commitment in fetal hematopoiesis

While fetal liver (FL) HSCs show major similarities to adult HSCs, some phenotypic and functional differences exist [186,316–318]. Remarkably, FL-HSCs possess some cell fate potentials such as the generation of $V\gamma3^+$ and $V\gamma4^+$ T cells [190] and B-1a lymphocytes [319,320] that are not detectable in adult HSCs. Therefore, it was important to determine the phenotype and developmental capacities of fetal liver CLP and CMP. We identified FL-CLPs ($Lin^-IL\text{-}7R\alpha^+B220^{-/lo}Sca\text{-}1^{lo}c\text{-}kit^{lo}$) [305], and the myeloid progenitors FL-CMPs ($Lin^-IL\text{-}7R\alpha^-Sca\text{-}1^-c\text{-}kit^+AA4.1^-FcgR^{lo}CD34^+$), FL-GMPs ($Lin^-IL\text{-}7R\alpha^-Sca\text{-}1^-c\text{-}kit^+AA4.1^-FcgR^{hi}CD34^+$), and FL-MEPs ($Lin^-IL\text{-}7R\alpha^-Sca\text{-}1^-c\text{-}kit^+AA4.1^-FcgR^{lo}CD34^-$) [321] that show high similarities to their adult counterparts. However, some significant differences in proliferative potential colony-forming activity and lineage differentiation capacities exist. Of special note, FL-CLPs generate $CD45^+CD4^+CD3^-LTb^+$ cells that are initiators of lymph node and Peyer's patch formation

[322–325]; these represent an innate lymphoid cell unrelated to NK cells. About 5% of these IL-7R$^+$ FL-CLPs differentiated into macrophages and B cells, at least in vitro. Interestingly, the FL-CLPs fail to express Pax-5, a suppressor of macrophage development [323] while adult marrow CLPs that lack myeloid potential are Pax-5$^+$ [305]. Conversely, although FL-CMP and downstream FL-MEPs or FL-GMPs do not give rise to T cells, FL-CMPs possess some in vitro and in vivo B-cell potential. A close relation between B-cell and macrophage development for adult and fetal hematopoiesis was suggested before (see later). Therefore, during commitment to the lymphoid or myeloid lineage in FL hematopoiesis, the developmental capacity for macrophages and B cells might be the last to be lost in commitment to either the lymphoid or myeloid lineage.

DC development

DCs were initially defined as bone marrow-derived non-macrophage leukocyte populations with high antigen presentation capacities and the ability to prime naïve T cells [326–328]. Multiple DC subpopulations that differ in tissue distribution, surface-marker expression and functional capacities have been described in humans and mice (reviewed in [329,330] and Chapter 17), and additional subpopulations continue to be identified. From an evolutionary point of view, DCs may belong to the myeloid and lymphoid lineage because they link the innate and adaptive immune responses. Based on current data, two opposing models of DC ontogeny can be suggested: either the mature DC subpopulation is determined at the level of an early hematopoietic progenitor or, alternatively, immature DCs retain the capacity to mature to distinct DC subpopulations in response to different environmental instructions.

In mice, two major DC subpopulations are distinguishable by their expression of the CD8α chain [331]. Early intrathymic progenitors (TPs) (CD4^{lo}CD44$^+$CD25$^-$c-kit$^+$) and thymic pro-T cells (CD44$^+$CD25$^+$c-kit$^+$) reportedly contain precursors able to differentiate into CD8α$^+$ DCs in vivo [274,332]. Therefore, and because the majority of thymic DCs and only a minority of secondary lymphoid organ DCs express CD8α$^+$, the CD8α$^+$ DCs had been considered to be related to the T-lymphoid lineage and were therefore termed "lymphoid DCs" in contrast to the CD8a$^-$ "myeloid DCs." This model seemed to be supported, but not directly demonstrated, in mice deficient for the transcription factors RelB [333], PU.1 [334], and Ikaros [335] that lack only CD8a$^-$ DC. However, other mutants, such as c-*kit*$^{-/-}$, γc$^{-/-}$ [336], and Notch-1$^{-/-}$ [337] mice, showed a developmental dissociation of CD8α$^+$ DC and T cells. We and others therefore tested DC developmental potentials in adult and fetal lymphoid and myeloid committed progenitors. Surprisingly, CD8α$^+$ and CD8α$^-$ DCs were produced both by highly purified CLPs and CMPs with similar efficacy on a per cell basis, therefore disproving the concept of CD8α$^+$ "lymphoid"- and CD8α$^-$ "myeloid"-derived DCs in mice [305,337–340]. Furthermore, beyond CLPs and CMPs, DC developmental potential is conserved in the lymphoid lineage in pro-T cells and in the myeloid lineage in GMPs, but it is lost once B-cell or megakaryocyte/erythrocyte commitment occurs [337,338].

In contrast to mice DCs or their precursors, which are usually isolated from the lymphoid organs or bone marrow, human DCs and their precursors have usually been isolated from peripheral blood and only in rare cases from blood-forming organs, making direct comparisons difficult (for a review see [330]). However, DCs could be generated from total CD34$^+$ progenitor populations, lymphoid-restricted progenitor populations, and peripheral blood monocytes, suggesting that DC developmental potential in human is analogous to that in mouse and is conserved in myeloid and lymphoid-restricted lineages [283,340–345]. An immediate DC precursor population that, upon stimulation, can produce large amounts of interferon-α, displays a set of distinct pattern recognition receptors and can mature into DCs in vitro, was identified in humans [346–349] and later in mice [350–352]. This DC subpopulation was termed plasmacytoid DCs, DC2 and interferon-α-producing cells [329]. These cells can derive from both myeloid and lymphoid progenitors (reviewed in [81]) but are predominantly of myeloid origin [353].

In summary, DCs develop along a lymphoid and myeloid pathway in vitro and in vivo. By using simple assays – such as immunophenotyping, or functional readout as mixed lymphocyte reaction and cytokine production – we did not detect differences in lymphoid precursor- and myeloid precursor-derived CD8α$^+$ DC fraction DCs [338]. This is an unexpected finding, because it suggests developmental redundancy for DCs. More likely is that CLP- versus CMP-derived DCs have functionally distinct roles. Of note, DC developmental capacities in CLPs and pro-T cells correlate with their latent myeloid developmental potential that can be "rescued" by artificial introduction and activation of the IL-2β or GM-CSF receptor [301,354]. It would therefore be important to characterize the signals that are involved in retaining DC developmental capacities in the otherwise exclusively lymphoid or myeloid committed progenitor cells. Activation of the Flt3 tyrosine kinase could be one of these signals [255].

Gene expression profiles of HSCs and their oligolineage progenitors: Gene Expression Commons

Several powerful technologies have allowed the characterization of transcripts expressed in defined cells, populations of cells, tissues, and organs. These technical advances allow for reductionist approaches to understand the complexity of RNA transcripts found in these cells or tissues, and how the transcriptional profile changes upon differentiation, self-renewal, or other functional events. One of the most powerful technologies is microarray analysis, wherein the expression profile of transcripts for a high fraction of all known genes in the species can be ascertained for purified cell populations. Several groups have performed microarray expression profiling of hematopoietic stem and progenitors cells from fetal, adult, and aged mice [74,75,78,79,256,295,355–357]. These studies have identified several genes that have been subsequently validated as important for HSC function, such as *BMI1* [78], or useful for HSC purification, such as *Slamf1* [75,355]. Gene subtractions or other differential expression techniques have revealed a subset of genes that appear to be expressed in HSCs, but not broadly elsewhere. In fact, some genes have been found that are expressed in HSCs and neurosphere cultures (highly enriched for CNS stem cells) [130], or HSCs and ES cells, or all three [130,358].

We have recently published a user-friendly set of microarrays from all known mouse and human hematopoietic subsets, called Gene Expression Commons [104]. Shown in Figure 4.6 are four examples of the utility of this absolute comparison of the array data for any particular cell type versus the entire RNA on the array. In the examples shown, four genes (*c-Mpl*, *EpoR*, *IL-7R*α, and *c-EBP*α) were entered into the database, and the absolute comparison of each hematopoietic subset is shown.

These gene expression arrays cannot detect important events that are essentially post-translational, for example the phosphorylation

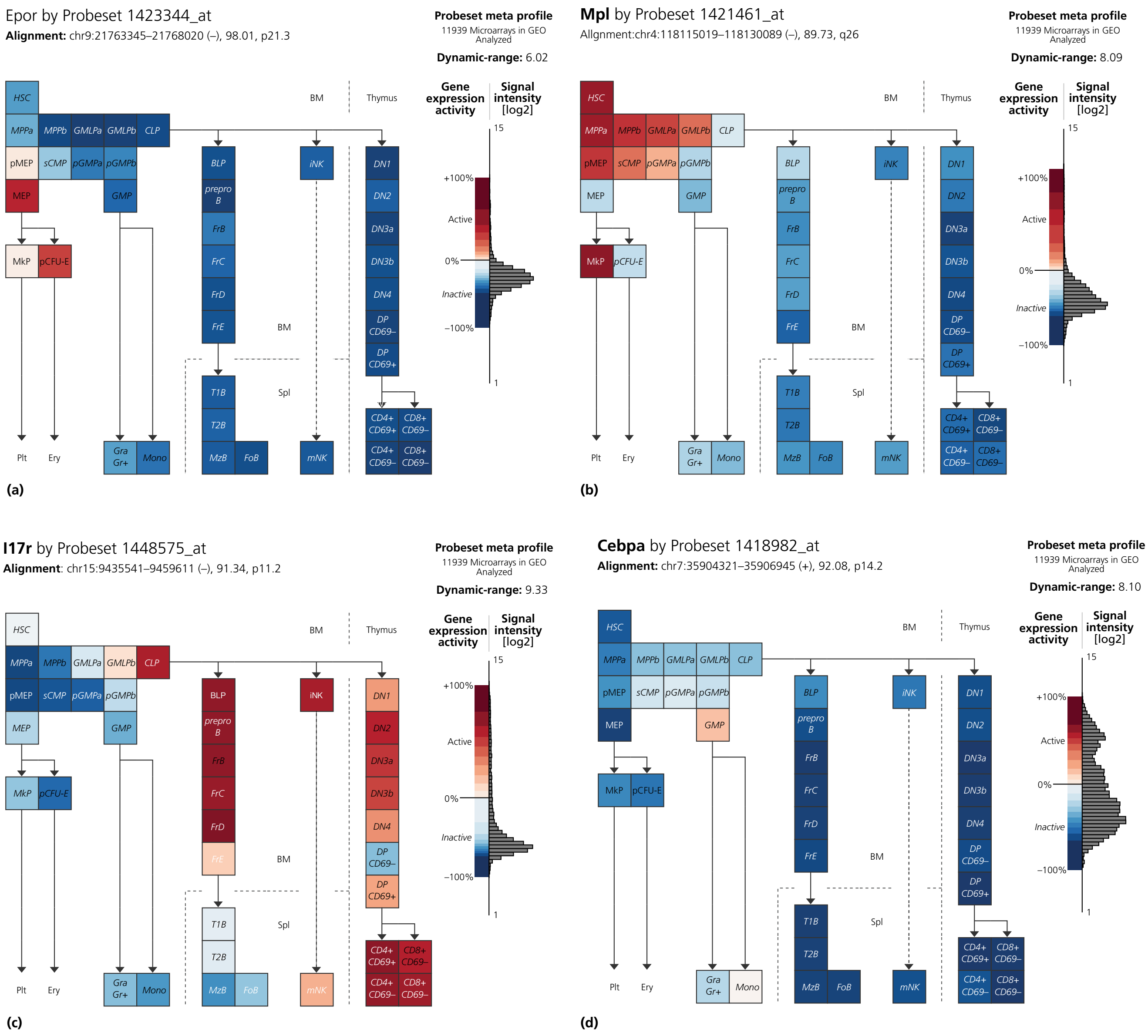


Figure 4.6 Images from Gene Expression Commons (https://gexc.stanford.edu/) showing absolute comparison of the array data for particular genes (*EpoR*, *c-Mpl*, *IL-7Rα*, and *c-Ebpα*). Raw microarray data are individually normalized against a large-scale common reference, then mapped onto each probe-set metaprofile. This strategy enables profiling of absolute expression levels of all genes on the microarray. (For a color version of this figure, please see the color plate section.)

and dephosphorylation of proteins in signal transduction pathways, or the changes in subcellular locations of proteins, such as β-catenin, under conditions when they are held within the degradation complex, or attached to the cytoplasmic face of cadherins, or allowed to be free in the cytoplasm and appear in the nucleus [112]. Most of the oligolineage progenitors downstream of mouse HSCs [102,248] and human HSCs [41,283,287,340] have been isolated to homogeneity, and in some instances a comparison of their gene expression profiles reveals important clues as to genes responsible for particular commitments to differentiated fates [130,357] (see later). The reductionist approach to cataloging genes in this way will likely reveal many new clues about stem cell behaviors, but a similar cataloging of cellular proteins (proteomics) will be required to reveal cell protein modifications that may help identify important pathways in stem cell behaviors.

Promiscuous expression of multiple myeloid or lymphoid genes in hematopoietic branchpoints

Prospectively purified LT-HSCs, oligopotent progenitors, and lineage-committed progenitors have been used for a definitive sampling of the transcriptional profiles of cells at these particular stages of physiologic hematopoiesis. The expression patterns of lineage-affiliated genes in each stage of hematopoiesis are largely consistent with their known functions. Semiquantitative RT-PCR assays targeted for each purified population provided the following results. *SCL* (stem cell leukemia) [359,360] is expressed in HSCs and all myeloid progenitors. *GATA-2* [361] and *c-Mpl* are expressed in HSCs, CMPs, and MEPs, but not in GMPs [74,102,103]. Other megakaryocyte/erythroid (MegE)-related genes such as *NF-E2* [360], *GATA-1* [362,363], and the erythropoietin receptor are expressed in CMPs and MEPs but not in GMPs, and their expression levels are highest in MEPs [103,357]. Granulocyte-affiliated transcription factor, C/EBPα [364] is expressed in HSCs, CMPs, and GMPs, but not in MEPs, and its expression level is highest in GMPs [103,357]. All of these genes are expressed in CMPs, but not in CLPs or other late lymphoid progenitors, suggesting potential roles for each in myeloid-specific cell fate determinations. In contrast, the lymphoid-related transcription factor Aiolos [365] is not expressed in any myeloid progenitors, nor in HSCs. Ikaros, an Aiolos partner nuclear factor, thought initially to be a lymphoid-specific transcription factor [212,366], is expressed in HSCs and all stages of hematopoiesis [367]. Aiolos first appears at the CLP stage, and is highest in T- and B-cell progenitors [102,103]. Lymphoid-related GATA-3 [368] and Pax-5 [369] are expressed at a low level in adult marrow CLPs but not in myeloid progenitors and fetal liver CLPs [102,305].

These data provide a clearer view of gene expression regulation in hematopoietic development. First, the expression of many myeloid and lymphoid genes is mutually exclusive in each pathway. Second, CMPs express both MegE and GM-affiliated genes, and CLPs express both T- and B-lymphoid genes, although CMPs and CLPs have not committed to myeloid and lymphoid sublineages, respectively [102,103]. These data are consistent both with the priming hypothesis [370] and a model wherein chromatin is open prior to commitment, but the transcripts from that open chromatin are not critical for maturation [103].

The view of myeloid or lymphoid promiscuity has been significantly extended by using an oligonucleotide microarray analysis targeted for purified CMPs and CLPs. Genome-wide profiling by using microarray methods revealed that CMPs and CLPs co-express a vast majority of GM and MegE-affiliated genes, and T, B, and NK lymphoid genes, respectively [74,357]. Furthermore, co-expression of both GM and MegE-related genes in CMPs and of both T and B lymphoid genes in CLPs have been formally demonstrated at the level of single cells [74]. RT-PCR analysis targeted for single cells has been used to determine that CMPs co-express MegE-related β-globin, EpoR, NF-E2, and GM-related MPO, G-CSFR, and PU.1, while CLPs co-express B-cell-related l5 and/or Pax-5 and T-cell-related CD3d and/or GATA-3 [103]. These results indicate that the expression of lineage-related genes can precede commitment, and suggest that the "promiscuous" expression of multiple lineage-related genes may allow a flexibility of commitment at these oligopotent stages: for differentiation towards T- and B-cell lineages in CLPs, and towards GM and MegE lineages in CMPs.

It is still unclear whether the primary mechanism of hematopoietic commitment could be operated first by opening chromatin at programmed sites, or whether the "priming" seen in promiscuous transcription patterns results from the presence of open chromatin and transcription factors that can form "sterile" transcripts. Nonetheless, in priming stages, multiple differentiation programs may collaborate or compete with each other until one becomes dominant. These programs should include cross-talk among transcription factors or transcriptional complexes, because transcription factor activity can be potentiated [371] or suppressed [372,373] by interaction with other transcription factors. Changes in levels of key transcription factors [374,375] may also be critical for the lineage decision. Cross-talk between different cytokine signals may also be important, as single progenitors co-express multiple cytokine receptors. Furthermore, fluctuations of gene expression of multiple differentiation programs in progenitors may lead to different outcomes depending on the microenvironment the cell inhabits. In this context, both intrinsic and extrinsic factors could play a part in lineage determination. However, data comparing HSCs from young and old mice show an intrinsic change in their differentiation potential with decreasing lymphoid potential with age, even when transplanted into a young mouse [77]. These data suggest that at least some portion of lineage determination is independent of the environment or that aging HSCs irreversibly lose responsiveness to signals provided by it.

One way to view the hematopoietic system is to assume that at or around the stage of yolk sac hematopoiesis, a pool of HSCs are created that are the only source of hematopoiesis for life. Each early HSC then is a clonogenic precursor of other HSCs, which self-renew, and their progeny, which do not. Over time and perhaps with a cell division counting developmental clock, the clones begin to diversify in terms of responsive potential; these intrinsic epigenetic and rarely genetic changes prime the clones for their responsiveness to normal cues, such as diffusible or membrane-bound factors, or to pathologic cues, such as bacterial endotoxins. At any given point, the clones that are present represent clonal competitions up to that point. In this way intrinsic programs can select certain clones over others, but within an inbred colony of pretty clean mice the clones chosen tend to be consistent, mouse-to-mouse. As we have discussed, this model would have to account for the clonal hematopoiesis model shown in Figure 4.5. We later discuss changes that not only enable clonal selection but are also proto-oncogenic in nature, so that the long process of preleukemic clonal progression is initiated and spread through the HSC clones.

Downregulation of genes irrelevant to committed lineages as a critical mechanism of lineage restriction

According to the "priming" model, commitment could be dependent on two independent molecular events: upregulation of differentiation programs related to selected lineages and the transcriptional abrogation of master control genes in the unselected lineages. A striking example is Pax-5, a key molecule for restriction to B-cell lineage [369]. In normal hematopoiesis, Pax-5 expression initiates at the CLP stage, and is upregulated in pro-B cells, but undetectable in pro-T cells or myeloid cells. Expression of Pax-5 in pro-B cells is important not to differentiate into other lineage cells, because pro-B cells from Pax-5-deficient mice (Pax-5$^{-/-}$ pro-B cells) can differentiate into T cells, NK cells, granulocytes, macrophages, and osteoclasts as well as B cells [369]. Pax-5$^{-/-}$ pro-B cells rearrange the D–J locus of IgH genes and express other pro-B related genes. Thus, even after a set of B lineage-associated genes begins to be transcribed, differentiation programs for other lineages are still accessible. Conversely, enforced Pax-5 expression in myeloid progenitors suppresses myeloid development, presumably blocking signals from myelomonocytic cytokine receptors such as G-CSFR and GM-CSFR [376].

Similarly, enforced expression of PU.1 in pro-T cells can block T-cell differentiation [377]. Ectopic GATA-1 expression in GMPs also inhibits differentiation into granulocyte/monocyte lineages, but induces transdifferentiation into megakaryocytes and erythrocytes [378]. A similar phenomenon is observed in ectopic cytokine receptors. Ectopic expression of IL-2Rβ or GM-CSFR in CLPs can deliver signals that redirect cells to myelomonocytic differentiation [301]. Further characterization of this latent myeloid potential revealed that CEBPα is upregulated in response to the ectopic IL-2 signal and leads to GM-CSFR expression and further myeloid differentiation [379]. This study also revealed that enforced expression of CEBPα in CLP is itself sufficient to induce the myeloid outcome and conversely, enforced Pax-5 expression blocks the myeloid differentiation induced from IL-2Rβ. Similarly, Laiosa et al. showed that committed T cell progenitors could be induced to develop into macrophages or dendritic cells by enforced expression of CEBPα or PU.1, respectively. These data suggest that committed T cell progenitors are also susceptible to the lineage instructive effects of myeloid transcription factors, but that T lineage development occurs by the normal role of Notch signaling downregulating CEBPα and PU.1 in multilineage precursors.

These data indicate that termination of lineage-related gene expression is equally important for completion of lineage commitment. First, in committed progenitors, abrogated differentiation programs for unselected lineages are not totally erased immediately after commitment but could be reactivated by "instructive" signals mediated by ectopic cytokine receptors and transcription factors. For example, GMPs may be restricted to GM lineages simply because GATA-1 is downregulated, and CLPs are likely lymphoid-restricted because they downregulate myeloid cytokine receptors. Second, differentiation programs into committed lineages can be operated on condition that transcription factors for unselected lineages are downregulated, as Pax-5 and PU.1 inhibit myeloid and T-cell differentiation programs, respectively. Thus, a hierarchy of transcription factors and cytokine receptors organized at the epigenetic level is critical to maintain hematopoietic homeostasis.

Changes in chromatin structure, allowing access for RNA polymerase to initiate transcription, is essential for genetic programs to be transcribed [380,381]. The activation of chromatin structure can occur prior to significant expression of genes [382]. It has been hypothesized that a wide-open chromatin structure is maintained in early hematopoietic progenitors, enabling multilineage-affiliated programs to be accessible [383]. This phenomenon of open chromatin in early hematopoietic progenitors could be the mechanism by which lineage priming occurs in stem or progenitor cells prior to their lineage determination [130,371,383,384].

Standard methods for studying chromatin structure require large numbers of cells. This requirement has made application of these methods to stem cell biology difficult, but we and others have made technical advances allowing chromatin immunoprecipitation on rare cells [385–387]. This technique can be used to determine whether the epigenetic marks in control regions of lineage-directing genes are modulated during hematopoiesis. Specifically, we analyzed histone modifications and DNA methylation patterns within the regulatory regions of erythroid (*Gata-1* and β-*globin*), myeloid (*c-fms*), and lymphoid lineage-affiliated genes (*Gata-3* and *Ptcr*α) in 50,000 prospectively purified stem and progenitor cells. By performing bisulfite sequencing analysis, we found that methylated H3K4 and AcH3 and unmethylated CpG dinucleotides localized at defined regulatory regions of these lineage-affiliated genes in HSCs. These histone modifications are epigenetic markers of active transcription and either accumulated or were replaced by increased DNA methylation and H3K27 trimethylation in committed progenitors, consistent with the pattern of gene expression. We also observed bivalent domains at lymphoid-affiliated genes in HSCs and downstream transit-amplifying progenitors. Bivalent domains described by Bernstein et al. contain both activating and silencing histone modifications [388] and are associated with genes such as transcription factors that are expressed at low levels and affect developmental decisions. These data support the role of epigenetic modifications as being reflective of HSC multipotency and suggest that the remodeling of chromatin is an important mechanism of driving gene expression changes associated with developmental decisions.

Transplantation of HSCs in mouse and human

It is fair to say that the field of bone marrow transplantation, now more accurately called hematopoietic cell transplantation (HCT), has provided one of the most striking medical advances in our lifetime and, similarly, the desire to understand cells involved in such transplants has opened the field of stem cell biology, first to HSCs, but then to other tissue-specific stem cells. It is important at the outset, however, to make sure that the scientific and clinical community uses the appropriate nomenclature for the type of transplant used, as misconceptions regarding the cell type transplanted lead to misconceptions about the scientific or clinical results [389,390]. Thus, the transplantation of complex mixtures of hematopoietic cells in bone marrow or mobilized peripheral blood is not the same as transplantation of purified HSCs.

Clinical HCTs have been used for a variety of purposes, but the primary use is to regenerate hematopoietic tissues in a cancer patient who has received otherwise lethal doses of a high-dose regimen of chemoradiotherapy [391,392]. Other uses have been the correction of inherited or acquired hematopoietic cell defects and dysplasias; in animals, the induction of transplantation tolerance to other cells tissues or organs co-transplanted from the donor of HSCs or hematopoietic cells; and the reversal of autoimmune disease [393] (see Chapter 18).

Whether purified HSC transplantation could replace HCT was not clear, as it was unknown whether a rapid and sustained regeneration of a particular hematopoietic outcome could be accomplished with the primitive HSCs alone, without having oligolineage progenitors in the transplant. Several experiments demonstrated in mice and humans that HSCs are the principal cells that are functional in the transplant setting, and that by varying cell dose one could achieve both rapid and sustained regeneration of hematopoietic tissues [394]. Prospective isolation of HSCs with multiple markers allows the possibility that grafts would consist solely of HSCs, and of no other contaminating cells. There are two important advantages, at least theoretically, to the transplantation of HSCs rather than HCT: removal of cancer cells and removal of T cells. Autologous HCT in cancer patients can be accompanied by transplantation of the contaminating cancer cells; in some diseases, such as stage IV breast cancer and many lymphomas, about 50% of the hematopoietic cell populations to be transplanted are contaminated with cancer cells [395–397]. In patients with multiple myeloma, contamination of the graft is the rule and not the exception [398]. Therefore, it was important to test whether positive selection of HSCs would effectively eliminate or purge cancer cells from the transplant.

Deliberate spiking of MPB with cell lines representing human breast cancer, human non-Hodgkin lymphoma (NHL) and human myeloma was performed, and four methods of HCT/HSC isolation were carried out to determine the full reduction of contaminating cancer cells. Two independent methods to identify breast cancer cells were used: microscopy to detect cells carrying breast cell cytokeratins, and a flow cytometry-based method. Myeloma and B-cell lymphoma representative cells were detected by a PCR specific for the immunoglobin heavy chain rearrangements in these B lineage cells. All three techniques have a sensitivity of between 10^{-5} and 10^{-6}.

Over 10^5-fold reductions of contaminating cancer cells were accomplished with the multiparameter cell sorting of $CD34^+Thy^+$ cells from MPB, whereas techniques based on CD34 alone were at least two to three orders of magnitude less efficient (Figure 4.7). The time to reach engraftment with purified HSCs (measured by absolute neutrophil count (ANC) >500 and platelets >20,000/mL of

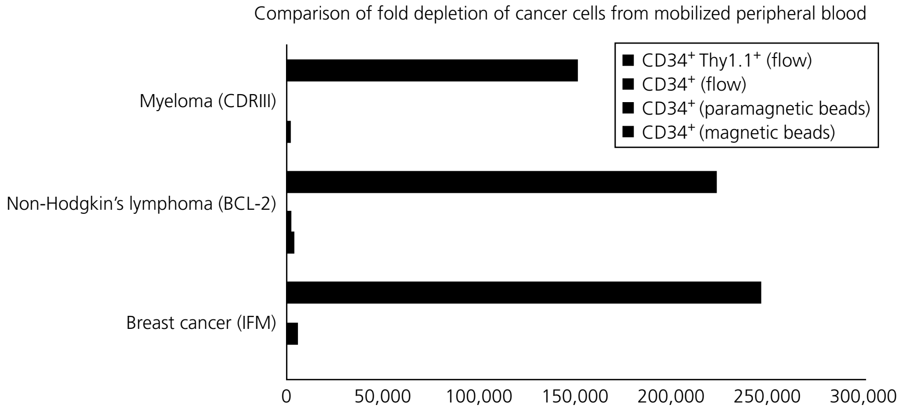


Figure 4.7 Comparison between CD34 only and CD34$^+$ Thy-1 isolation of human stem and progenitor cells in terms of fold depletion of human cancer cells. Mobilized peripheral blood (1.6×10^{10}) was spiked with 1.2×10^8 cancer cells (T47D breast cancer, SU DHL 6 non-Hodgkin lymphoma (NHL) cells, or RPMI-LAV myeloma cells) prior to cell sorting. The CD34$^+$ Thy-1 cells were flow sorted with a modified high-flow cytometer, as were the first CD34 flow sort test. Breast cancer cells were analyzed by immunofluorescence microscopy for cytokeratin, the NHL by Igh-BCL-2 real-time polymerase chain reaction (PCR), and the myeloma by CDR III real-time PCR. The data are represented as fold reduction of cancer cells from the sorted CD34 or CD34$^+$ Thy-1 cells. (E. Hanania, unpublished data.)

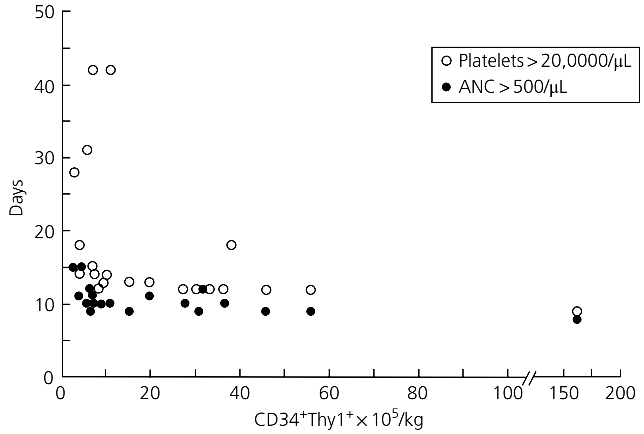


Figure 4.8 Transplantation of highly purified human hematopoietic stem cells (HSCs) in patients with metastatic breast cancer. Shown are the times to engraftment of neutrophils (absolute neutrophil count (ANC > 500/mL)) following transplantation with purified CD34$^+$ Thy1$^-$1$^+$ hematopoietic stem cells. Source: Negrin et al. 2000 [395]. Reproduced with permission of Elsevier.

blood [395,396,399]) (Figure 4.8), is very similar to the times obtained when unmanipulated MPB is used as the HCT transplant, and similar to the dose–response observed for mouse HSCs in syngeneic transplants [394]. Even in acute myeloid leukemia (AML), HSCs can be separated from leukemic stem cells by surface markers [400]. We have shown that cancer-free MPB CD34$^+$90$^+$-enriched HSCs from women with metastatic breast cancer engraft rapidly in autologous transplantation following high-dose chemotherapy. About 15 years after transplantation, the overall survival rate was 33% in patients who received cancer-free HSCs but only 9% in a retrospective cohort of patients who received unpurified MPB [401] (Figure 4.9). Because these groups were not prospectively randomized, a larger trial with stage IV or other breast cancer patients with aggressive phenotype cancers or circulating cancer cells should be performed. Also, the stage is set to test whether autologous transplantation of cancer-depleted HSCs from patients with non-Hodgkin lymphoma or multiple myeloma will lead to clinical benefits when compared to transplantation of cancer-containing unmanipulated hematopoietic grafts.

Allogeneic hematopoietic cell transplants have been plagued from the beginning by the appearance of graft-versus-host disease (GVHD) as a result of T cells in the graft [402]. Mice receiving allogeneic HSCs have little or no contaminating T cells, and never develop GVHD [394,403]. Dose–response comparisons of purified HSC transplants in syngeneic, in matched unrelated donor (URD) allogeneic, and in fully allogeneic circumstances have been carried out and, for the most part, doses of HSCs sufficient to allow rapid and sustained engraftment can be achieved without contaminating T cells (Figure 4.10) [403] (also see Chapter 18). However, the number of HSCs required for engraftment can be 10 times higher in the allogeneic than in the syngeneic setting (Figure 4.10) [394].

Graft engineering

One can achieve nearly equal levels of engraftment at the same HSC dose with syngeneic and allogeneic models if the allogeneic hosts are preconditioned with high-dose conditioning regimens that include either facilitator cells (FCs) [404–406] from the donor or monoclonal antibodies that eliminate NK- and T-cell populations [403,407]. High-dose conditioning alone results in donor B cell and myeloid regeneration from the donor alone [403–407] but persistent donor and host T-cell chimerism requires elimination of the host residual T and NK cells with FC or antibodies.

There are two kinds of FCs found in mouse bone marrow and lymphoid tissues: classic CD8α$^+$TCR$^+$CD3$^+$ T cells and CD8α$^+$TCR$^-$ CD3$^-$ FCs [404–406]. The immunocompetent CD8α$^+$TCR$^+$ T cells have some capacity to elicit GVHD disease, and so there is great interest in understanding the nature and function of CD8α$^+$ TCR$^-$CD3$^-$ FCs [404]. There are two other kinds of cells that share this phenotype: CD8α positive immature DCs and veto cells [404,408,409]. We have proposed that all three cell types are in fact a single cell class, and only the assays give the impression that they are different lymphoid cell subsets [404]. This view is strengthened by the findings of Steinman et al. [410] that CD8α$^+$ DCs that do not have active or appropriate co-stimulation can in fact lead to the inactivation or disappearance of T cells (and, we propose, NK cells)

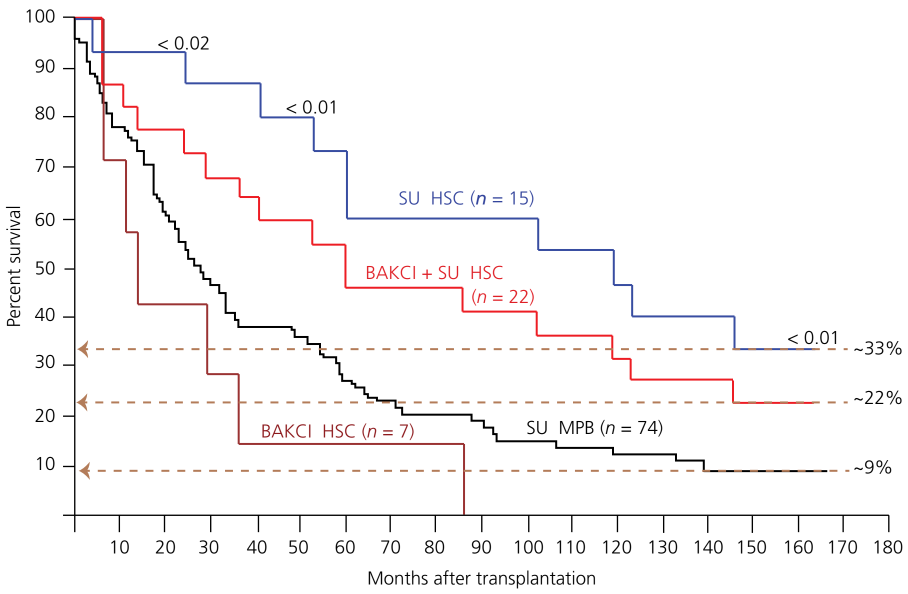


Figure 4.9 Results of several contemporary phase I/II clinical trials of autologous hematopoietic cell transplant rescue in patients with stage IV breast cancer following high-dose chemotherapy (STAMP 1 protocol). All studies were completed between 1995 and 1999. For 22 patients, mobilized peripheral blood (MPB) was initially purified by CD34$^+$ enrichment on Baxter Isolex beads and then sorted by FACS with anti-CD34 and anti-CD90 antibodies. Although ~50% of the CD34$^+$-enriched cells were keratinocyte-positive, none were positive after FACS. Two groups of patients received sorted CD34$^+$90$^+$ HSCs: (1) a group of 7 patients at Barbara Ann Karmanos Cancer Institute (BAKCI HSC), 100% of whom had stable disease after chemotherapy; (2) a group of 15 patients at Stanford University (SU HSC), 73% of whom had a complete or partial response and 27% had a stable disease after chemotherapy. A third group (SU MPB) consisted of 74 patients at SU who received unmanipulated MPB and resembled the SU HSC group, that is, 87% had a partial or complete response, 9% had stable disease, and 4% had progressive disease after chemotherapy. The estrogen- and progesterone-receptor profiles of tumors did not differ significantly among the patients in the three groups. Of the 15 Stanford patients at the last time point, 10 had died with relapse, 4 were disease free, and 1 was alive with relapse. All of the 7 BAKCI HSC patients died with relapse. Of the 74 patients at Stanford receiving MPB, 7 were alive, 5 without disease. The p values above the Stanford HSC curve were determined by 2 × 2 chi-square analysis of alive versus not alive patients at each time point, comparing Stanford HSC and MPB. At the last time point, in addition to $p < 0.01$ for overall survival, the chi-square for progression-free survival was $p = 0.04$.

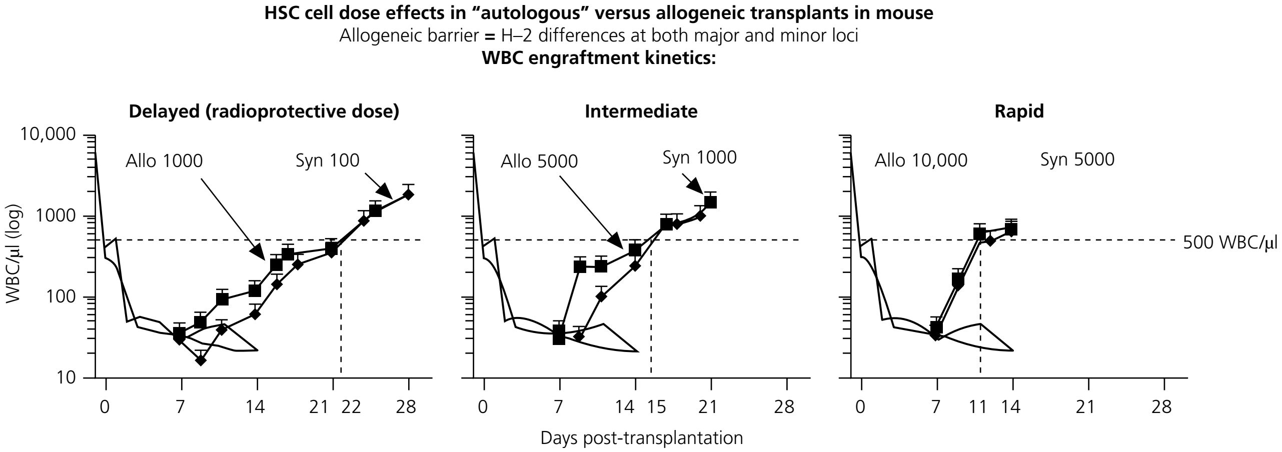


Figure 4.10 Engraftment kinetics of allogeneic versus congeneic purified hematopoietic stem cells (HSCs). Comparison of stem-cell dose required to achieve early hematopoietic recovery in syngeneic versus allogeneic irradiated mice. Syngeneic or allogeneic mice were transplanted with purified Thy-1^{lo}Sca-1$^+$Lin$^{-/lo}$c-kit$^+$ (KTLS) cells and bled on sequential days post-transplant. The kinetics of white blood cell (WBC) recovery were categorized into delayed, intermediate, and rapid recovery. Source: Adapted from Uchida et al. 1998 [394]. Reproduced with permission of John Wiley & Sons Ltd.

that have receptors which recognize them. Thus, a cell that facilitates engraftment of allogeneic HSCs might be a cell that presents MHC/peptide antigens to reactive T and NK cells without delivering a co-stimulatory signal, or induces the cells to undergo activation-induced cell death; and these same cells may be responsible for the veto of active CD4 and CD8 T cells that recognize and respond to them.

The immunosuppression of allogeneic HCT is largely due to the donor T-cell-mediated GVHD, which is by itself immunosuppressive. As discussed in Chapter 20, decreasing the number of T cells added to pure HSC allotransplants in a matched, unrelated donor (MUD) setting reveals no positive gain from added T cells either in antibody formation to new antigens, or cell-mediated immunity to a lethal viral infection in mice, that is, mouse cytomegalovirus (mCMV) [411–413]. At doses of T cells that give systemic GVHD, the host has little responsiveness, and less if the GVHD is ameliorated with immunosuppressive drugs. If the T-cell dose is under the level that gives systemic GVHD, T cells still home to lymph nodes via CD62L [414], and Peyer's patches and mucosal lymphoid tissue via integrin $\alpha_4\beta_7$ [415], where they are activated and carry out immune reactions that destroy these peripheral lymphoid organs as sites of immune responsiveness [412,413]. Activated T cells then home to the thymus, destroying the site of T-cell generation. It was not surprising, therefore, that the best antibody and T-cell responses occurred in recipients of pure HSC transplants; even better were responses when pure HSCs plus CMV-reactive T cells were co-transplanted [411].

The bane of clinical HCT in terms of infectious disease is the often lethal infections with *Aspergillus fumigatus*, CMV, and *Pseudomonas* [416]. Small numbers of each of these infectious organisms given to mice that have received HSC transplants can result in the morbidity and mortality that occurs in patients [416]. With the isolation of clonal CLPs, CMPs, and GMPs, cellular therapies for each of these disorders has been investigated. Co-transplantation of HSCs and CLPs can render mCMV infection non-lethal in both syngeneic and allogeneic protocols [279]. Co-transplantation of HSCs and CMPs and GMPs can prevent the lethal consequences of *Aspergillus* infections if the *Aspergillus* is given on day 9, 11, or 14 after HSC transplantation, and addition of G-CSF to these HSC/CMP/GMP transplant protocols prevents *Aspergillus* infections given just 3 days after transplantation [416]. Similar CMP *plus* GMP protocols are sufficient to eliminate *Pseudomonas* infections, and in both cases these treatments work in both the syngeneic and allogeneic settings.

Immunologic tolerance and HSC transplantation

It has been known since the pioneering work of Main and Prehn that bone marrow chimeras are usually transplantation tolerant to cells, tissues, or organs from the hematopoietic cell donor [372]. This method of induction of transplantation tolerance has been substantiated with pure HSC transplants [404,407], either in the high-dose or reduced-intensity setting, and whether the transplant is concurrent with the HSC transplantation or occurs months later [404,407]. Hematopoietic chimeras in humans are also usually specifically transplant tolerant of donor tissues, and trials for inducing tolerance with bone marrow cells in a reduced intensity but lymphoablative setting along with organ allografts from the bone marrow donor [417] should be followed with purified HSCs that are incapable of GVHD induction.

Reversing autoimmune diseases with disease-resistant HSCs

Partial or complete replacement of an autoimmune diabetes-prone hematopoietic system in mice with purified HSCs from diabetes-resistant strains of mice in the prediabetic period completely precludes progression to diabetes (see Chapter 18), whereas the same treatment of already diabetic hosts along with donor strain islet cell transplants cures the small numbers of mice undergoing this protocol [418]. These cures have also been accomplished in both the high-dose and reduced-intensity settings. Additionally, in a mouse model of systemic lupus erythematosis, Smith-Berdan et al. were able to achieve greatly increased overall survival by treating mice with established disease with reduced-intensity conditioning and transplants of MHC haplomismatched allogeneic HSCs [393]. Compared to control mice, those that received transplants exhibited stabilization or reversal of their lupus, including proteinuria and elevated circulating immune complexes or autoantibodies. These results indicate that induction of durable mixed chimerism by transplantation of purified allogeneic HSCs after reduced-intensity conditioning has the potential to reverse symptoms of established autoimmune disease.

The future of HSC transplantation: replacing myeloablative conditioning with selective depletion of endogenous HSCs and living donors with cell lines as donors

We have found that we can deplete not only T cells and NK cells with monoclonal antibodies, but also HSCs and some progenitors. Mouse HSCs require constant in vivo signaling of their c-kit receptors with endogenous, likely membrane-bound, kit ligand (KitL, SLF, or SCF) [70]. Treatment of immune-deficient mice with a blocking anti-c-kit antibody, called ACK2, leads to loss of HSCs, MPPs, CMPs, and so on, as well as precursors of melanocytes [101]. Following HSC depletion, transplantation of nearly syngeneic mice (i.e., of the same strain but with a green fluorescent protein (GFP) insert or strains congenic for CD45 alleles) resulted in ~15% chimerism, rather than the ~0.5% seen in untreated hosts. Repeating the antibody and HSC injections three times results in ~85% lifelong chimerism with donor cells [101]. Therefore, lymphoablative and HSC/MPP antibody depletions could replace chemoradiotherapy to create chimeras where the donor cells are healthy HSCs but not the host's HSCs.

Eventually, if we can generate transplantable HSCs from pluripotent stem cell lines, and other tissue stem cells from the same line, the HSCs could induce tolerance of the tissue that the other tissue stem cell produces. We are within years, rather than decades, of achieving these goals, at which time we will be in the era of regenerative medicine from stem cell lines.

Stem cell competition and natural chimeras

In some species, individuals can be linked together physically and exist as natural chimeras. Sponges are an example, but colonial tunicates are the best-studied example. These organisms are protochordates in the phylum urochordata, the most advanced of the invertebrates. When the individual is generated by sexual reproduction, the oozoid (egg-derived) embryo goes through the classical chordate intermediates of blastula, gastrula, and neurula, and is released from the mother colony as a tadpole with head, eyes, notochord, neural tube, and segmented musculature making up the tail. The tadpole swims to a nearby subtidal surface and metamorphoses to an invertebrate body plan, losing notochord, neural tube, and segmented musculature by apoptosis. The dying tissues are cleared by blood and tissue macrophages [419]. The oozoid, with a heart, gill arches, oral and anal siphons connected to a gastrointestinal organ, and a number of distinct blood cell types, is covered with a gelatinous tunic, and immediately begins to bud from its body wall a collection of cells, now known to be mainly somatic stem cells [420]. This group of stem cells organize without reproducing the blastula, gastrula, or neurula stages, into a blastozoid individual with an identical body plan to the mother from which it budded. The blastozoid undergoes vasculogenesis from the intracorporeal heart and blood vessels to form an extracorporeal blood system that connects, in the tunic, with the blood vessels from all other blastozoids in the common tunic. When two or more oozoids or blastozooid colonies on the same subtidal surface contact tunics, the ends of the extracorporeal blood vessels, called ampullae, extend and contact the blood vessels and ampullae of the other colony. Within a day of this contact, either the vessels anastomose to form a common circulation between colonies or an immune-based rejection between the vessels occurs, resulting in scar formation [421]. The fusion or rejection is mediated by a single highly polymorphic gene locus with hundreds of alleles [421]. Sharing one or both alleles leads to chimera formation, sharing none leads to scars between the colonies. These living natural parabionts undergo the budding cycle approximately every week, and the full life span of an individual from bud to senescence and death is about 3 weeks.

These colonies exchange somatic stem cells for each of the budding cycles, so that the individuals are themselves chimeras, but when the colonies begin the process of preparing for sexual reproduction by forming an ovary and a testis in each individual of a single individual derived or fused chimeric colony, the germline that develops from germline stem cells is usually dominated by one genotype rather than both genotypes in a megacolony between genetically distinct individuals [422]. Because of the high polymorphism of the histocompatibility locus, only kin – and usually siblings – share alleles and fuse vessels. The competition between somatic and between germline stem cells is therefore within a kindred. The full genome sequencing of the *Botryllus schlosseri* colonial protochordates has been completed, and the genomic sequence relationship of the blood system and other tissues is most like the vertebrate branches of the phylogenetic trees [423]. Therefore, in perhaps the best invertebrate to vertebrate link known, stem cells for soma and for germline exist and compete. Can this happen in the vertebrates?

In 1945 Ray Owen published that in freemartin cattle, wherein male and female twins can share a placenta, the offspring for life have blood systems from each other [424]. This finding underpins much of the research concerning both blood-forming stem cells and the development of self-non-self immune recognition that allowed the twins to “tolerate” each other’s blood cells (and as Medawar later showed, skin grafts) [425]. These findings led Burnet to propose the clonal selection theory of immunity, where each immunocompetent lymphocyte would be restricted to produce only a single type (but thousands of) immune receptors, so that anti-self clones could be eliminated during fetal development, and anti-non-self clones could be amplified by immunization to become “memory” cells [426].

The fact of tunicate stem cell competitions led us to search for HSC competitions, and the emergence of some clones that are selected for or against in aging [192]. We also tested and showed germline stem cell competitions in the developing mouse testis [427]. How, then, could competing stem cell clones play a role in the development of leukemias and other cancers?

Myelogenous leukemias are driven by leukemia stem cells (LSCs), which are at the oligolineage progenitor stages

Poorly regulated or unregulated self-renewal is a property that all cancers have, and we call those cells within a cancer that have self-renewal capacity cancer stem cells [112]. Therefore, the major distinguishing property of normal and neoplastic stem cells is their capability of self-renewal, so it becomes important to understand whether the pathways that lead to self-renewal are novel in normal HSCs or might be shared among different classes of tissue-specific multipotent stem cells, and might be acquired by cancer cell progenitors as they emerge as cancer stem cells [112].

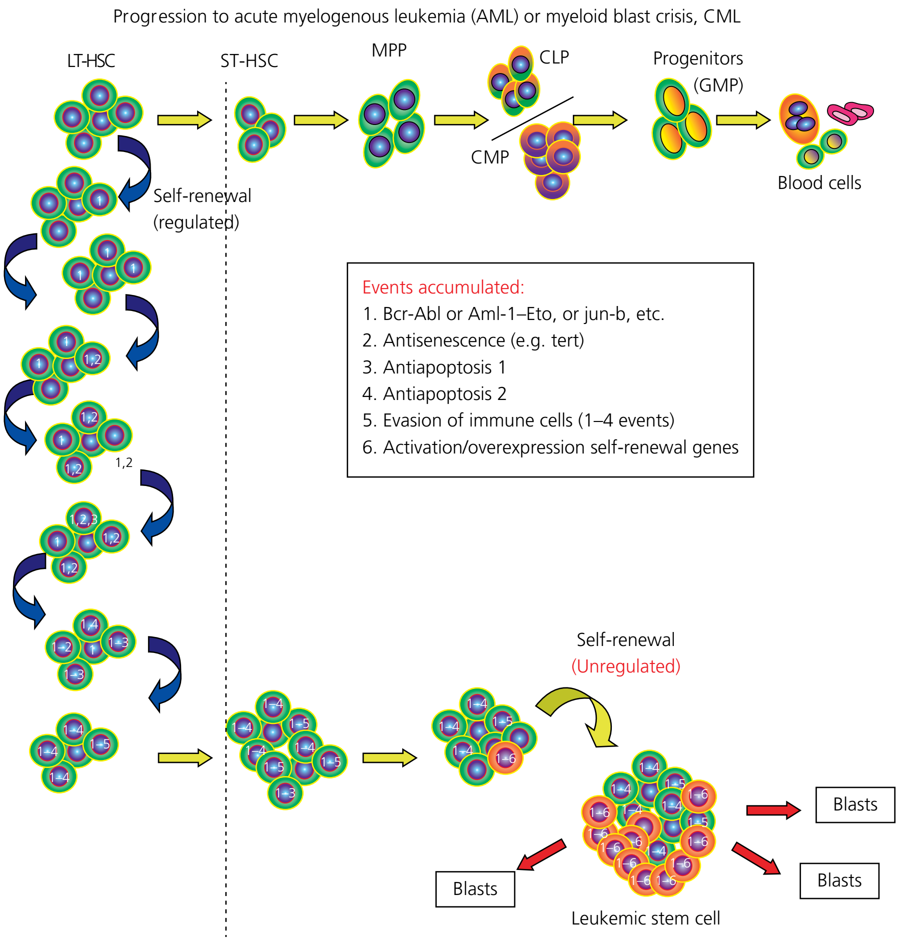
To understand the mechanisms of leukemic transformation, it is important to understand the life history of leukemic progression (Figure 4.11). If one views leukemia development as a series of steps in malignant progression, malignant self-renewal may be obtained through several transformation steps that include a block in differentiation, the retention or acquisition of self-renewal, and/or avoidance of programmed cell death by several independent cell-intrinsic pathways and by immunosurveillance of the intrinsic and adaptive immune systems.

A set of early stem/progenitor cells with extensive proliferation, or with reduced programmed cell death that are not yet malignant, may form “preleukemic” clones, and these clones may undergo additional oncogenic events that induce differentiation blocks at a progenitor stage to become leukemia. Many of the genes overexpressed in leukemias or cancers are driver genes for normal stem cell self-renewal without differentiation, such as *BMI1*, *PTEN*, *MLL*, *RUNX1* (for review see [428]).

Progression to myelogenous leukemias occurs in competitive HSC clones in a stepwise fashion involving both mutations and epigenetic events

Molecular markers for leukemia, including oncogenic fusion proteins resulting from leukemia-specific chromosomal translocations, have disclosed the multistep nature of acute leukemia development. A typical case is chronic myelogenous leukemia (CML). The CML-specific t(9;22) Philadelphia chromosome and its resultant *Bcr–Abl* fusion gene is present in all myeloid cells, erythroid progenitors, B cells, and rare T cells; thus it is a multipotent progenitor or HSC. We have shown that it is HSC clones which, during the myeloproliferative stage, outcompete normal HSC clones in the same patient. When the chronic phase of CML converts to myeloid blast crisis, the LSC is at the stage of the GMP in all cases studies [429]. These GMPs are driven by the β-catenin signaling pathway, and can be inhibited in their proliferation by transfection of axin, the natural inhibitor of signaling in this pathway. In 4 of 7 patients, GSK3β – the enzyme that normally phosphorylates β-catenin (in a complex with the adenomatous polyposis coli gene product and axin) in the cytoplasm – is misspliced so that it lacks the kinase domain in all GMP LSCs (and some HSCs). Furthermore, only these GMP clones, and not the HSCs, spread the disease and, in experiments, transplant the disease to immunodeficient mice [430].

There are many other examples of human adult and childhood leukemias that support the early acquisition of predisposing mutations that occur in HSCs which require additional events to result in leukemic transformation. The AML1-ETO chimeric protein that is the product of the t(8;21) translocate genomic region is continuously detectable in t(8;21) AML patients who maintain long-term remission for more than 10 years. Although these patients are clinically “cured,” AML1-ETO remains detectable in HSCs (Thy-1$^+$CD34$^+$Lin$^-$CD38), B cells, and erythroid and megakaryocyte colony-forming cells [400]. In all of these cells, only HSCs self-renew, setting up clones that can acquire additional proto-oncogenic events. In patients with childhood t(8;21) AML, AML1–ETO fusions are retrospectively detected in neonatal Guthrie spots [431], further suggesting that AML1–ETO fusion is at the level of HSCs (in some cases in utero). Acquisition of chromosomal translocations in utero is also demonstrated in infant t(4;11) and childhood t(12;21) acute leukemias. Clonotypic MLL–AF4 and TEL–AML1 genomic fusion sequences were demonstrated in neonatal blood spots from patients diagnosed with ALL [431–433]



Events accumulated:
1. Bcr-Abl or Aml-1–Eto, or jun-b, etc.
2. Antisenescence (e.g. tert)
3. Antiapoptosis 1
4. Antiapoptosis 2
5. Evasion of immune cells (1–4 events)
6. Activation/overexpression self-renewal genes

Figure 4.11 Comparison of stem cell self-renewal during normal hematopoiesis and leukemic transformation. During normal hematopoiesis, the signaling pathways that regulate self-renewal are tightly controlled, allowing proper differentiation to each of the mature blood cell lineages (top). Accumulation of mutations in the self-renewing hematopoietic stem cells (HSCs) over time until dysregulated self-renewal ability is attained and leukemia propagation is initiated. Dysregulation of self-renewal mechanisms in transformed cancer stem cells leads to uncontrolled self-renewal and the production of leukemic blast cells. The leukemic population capable of sustaining disease is often distinct from the blast population, and may reside in the stem cell or progenitor cell compartment. Importantly, if the transformation event occurs in a progenitor cell, it must endow the progenitor cell with the self-renewal, because the progenitors would otherwise differentiate. (For a color version of this figure, please see the color plate section.)

and in identical twins whose genomic fusion sequence was identical [382]. In these cases, t(12;21) ALL developed at ~10 years of age, indicating that cells with TEL-AML1 were present for more than 10 years before leukemic transformation. Further, TEL-AML1$^+$ ALL cells in twins sometimes possess identical IgH rearrangement, implicating in utero transfer of preleukemic cells. In childhood ALL, preleukemic B- or T-cell progenitors appear to persist over the long term: “leukemic clones” possessing clonal T-cell receptor or IgH rearrangements are reported to persist more than 3 years after onset of complete remission [434]; these might involve the only other blood cells that self-renew – memory T and B cells. These data strongly suggest that leukemic transformation commonly occurs by a multistep process. In mice and humans, the only cell in the myeloid lineage that self-renews over long periods of time is the LT-HSC [1]. The preleukemic clone should exist in the LT-HSC to sustain multiple mutational events over time. Therefore, it appears that the accumulation of mutations occurs as a precondition for leukemia, at the level of HSCs or progenitors that have gained the capacity of self-renewal.

We have shown that in 5 patients with AML and an internal tandem repeat mutation of the *FLT3* gene, individual HSCs can be found with 1, or 1 + 2, or 1 + 2 + 3, and so on genomic mutations (Figure 4.12) [84]. In all five cases the *FLT3* internal tandem repeat mutation is the penultimate event in the preleukemic HSC clone, with the leukemias emerging with LSCs at the MPP or LGMP stages [84] (see below). It is very interesting that in at least 2 of the 5 patients the first mutation was in one allele of *TET2*, and in both patients the second mutation to appear was in the other allele of *TET2* [84]. These mutations are in genes whose primary known functions are the oxidative hydroxymethylation of 5-methyl cytosine in DNA, an epigenetic mark, in the immediate vicinity of a gene, which usually prevents its transcription. Thus the ability of opening DNA sites that had been silenced is likely lost in these cells. Full analysis of the epigenome in these cells is currently not possible, as these early clones in preleukemic progression are too rare to elucidate with current biochemical techniques. Nevertheless, if the silenced loci are part of the differentiation program that operates in stem and progenitor cell undergoing continued proliferation, their silencing is likely to cause HSCs to expand.

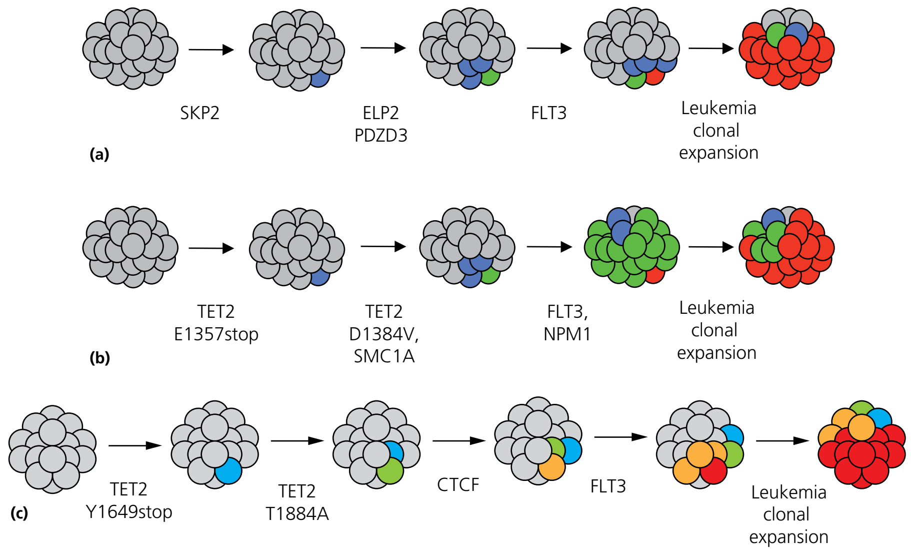


Figure 4.12 Clonal progression of preleukemic mutations in hematopoietic stem cell (HSC) clones, followed by emergence of the leukemia stem cell (LSC) in the multipotent progenitor (MPP) stage in three cases of FLT3td acute myeloid leukemia (AML) [84]. Exomic sequencing of the AML cells and T cells from the same patients allow listing of the mutations specific to the AML. Individual HSCs were isolated from the 3 patients and grown to colonies with hematopoietic growth factors, and the colonies analyzed with DNA primers specific for each of the mutations in the LSCs. Shown are the normal HSC clones (grey) and mutant HSC clones (with each successive color – blue, green, yellow – indicating the addition of a new mutation). Leukemic HSC clones (red) finally have all mutations. A TET2 loss of function mutation via a stop codon was the earliest preleukemic HSC event in 2 of the 3 patients (b) and (c). In the same individuals, the next event is the loss of TET2 by another stop codon in the other TET2 allele. Following that are clones with 3, or 4 or *n* mutations in the 3 patients. In all 3 the FLT3 internal tandem repeat occurred as the final mutation before the LSCs emerge. It is interesting that in 2 patients the loss of all TET2 function, which regulates CMEpG to hydroxymethyl cytosine, an epigenetic modifier of the CpG methylome, occurs before any of the other mutations. Source: Jan et al. 2012 [84]. Reproduced with permission of the American Association for the Advancement of Science. (For a color version of this figure, please see the color plate section.)

Final leukemic transformation can occur at the level of myeloid progenitors

Several lines of evidence show that final myeloid leukemic transformation can only occur at the level of progenitors that have acquired the property of self-renewal. In contrast, lymphoid cells include neoplastic cells with clonally rearranged T-cell receptor or immunoglobulin genes. Whereas myeloid progenitors and myelomonocytic cells are not self-renewing, lymphocytes that contribute to the memory pool are self-renewing. Thus, the final transformation to lymphatic neoplasms can occur in cells that already have regulated self-renewal capacity.

The search for LSCs involves the one property shared by all malignancies, the ability to initiate and spread the disease. Transfer of human AML to immunodeficient mice was first demonstrated to be limited to CD34$^+$CD38$^-$ or CD34$^+$CD71$^-$HLA$^-$DR$^-$ cells, and attributed to early progenitors such as HSCs [435]. We [400] and others have shown that in at least some AMLs, the LSCs lack CD90 expression. In our study of AML1/ETO AML, LSCs were in the MPP phenotype (CD34$^+$38^{lo}90$^-$Lin$^-$) [400]. Surprisingly, in the same patients, 1–40% of HSCs were t8;21$^+$, but these HSCs gave rise in vitro to normal myeloerythroid colonies, not leukemic blasts. As shown in Figure 4.11, these represent preleukemic clones; thus AML1/ETO may be necessary but not sufficient for full leukemic transformation.

A mouse model that expresses AML1/ETO driven by the MRP8 (GMP-, granulocyte-, and monocyte-specific) promoter did not develop leukemia. After the injection of a DNA alkylating mutagen, *N*-ethyl-*N*-nitrosourea (ENU), however, almost 55% of mice develop AML-M2 [436]. Similarly, PML/RARα is a product of the t(15;17) translocation, and MRP8$^-$PML/RARα transgenic mice develop acute promyelocytic leukemia (APL)-like disorders [437]. The frequency and rapidity of PML-RARα leukemias increases when they are crossed to MRP8–Bcl-2 mice [438]. In these mice the proto-oncogenes were present in all cells of the GMP lineage, allowing rare subsequent events to be fixed.

Inhibition of apoptotic pathways alone can allow leukemia development at progenitor stages. MRP8–Bcl-2 mice display increased numbers of mature monocytes, resembling CMML, but do not develop acute leukemia. However, an additional introduction of Fas (*lpr/lpr*) mutations into these mice results in transformation into AML in about 15% of progeny, but only if leukemia emerges by age 8 weeks [439]. The transplantable GMP stage leukemias are TERT^{++}, and overexpress CD47, and have acquired the property of self-renewal, implicating a multistep process. Even MRP8–Bcr-Abl mice have increased frequencies of conversions to acute blastic leukemias if crossed to MRP8–Bcl-2 strains.

The general rules so far are that mouse AML LSCs induced as described are at the stage of the GMP, as is human CML myeloid blast crisis [429,440–442]. Most human AMLs are at the level of the MPP and/or LGMP. No HSC-stage LSC has been found.

The identification and characterization of LSCs is crucial to the development of more effective treatment strategies. Ideal therapies will be those that target the LSC while sparing normal HSC, so identifying LSC-specific markers is an important strategy. To date we have identified several candidate LSC-specific markers, including CD96 and CD47 [149,443]. FACS analysis indicates that in many cases CD96 is expressed on the majority of CD34$^+$CD38$^-$ AML cells but on few, if any, normal HSCs (Lin$^-$ CD34$^+$CD38$^-$ CD90$^+$) [443]. Further, in four of five samples, only CD96$^+$ cells were able to propagate the leukemia in recipient immunocompromised mice, supporting CD96 expression on LSCs and its usefulness as a potential therapeutic target.

We identified CD47 in the mouse AML microarray screen, and have verified it as specifically upregulated in human AML. Subsequently, we found it to be expressed in all mouse models of myeloid leukemia we have examined. Most importantly, it is also expressed in LSCs and all leukemia daughter cells in human AML and blast crisis CML [41,149,444,447]. Oldenborg et al. have shown that CD47 is an age marker on mouse erythrocytes, acting as a "don't eat me" signal to macrophages [445]. The CD47 on cells signals macrophages via their SIRPα receptor, which upon ligation activates its ITIM motifs with tyrosine phosphorylation, triggering SH2-motif positive SHP1 and 2 tyrosine phosphatases. This activation results in the loss of cytoskeletal phosphotyrosines and their failure to enforce engulfment of particles or cells bound to the macrophage [446]. Transfected expression of CD47 is sufficient to allow a human myeloid leukemia cell line to evade phagocytosis by mouse macrophages in vitro, and to efficiently engraft immunodeficient mice in their hematopoietic tissues and develop into large tumors; control cells fail to engraft in immunocompromised $Rag2^{-/-}\gamma_c^{-/-}$[149]. Thus, CD47 does seem to play a role in the spread and/or life span of AML LSCs. These data also suggest that macrophage-mediated killing may be a significant player in leukemia surveillance. CD47, found to be overexpressed in mouse AML LSCs first, is now known to be overexpressed in all cancer stem cells and all human cancers tested [448–455].

There are increasing descriptions of mutations that occur at the post-translational and epigenetic levels and would not be detected by gene expression profiling. A study of 100 CML blood and marrow samples has demonstrated that, in comparison to normal GMPs, those from patients with CML blast crisis have higher levels of the active β-catenin in the nucleus and exhibit higher degrees of self-renewal in in vitro replating assays [429]. Dysregulation of microRNAs, small RNAs that bind to specific transcripts and either suppress their translation or target them for deletion, is another potential leukemogenic mechanism [457–460].

The important point here, as outlined in Figure 4.11, is that the elucidation of the phenotypes of normal HSCs and hematopoietic progenitors allows isolation of pure cell types, usually rare in the entire tissue, which can be assayed for their particular gene expression profiles, proteomes, histone changes, and DNA CpG methylation. These studies cannot be done with crude tissues such as whole leukemic bone marrow, $CD34^+$ leukemic, or MDS samples, etc. When done properly with highly purified subsets, the classification of the various types of LSCs, and the comparison of genes and gene transcripts between normal cells and their neoplastic counterparts have revealed a plethora of new and perhaps useful diagnostic and therapeutic targets undetected when whole blood or marrow populations are analyzed.

Programmed cell death and programmed cell removal are initiated by proto-oncogenic events, and all leukemias (and cancers) overcome both to become LSCs (CSCs)

Many terminally differentiated blood cells have a limited life span, for example, ~1–2 days for neutrophils. In mice with neutrophils that have extended life spans due to enforced expression of Bcl-2, surprisingly the homeostatic level of blood and tissue neutrophils does not rise with time, whereas $Bcl\text{-}2^+$ monocytes accumulate in the blood and spleen in a syndrome reminiscent of chronic myelomonocytic leukemia. If the neutrophils cannot die, how is their relative number maintained? It turns out that about 12–20 hours after migration of newborn bone-marrow neutrophils to an inflammatory site (e.g., the peritoneum after infusion of thioglycollate broth), wild-type neutrophils begin to undergo the intracellular events of apoptosis (e.g., DNA strand breaks). However, at this point, these cells have not yet "popped" open to release their cytoplasmic and nuclear contents (as is observed with isolated neutrophils in vitro) [51,461]. Nevertheless, Bcl-2 neutrophils not undergoing the early apoptotic events and wild-type neutrophils undergoing DNA strand breaks begin to be phagocytosed by macrophages 12 hours after migration. These cells are the population that follows neutrophils of acute inflammation [461]. We call this macrophage phagocytosis *programmed cell removal* (PCR), an event for preventing inflammation around dying cells, insofar as they pop inside macrophage phagolysosomes [52], not in tissue spaces. PCR is mediated by cell surface alterations on the target cell that express "eat me" signals such as phosphatidyl serine, calreticulin [462], and/or asialyglycoproteins for which macrophages have prophagocytic receptors. Adaptive immune responses can also bind tumors with antibodies or complement components that also serve as "eat me" signals. For example, human IgG1 and mouse IgG2 antibodies have Fc regions that are bound by the high affinity FcR found on macrophages (and NK cells). In summary, cells that are triggered to undergo programmed cell death are also triggered in a Bcl-2-independent process to undergo PCR [461]. Such triggers can be genotoxic stress, unbalanced protein synthesis, endoplasmic reticulum stress – events that can occur in the progression to cancer. We propose that cells involved in cancer progression (e.g., HSCs and myeloid progenitors in AML) sustain these lesions at a particular differentiation stage, and usually die from apoptosis. However, some HSC clones may have activated the antiapoptotic genes such as Bcl-2, and these clones would survive, except that they still could express eat me signals and undergo PCR. As stated earlier, all leukemias and cancers tested overexpress CD47, the "don't eat me" signal that overrides the "eat me" signals for PCR. At that point, the cells have not only escaped death, but also are free to migrate through tissue fields containing macrophages [452]. The upregulation of CD47 appears to be a late event in leukemogenesis.

Myelodysplastic syndrome is a stage of leukemic progression before programmed cell removal is overcome

Myelodysplastic syndromes (MDS) are a heterogeneous set of diseases wherein the patient, usually elderly, loses the ability to produce one or more terminal blood cell types but maintains highly proliferative bone marrow. MDS commonly progresses to AML. It has been associated with a variety of mutations such as $5q^-$, monosomy 7, mutations in ribosomal proteins, and so on (for review see [463]). It has been a mystery how such a diverse set of causes could lead to the same dysplasia with, for example, anemia or thrombocytopenia. We investigated a subset of MDS patients, mainly with monosomy 7, and discovered that MDS HSCs outcompete normal HSCs in patients and in NSG immune-deficient mice that receive transplants of MDS bone marrow or HSCs [464]. Thus, the MDS HSC clone is outcompeting the normal clone at the stem cell level. However, a hallmark of MDS is the depletion of progenitors such as the GMPs, erythroid progenitors, and megakaryocyte progenitors [464]. These $CD34^+38^+$ progenitors express high levels of calreticulin on their surfaces and are phagocytosed in vitro when co-incubated with macrophages. Phagocytosis could be inhibited completely with calreticulin peptides that block LRP1-mediated macrophage phagocytosis. These progenitors do not express CD47, but blasts in both refractory anemia with excess blasts and AML secondary to MDS express CD47 at high levels, allowing these cells to evade phagocytosis [464].

CD47, the "don't eat me" signal that overcomes programmed cell removal, is a target for antileukemia and anticancer therapies

As described earlier, CD47 provides a "don't eat me" signal to macrophages by acting as a ligand for the SIRPα receptor on macrophages [84,444,447–452,456,465–469]. We have obtained, made, and humanized blocking anti-CD47 antibodies that prevent CD47 ligation of macrophages. If the blocked CD47 is on a tumor cell adherent to a macrophage, the result is tumor cell phagocytosis and death in the phagolysosome [447]. This occurs both in vitro and, apparently, in vivo, as we have observed, in immune-deficient mice, the reduction in size or growth rate of primary human leukemia or cancer xenografts, ranging from breast cancer to glioblastomas [84,444,447–452,456,465–469]. CD47 was first discovered by comparing gene expression patterns between highly purified HSCs, MPPs, and LSCs at the MPP stage. Indeed, anti-CD47 is one of the earliest therapies coming from the identification, purification, and isolation of cell surface proteins selectively expressed on LSCs, and also CSCs [84,444,447–452,456,465–469]. At time of writing, various forms of CD47 blocking antibodies and proteins are being readied for early phase clinical trials. In addition, in a single experimental system, anti-CD47-enabled phagocytosis of a colorectal cancer cell line (DLD1, transfected with the chicken ovalbumin gene to be expressed in the cytoplasm) allows the macrophages to digest and cross-present the SIINFEKL peptide on mouse H2Kb macrophage cell surfaces in a way that cross-presents it to OT1 CD8 killer T cells in vitro and in vivo, but not to OT2 CD4 T cells [466]. This implies that CD47 allows cells to evade both macrophages of the innate immune system and T cells of the adaptive immune system. Whether monotherapy with anti-CD47 antibody will enable both arms of the immune system to target malignant cells will be examined in a subset of patients in phase I trials.

Conclusions

The development of a general method to prospectively isolate hematopoietic stem and progenitor cells [1,5,30] has made it possible to construct increasingly detailed lineage pathways from LT-HSC to mature blood cells. With the exception of memory T and B lymphocytes in the entire hematopoietic lineage, only LT-HSC self-renew, and this self-renewal capacity is tightly regulated [1]. The genetic regulation of HSC self-renewal includes regulation of programmed cell death, regulation of cell-division frequency, and regulation of transition to multipotent and to oligopotent progenitors. Gene expression analyses of prospectively isolated HSCs and progenitors provide insights into the mechanisms of self-renewal and lineage commitment. These same isolated HSCs and progenitors are increasingly useful in clinical HCT, whether to regenerate the myeloablated hematopoietic system in cancer patients, to condition allogeneic hosts for donor tissue or organ grafts, or even to replace an autoimmune-prone hematolymphoid system with a non-autoreactive donor population. Finally, the concept of self-renewal as the critical regulated event limited to HSCs in normal hematopoiesis has now been extended to the hypothesis that within leukemias and cancers are relatively infrequent populations of LSCs and CSCs that undergo poorly regulated self-renewal. Prospective isolation of LSCs should allow more direct approaches whereby immune and drug therapies target molecules restricted to these malignant stem cells. Both the preleukemic phase and the leukemic phase of the myeloid leukemias are shown to involve stem cell competitions, the early phase HSCs almost certainly for niche occupancy, and the later phase frank AML LSCs for ever more successful and drug-resistant clones. Thus, in the field of stem cells, the principal properties are self-renewal, regulated differentiation, migration, longevity even after many doublings, and competition for niches. This is true for normal HSC, abnormal HSC, and frank LSC clones.

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CHAPTER 5

Marrow Microenvironment and Biology of Mobilization of Stem Cells

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Introduction

The habitat for hematopoietic cells within the bone marrow has now been dissected to the single cell level [1–4]. We are just beginning to understand the intricate interactions of cellular and non-cellular components of the microenvironment and hematopoietic stem and progenitor cells (HSPCs), which when normal are critical for health and when dysfunctional lead to disease. An understanding of the networks of interactions within this bone marrow microenvironment has provided important mechanistic information on normal hematopoietic and stem cell trafficking to, and egress from this environment. This understanding has led to improvements in approaches to hematopoietic stem cell mobilization and a better understanding of the pathogenesis of diseases such as acute myeloid leukemia and myelodysplastic syndrome.

Adult hematopoietic stem cells (HSCs) predominately reside in the bone marrow within a niche that is critical for hematopoiesis. Chemokines and cell surface molecules are critical intermediaries for the normal physiologic process of homing and egress of HSCs to and from the bone marrow. Altering this normal process through administration of supraphysiologic doses of cytokines or small molecule antagonists of key pathways has been the recent goal of hematopoietic cell transplantation (HCT) by using mobilized peripheral blood stem cells to engraft recipients. Currently, granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), and the small molecule antagonist of the chemokine receptor CXCR4, plerixafor (AMD3100/Mozobil), are being used clinically to increase circulating peripheral blood stem cells with varying kinetics.

Under steady-state conditions, BM-derived HSPCs periodically leave the bone marrow niche and travel from the blood into multiple peripheral tissues, from the tissues into the lymphatic system, and from the lymph back into the blood, where they can either re-enter the BM or repeat the peripheral migration cycle [5,6]. The presence of small numbers of circulating HSPCs was first reported in the early 1960s [7,8] and the number of circulating HSPCs has been found to increase significantly in response to different stressful situations such as inflammation [9], organ/tissue damage [10–12], or strenuous exercise [13]. Pharmacologically mediated enforced egress of HSPCs from the BM microenvironment into the peripheral blood is called "mobilization," and this phenomenon has been exploited in HCT as a means to obtain HSPCs for hematopoietic reconstitution.

The term "mobilization" was first used in 1977 to describe a fourfold increase of peripheral blood granulocyte–macrophage colony-forming units (CFU-GM) after the administration of endotoxin to healthy volunteers [14]. One year earlier, high levels of circulating CFU-GM were observed in patients recovering from myelosuppressive chemotherapy [15]. Subsequent studies during the 1980s led to the development of a single high dose of cyclophosphamide as a generic mobilizing agent and the use of chemotherapy-mobilized HSPCs for transplantation [16–19]. During this same time period, while treating patients with recombinant human G-CSF to accelerate recovery from neutropenia after myelosuppressive chemotherapy, investigators observed that G-CSF, in addition to increasing circulating granulocytes, also mobilized HSPCs into the peripheral blood that were capable of providing long-term multilineage reconstitution capacity following transplantation [20–22]. The labeled indications for G-CSF were subsequently expanded to include the mobilization of HSPCs into the peripheral blood for collection and HCT.

In order to understand the biology of mobilization an understanding of HSPC homing and the environment that nurtures HSPCs is required. Using an anatomical approach, location near endosteum or endothelium has come to define two niches. This simplified approach likely underestimates the plastic and overlapping nature of the environment. Some of the critical components involved in trafficking and homing of HSPCs to these niches are chemokines, integrins, and selectins. This chapter will review the critical components of the stem cell niche and how stem cells home to, interact with, and are released from the marrow microenvironment during mobilization. The mechanism of mobilization and critical axes such as CXCR4/CXCL12, VLA-4/VCAM-1, stem cell factor, proteases, complement, and bioactive sphingolipids will be discussed.

Stem cell homeostasis and the components of the bone marrow niche

Niche is used to refer to the in vivo microenvironment that harbors adult hematopoietic stem cells, and regulates their renewal, differentiation, and survival [23]. The concept of a niche was initially proposed in 1978 by Schofield [24]. The idea that the environment could govern the fate of hematopoietic stem cells was controversial at the time and was counter to the argument that stem cell behavior was cell autonomous [25]. Numerous groups have confirmed the importance of a heterogeneous group

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of cells, generically referred to as stroma, and extracellular matrix (ECM), which specializes in governing stem cell behavior. Just as the heart or lungs have a scaffolding of stroma and ECM to allow the normal development of the mature functional organ, the bone marrow contains a scaffold of stromal cells and ECM that are critical for the development of normal blood and maintenance and regeneration of hematopoietic stem cells. Modification of certain molecules or cells within the microenvironment can lead to alterations in proliferation, differentiation, and egress of hematopoietic stem and progenitor cells [26–28].

Regional differences within the bone marrow

The components of a niche

The specialized microenvironment of the bone marrow contains a number of cellular and non-cellular components that make it hospitable for HSPCs. Bone marrow stroma is made up of adipocytes, macrophages, vascular endothelial cells, mesenchymal stem cells, smooth muscle cells, and CXCL12-abundant reticular cells. These stromal cells produce components of the ECM such as fibronectin, hyaluronan, collagen, laminin, chondroitin sulfate, heparin sulfate, and glycosaminoglycans. The stroma and ECM interact with HSPCs through direct contact with cell surface receptors, secreted factors such as cytokines, and adhesive ligands such as hyaluronan. The major known components of the niche and their role in HSPC homeostasis and mobilization are outlined in Table 5.1. Interactions of HSPCs with niche components can be physical, structural, humoral, paracrine, metabolic, and neural.

Classically the marrow environment has been separated anatomically into two niches: perivascular [29,30] and endosteal [31–33] (reviewed in [30,34–37]). Location alone is not sufficient to be considered a niche, but functional properties to enable stem cells to survive, proliferate, and renew are essential to defining the niche. There is a symbiotic relationship that exists between stem cell and niche components. The dynamics between HSC and niche have been recently demonstrated using real-time imaging [2,3,38,39]. Location of HSPCs seems to depend on the degree of maturation with more immature cells and those that respond to damage signals located near the endosteum [2,3]. Within the unprovoked niche, most HSCs are quiescent (some estimates indicated up to one quarter of long-term HSCs can be cycling at any given time [40]), but after irradiation and transplantation HSPC cycling increases to as much as 50% [41]. Entry into the cell cycle is regulated by a number of mechanisms. Regulation of HSC differentiation is critical to prevent stem cell pool exhaustion [42]. Contact with osteoblasts and extracellular matrix glycoprotein, such as osteopontin, prevents exhaustion by inducing quiescence [33,43,44]. When HSPCs leave the bone marrow as occurs with peripheral blood stem cell mobilization, circulating HSPCs may be induced to cycle and lose their long-term repopulating capacity; however, the majority remain quiescent and retain the ability to engraft NOD/SCID mice [40,45,46]. On average, 1–5% of the stem cell pool enters the circulation daily [47]. Intriguing data suggests that there may be competition for a limited number of available niches [47]. The availability of these niches can be affected by mobilization using plerixafor alone or G-CSF + stem cell factor (SCF) [48]. Normal HSCs may also have to compete for the niche with malignant cells and this could affect mobilization and stem cell reserve [38,39]. The exact series of events that occur to result in HSPC mobilization under resting and provoked conditions, such as inflammation and peripheral blood stem cell mobilization, are just beginning to be understood and exploited for clinical use.

Perivascular niche

The bone marrow is a rich network of sinusoids. The vascular niche is in proximity to vascular endothelium. Cellular components of this niche in addition to endothelial cells include nestin$^+$ MSCs, *Lepr*-expressing perivascular stromal cells, sympathetic neurons, and CXCL12+ adventitial reticular cells (CARs) [29,49–53]. The concept of a vascular niche is supported by the observation that sites of extramedullary hematopoiesis such as the spleen and liver are devoid of bone yet can maintain HSCs. In addition, endothelial cells isolated from non-hematopoietic organs grown in culture can maintain stem cells [54]. In mobilized mice, HSCs, defined by CD150$^+$CD48$^-$, associate with sinusoidal endothelium in both bone marrow and spleens [29]. Finally, in non-irradiated mice, HSCs are in close proximity to vascular endothelium [2,3,39].

The divide between vascular and endosteal niche is arbitrary and the highly vascular endosteum supports HSCs that reside adjacent to endothelium which also associates with N-cadherin$^+$ pre-osteoblasts [2]. Alterations in this niche through damaging ionizing radiation result in leaky sinusoids and increased CXCL12 levels, and can significantly impact the engraftment of HSCs near bone or vasculature [2,3]. Non-irradiated recipients engraft HSPCs randomly between vascular and endosteal sites and irradiated recipients engraft preferentially near the endosteum. CXCL12, also known as stromal-derived factor-1 (SDF-1), is one of the best described chemokines that plays a central role in HSPC trafficking, homing, and retention in the bone marrow. A gradient of expression within the marrow between endosteum (high) and endothelium (low) can be altered for the purpose of mobilization through the administration of G-CSF [55] or manipulated through small molecule inhibitors of its receptor, CXCR4 (see mobilization section later).

Another component of the vascular niche are nestin$^+$ MSCs, which have been shown to associate with sinusoidal endothelium and are critical for HSC maintenance [50]. In close association with these cells are also CXCL12 adventitial reticular cells (CARs), which are perhaps the highest producers of CXCL12 and have been identified within the endovascular and endosteal niche [30]. CAR cells appear closely related to a CD146$^+$ adventitial reticular cell associated with sinusoidal endothelium [56]. Evidence suggests that perhaps these cells are coordinated through the sympathetic nervous system, which is closely associated with endosteal and perivascular niches [52]. Normal circadian oscillations in sympathetic tone and disease states such as diabetes may affect the sympathetic nervous system interaction with nestin$^+$ MSCs and alter mobilization through alterations in CXCL12 [52,57,58] (see mobilization section).

Although CXCL12 is a critical factor produced in the perivascular niche another component appears to be stem cell factor (SCF). Endothelial cells and newly described *Lepr*-expressing perivascular stromal cells are the primary producers of SCF, which is critical to the maintenance of stem cells [53]. Disruption of c-kit (receptor for SCF) is known to affect maintenance and engraftment of stem cells. Finally, other components important for normal leukocyte and HSPC migration, homing, and mobilization that are expressed on endothelium include selectins and integrins. The integrin VLA-4 has been perhaps one of the best studied. Both the α_4 and β_1 subunits of VLA-4 have been inactivated in mice. Lack of α_4 or β_1 integrin is embryonic lethal due to non-hematologic defects [59–61]. Conditional genetic ablation of α_4 integrin in both hematopoietic and non-hematopoietic compartments induced an eightfold increase in the number of circulating HSPCs (CFU-Cs) at 2 weeks

Table 5.1 Components of the niche and their role in mobilization

Cell / Factor / Receptor	Identity / Source	Role / Mechanism	Reference
Cells			
Osteoblast lineage	Endosteum lining cells N-cadherin+ CD45–	Decrease numbers results in decreased cellularity and extramedullary hematopoiesis. Alterations in CXCL12 expression leads to mobilization. Constitutive activation of PTH receptor increases HSC.	[31,33,55,68,69]
Osteocytes	Terminally differentiated osteoblasts	Knock out of G-protein receptor alpha from osteocytes leads to increased G-CSF-mediated myelopoiesis.	[67]
Osteoclasts	Endosteum lining cells resulting in bone resorption	Decrease results in increase trabecular bone formation, decreased # HSCs, decreased engraftment, and increased mobilization of HSC.	[70–72]
Macrophages	M-CSF receptor+, CD169+	Decrease leads to HSC mobilization.	[82,83,114]
CXCL12 adventitial reticular cell	Adipo-osteogenic bipotential progenitor CD45–, Ter119–, CXCL12++, CD44+, CD51+, VCAM-1+, PDGFRa and b+ ?CD146+	Apoptosis of CXCL12 expressing cells by diphtheria toxin treatment of transgenic mice leads to reduced HSC bone marrow numbers, increased HSC quiescence, and increased HSC in peripheral blood.	[49,56] [78]
Nestin+ MSC	Nestin+ peri-vascular cells CD45– CD31– CD34– VE-cadherin–	Regulated by SNS and parathyroid hormone. High expression of CXCL12. Decrease leads to decreased HSC number, decreased homing, increased EM hematopoiesis.	[50]
Lepr-expressing perivascular stromal cells	Nestin–, ***Lepr+***, peri-vascular cells		[53]
Sympathetic neuron		Beta adrenergic tone can regulate nestin+ MSCs. Inhibition increases mobilization.	[51,52,57,58]
Sinusoidal endothelial cell	CD45– CD31+ Sca-1+ Ter119–	Producer of SCF,	[29]
Adipocytes		Decrease increases trabecular bone, HSC numbers and engraftment.	[86]
Small molecules			
Oxygen	Oxygen tension near bone 10 mmHg	Hypoxia induces CXCL12 expression through HIF1α.	[89]
Calcium	Calcium ions concentrated near bone	Calcium ions are concentrated along bone and sensed through calcium receptors on HSCs.	[32]
Syphingosine-1-phosphate	Bioactive lipid, S1P levels highest in RBCs	S1P gradients may play a role in stem cell mobilization.	[272,278,280]
Cytokines / chemokines and other factors			
CXCL12 (SDF-1)	Osteoblasts, CAR cells, endothelial cells, nestin+ MSC	Gradient-induced chemotaxis and upregulation of integrins and CD44 on HSCs. Homing, retention and mobilization. HSC maintenance. Gradient affected by G-CSF.	[55,84,104,105]
IL-8 (CXCL2)/ (CXCR1 and 2)		Induces release of proteases.	[282,283]
Proteases (MMP-9, NE, Cathepsin K)		MMP-9: Cleaves mKitL and releases sKitL. NE: Cleaves CXCL12. Cathepsin K: Bone resorbing, cleaves CXCL12 and SCF.	[72,75,78,127,147,153]
Complement cascade	Classical (Ig-mediated) and non-classical pathways	C5 cleavage fragment and distal complement cascade is required for G-CSF and CXCR4 inhibitor mobilization.	[268–274,284]
Osteopontin		Produced by osteoblasts and binds CD44 on HSC to cause quiescence.	[43,44]
Stem cell factor (SCF) (soluble and membrane-bound c-kit)	Osteoblasts, endothelial cells, nestin+ MSC	HSC maintenance.	[53,285,286]
Thrombopoietin (TPO)	Osteoblasts	Stem cell quiescence and maintenance.	[287,288]
Angiopoietin-1	Osteoblasts and nestin+ MSC	Stem cell quiescence and maintenance.	[73]
Notch ligands	Endothelial cells, osteoblastic cells	Binds Notch on HSC to cause quiescence. Increased expression post-PTH causes increased HSC numbers. Not essential for HSC maintenance.	[33,289,290]
Prostaglandins	PGE2	HSC homing survival and proliferation. NSAIDs have been used to augment mobilization.	[291]
Cell surface receptors			
CD44		Binds hyaluronan and osteopontin.	[107–109]
CD62E and L (E- and L-selectin)		Expressed on endothelium (E) and leukocytes (L), essential for endothelial transmigration.	[101,292]
LFA-1 (CD18/CD11a)/ I-CAM		Integrin critical for IL-8-induced mobilization, mediates endothelial adhesion and transmigration.	[293]
VLA-4 (CD49d) / VCAM-1		Endothelial transmigration.	[101–103,110–112,166]
VLA-5 (CD49e) / VCAM-1			
CXCR4	Expressed on HSPCs, lymphocytes and megakaryocytes.	Binds the chemotactic factor CXCL12 (SDF1). Blocked by plerixafor.	

CAR, CXCL12 adventitial reticular cell; NE, neutrophil elastace; MMP-9, matrix metalloproteinase 9; NSAIDs, nonsteroidal anti-inflammatory drugs; Ig, immunoglobulin.

after deletion [62]. Similar HSPC mobilization was reported following conditional deletion of α_4 integrin in hematopoietic and endothelial cells [63]. In separate competitive transplant studies, $\alpha_4^{-/-}$ BM cells exhibited impaired homing to the BM, increased numbers of circulating CFU-Cs, and a competitive disadvantage in both short-term and long-term hematopoietic reconstitution compared to normal competitors [62–64]. Likewise, genetic ablation of β_1 in both the hematopoietic and non-hematopoietic systems of β_7-deficient mice resulted in an eightfold increase in the number of circulating HSPCs [65]. These data suggest that expression of α_4 integrin on HSPCs plays an important role in their retention within the BM microenvironment.

Endosteal niche

Osteoblasts support the growth of HSCs [66] and regulate their numbers [31,33]. The endosteal niche is defined by proximity to trabecular or cortical bone. One of the initial sites of HSC homing after transplant is near the endosteum [4], and this homing is influenced by radiation effects on the stroma [2,3]. The critical cellular components that create the endosteal niche include osteoblasts, osteocytes, osteoclasts, and nestin$^+$ MSCs [31,33,50,55,67–72]. HSCs interact with N-cadherin$^+$ osteoblasts [31] and these interactions are dependent on Angiopoietin-1/Tie2 tyrosine kinase receptor regulating stem cell quiescence [73] and Jagged 1/Notch signaling to increase HSC numbers [33]. Osteoblasts are critical for maintaining stem cell number and losses of osteoblasts result in decreased numbers of HSCs [31,33,74]. In addition, committed osteoblast cells known as osteocytes, which lie deep within the bone matrix, can affect hematopoiesis. Perturbations in osteocyte function can also alter the niche and affect HSC development through a G-CSF-mediated mechanism [67]. On the other hand, bone-resorbing osteoclasts, are important for stress-induced mobilization of HSCs through matrix metalloproteinase-9- (MMP-9) and CXCR4-dependent mechanisms [72]. Osteoclasts release the protease cathepsin K during inflammation resulting in cleavage and inactivation of SDF-1 and mobilization. Knockout of cathepsin K results in decreased bone marrow cellularity and extramedullary hematopoiesis [75]. Inhibition of osteoclast function through bisphosphonates decreases stem cell numbers and impairs engraftment [70]. Another critical role of osteoclasts is calcium production from bone, which maintains a gradient of high calcium near bone that is sensed by calcium receptors on HSCs [32]. Finally, nestin$^+$ MSCs have been found associated with endothelial sinusoids as mentioned earlier as well as lining bone consistent with their multilineage potential [50]. Mobilization with G-CSF directly alters osteoblast production of CXCL12 [55] and can affect nestin$^+$ MSCs as will be described later under mobilization.

The endosteal niche also includes non-cellular components including extracellular matrix and cytokines (Table 5.1). These extracellular components are important for regulating HSC numbers and proliferation. Osteopontin is a pleotropic glycoprotein produced by osteoblasts that negatively regulates HSC pool size [43,44]. It is through direct contact with receptors on the surface of HSCs such as CD44 and α_4 integrin that osteopontin mediates its effects. In addition, calcium ions that are concentrated near the bone surface can regulate HSC homing and engraftment through direct interaction with calcium receptors expressed on the surface of HSCs [32]. The loss of the calcium receptors on HSCs results in a decrease in bone marrow derived HSCs and affects homing to the endosteal niche. Other non-cellular factors regulating trafficking, proliferation, and survival include cytokines and proteases secreted by osteoblasts, endothelial cells, and stromal cells. A prototypical cytokine critical for stem cell maintenance and engraftment is stem cell factor (SCF) also known as Kit ligand or steel factor. Soluble SCF is secreted mostly by endothelial cells and specialized MSCs [53]. SCF exists as a soluble form (sKitL) and a membrane-bound form (mKitL). mKitL acts to bind HSCs and maintain quiescence and has antiapoptotic effects. SCF levels within the marrow microenvironment can be altered by a number of mechanisms. Cytokines such as G-CSF, IL-8, or vascular endothelial growth factor (VEGF), result in induction of matrix metalloproteinase-9 (MMP-9) which cleaves mKitL and leads to release of stem cells from the osteoblastic niche and increased proliferation [76–78]. Inhibition of SCF binding to HSCs allows engraftment of donor HSCs into previously occupied niches [79]. Other cytokines found in the microenvironment such as thrombopoietin [80], Flt3 ligand, IL-3, and the colony-stimulating factors G-CSF and GM-CSF, play critical roles in stem cell homeostasis, signaling differentiation and self-renewal and, as described later, can be involved in HSPC mobilization.

A unifying theory of niches

In reality the distance between vasculature and osteoblasts is in the order of 10–20 μm [3], this means as few as 1–2 cell diameters apart (recall a red blood cell is typically 6–8 μm). This proximity suggests the possibility for paracrine effects on HSPCs in either location. Coordinated, multidimensional interactions between vasculature, mesenchymal, and endosteal cells is likely to occur in the bone marrow, and future systems biology approaches may help to define the critical interactions between all components of the marrow microenvironment and HSCs. Critical interactions with the niche may allow for novel therapeutic interventions targeted to molecules and pathways.

Insight into the interdependency of the aforementioned niches comes from studies to find the common progenitor of the niche. In mice, the bone marrow niche does not develop without CD45$^-$ Tie2$^-$ CD105$^+$ Thy1.1$^-$ cells forming a cartilaginous matrix and undergoing ossification [81]. An ectopic niche can be generated by transplanting fetal bone isolated CD45$^-$ Tie2$^-$ CD105$^+$ Thy1.1$^-$ cells or via adult CD146$^+$ MSCs [56,81]. These ectopic niches require ossification and vascularization prior to engraftment of HSCs supporting the interdependency of niches.

One cell type common to both niches as mentioned earlier is the nestin$^+$ MSC. Nestin$^+$ MSCs have been reported to closely associate with sympathetic neurons and are located primarily perivascular and to a lesser extent near the endosteum. The sympathetic nervous system produces adrenergic signals that affect nestin$^+$ perivascular mesenchymal stem cells and regulates mobilization of HSCs [50–52]. The nestin$^+$ MSCs produce CXCL12 and their proliferation is inhibited by G-CSF administration. Expression of genes involved in HSC retention in the bone marrow such as CXCL12, SCF, and angiopoietin-1 are downregulated by G-CSF and adrenergic stimulation. Mobilization of HSPCs can occur through adrenergic signaling to nestin$^+$ MSCs that directly interact with HSCs. A further level of regulation bridging niches comes from CD169$^+$ bone marrow macrophages and monocytes which negatively regulate nestin$^+$ MSCs [82], express the G-CSF receptor and are important intermediates for G-CSF-induced mobilization [83]. One final cell type that deserves mention as an intermediary cell is the CXCL12-abundant reticular cell, which is the highest producer of CXCL12 and may be directly affected by stem cell mobilizing agents [84], and maintains early B-lymphoid progenitors [85].

As humans age the bone marrow is increasingly replaced by fat. The fat-producing adipocytes are not just fillers of space in the

Table 5.2 Mobilization agents and their mechanism

Agent	Mechanism	Kinetics	Reference
BIO5192	VLA-4 antagonist	<30 min	[264]
AMD3100/ Plerixafor	Bicyclam small molecule antagonist of CXCR4	Peak in 3–4 h	[207,221,294]
G-CSF	Downregulation of CXCL12 in BM osteoblasts, and minor release of proteases	Peak in 4–5 days	[68,153]
GM-CSF	Exact mechanism unknown but, alterations in adhesion molecules on HSCs may play a role	Peak in 5–7 days	[295–297]
Groβ/CXCL2	CXCR2 agonist and induces protease release, i.e., MMP-9	<15 min	[298,299]
IL-8/CXCL8	CXCR1 and CXCR2 agonist and results in release of proteases, i.e., MMP-9	<15 min	[282,283]
SCF	Binds and stimulates c-Kit	Mobilization in days to weeks, synergizes with G-CSF	[168–172]

marrow but have been shown to be another cellular mediator of HSPCs. As numbers of adipocytes increase in bone marrow the hematopoietic activity of the marrow decreases [86]. This effect may be partly related to adipocyte suppression of the endosteal niche by decreasing trabecular bone formation [86].

Non-cellular factors, acting in a humoral, paracrine or metabolic fashion, that play a role in retaining HSPCs in the bone marrow include oxygen, calcium ions, CXCL12, complement factors, and bioactive lipids: sphyngosine-1 phosphate and ceramide-1-phosphate. An oxygen gradient exists between the vasculature and the endosteum. Hypoxia is known to play an important role in HSC quiescence and long-term repopulating potential [87,88] and affects CXCL12 expression by stroma [89]. As oxygen tension increases HSCs are stimulated to cycle and expression of factors associated with quiescence decrease such as calcium receptor, N-cadherin and Notch-1. The key sensor of oxygen tension in the bone marrow is hypoxia-inducible factor-1 alpha (HIF1α). HIF1α directly regulates CXCL12 expression in endothelial cells [89]. Stabilization of HIF1α is a strategy that can improve stem cell mobilization [90]. The CXCL12/CXCR4 axis, complement, and S1P will be discussed extensively in more detail later and are summarized in Table 5.2.

In conclusion, a number of cellular and non-cellular factors acting through direct structural or physical ways or indirectly through humoral, paracrine, metabolic, or neurologic ways regulate the bone marrow environment and are targets for inducing HSPC mobilization, which will be discussed later.

HSC homing and engraftment

Homing to the bone marrow and mobilization can be thought of as interrelated processes sharing common pathways. The niche described earlier allows for entry and egress of HSPCs under homeostatic, supraphysiologic (such as mobilization), and pathologic (such as chronic inflammation) conditions. An understanding of what attracts HSPCs to the bone marrow has led to improvements in mobilizing HSPCs from the bone marrow.

The best-studied component of homing and mobilization is the CXCR4/CXCL12 axis. The chemokine CXCL12 (also known as stromal derived factor-1 (SDF-1)) is the ligand for the chemokine receptor CXCR4, which is expressed on stem cells, T cells, B cells, and megakaryocytes. A number of bone marrow stromal cells express CXCL12, which is essential for HSC homing to the bone marrow [85,91]. Alterations in the gradient of CXCL12 that exists in the marrow, or, direct pharmacologic blockade of its receptor, CXCR4, results in HSPC mobilization (see mobilization section).

Targeted disruption of either CXCL12 or CXCR4 is embryonically lethal in mice, resulting in very similar developmental defects, including the failure of HSPC migration from the fetal liver to the BM, defects in lymphoid and myeloid hematopoiesis, and cerebellar dysgenesis [92–95]. Mice transplanted with CXCR4-deficient progenitor cells exhibit increased numbers of circulating immature granulocytes, CFU-Cs (30-fold increase), and $\text{Lin}^{-}\text{Sca-1}^{+}\text{c-kit}^{+}$ (LSK) cells compared to animals injected with wild-type cells [55,91,96,97]. In separate competitive transplant studies, $\text{CXCR4}^{-/-}$ BM cells exhibited impaired engrafting capacity, with fivefold more $\text{CXCR4}^{-/-}$ BM cells being required to achieve engraftment levels similar to wild-type BM cells [98]. Consistent with the phenotype of mice reconstituted with $\text{CXCR4}^{-/-}$ cells, conditional ablation of CXCR4 in mice using the Cre-loxP recombination approach caused a severe defect in B-cell lymphopoiesis and a significant increase in the number of peripheral LSK stem cells [84,98]. In both of these conditional ablation studies, primitive hematopoietic stem cells (LSK cells) were retained in the bone marrow and became hyperproliferative following CXCR4 inactivation [84,98]. This observation demonstrates that HSPCs can be retained in the BM microenvironment through a CXCR4-independent mechanism. Furthermore, the hyperproliferative state of LSK cells following CXCR4 ablation indicates that CXCR4 acts intrinsically in primitive HSPCs to enforce quiescence [98].

The homing of stem cells to the bone marrow is a multistep process of rolling, adhesion, diapedesis, and transmigration [99–102]. Factors involved in the normal homing of HSCs after transplant include: selectins (E- and L-selectin) and integrins (VLA-4) for endothelial rolling and CXCL12 for transmigration [101–103]. These factors play dual roles in homing and mobilization. A gradient of CXCL12 causes chemotaxis of stem cells and homing and engraftment of HSCs in mice and is dependent on expression of the chemokine receptor CXCR4 [104,105]. Once in the bone marrow, exiting circulation (rolling and diapedesis) is mediated by CXCL12 signaling resulting in increased expression of VLA-4, VLA-5, and LFA-1 on stem cells [102,103,106]. Tropism of bone marrow sinusoidal endothelium specifically facilitating HSC diapedesis has been reported [106]. An additional mechanism facilitating HSC homing based on CXCL12 is a conformational change in the HSPC and enrichment of CD44 at the tip of cellular projections [107]. This selective expression of the glycoprotein receptor for hyaluronic acid (HA) can act as a ligand for E- and L-selectins [108,109]. HA is expressed on bone marrow endosteum and endothelium and may act as one of the initial tethers for HSCs expressing CD44 as they traffick to the bone marrow [107]. Finally, blockade of integrins (VLA-4 ($\alpha_4\beta_1$ integrin)), VCAM-1, or CD44 prevents homing of HSCs [110–112]. Studies using blocking antibodies have demonstrated that VLA-4 and VCAM-1 play an important role during the migration of HSPCs to, and retention in the bone marrow [110,111]. One final alteration that affects homing is damage to the microenvironment through irradiation, which results in increased CXCL12 mRNA levels within bone marrow-depleted trabecular bone areas. This results in preferential trafficking of HSCs to trabecular bone areas [2]. These areas are likely to be critical anatomic areas of HSC homing and expansion after transplant.

HSPC mobilization via ablation of niche cell subsets

Studies have shown that ablation of different niche components results in HSPC mobilization. Conditional ablation of endosteal osteoblasts in Col2.3Δ-TK transgenic mice by ganciclovir administration [69,113] or in osteocalcin-diphtheria toxin receptor (Oc-DTR) transgenic mice by diphtheria toxin treatment [57] resulted in mobilization of HSPCs and the depletion of a wide range of hematopoietic cells from the bone marrow, including HSPCs. Similarly, depletion of nestin$^+$ MSCs by inducible expression of diphtheria toxin receptor in nestin-expressing cells caused the mobilization of approximately 50% of HSPCs to the spleen [50]. Finally, two recent studies showed that ablation of bone marrow mononuclear phagocytes results in HSPC mobilization. First, Chow et al. [82] used four different models to deplete different types of monocyte/macrophage cell populations from mouse bone marrow. These models included clodronate-containing liposomes, an inducible c-fms promoter driven Fas-mediated cell depletion model (macrophage Fas-induced apoptosis (MAFIA)), and transgenic animals with DTR driven by CD11b or CD169. In each model, monocyte and/or macrophage ablation was associated with HSPC mobilization. Winkler et al. [114] observed similar HSPC mobilization following monocyte/macrophage depletion with the clodronate liposome or MAFIA mouse models. However, the mechanism(s) whereby monocyte/macrophage depletion induces HSPC mobilization is controversial. Chow et al. [82] observed decreased expression of CXCL12, SCF, Ang-1, and VCAM-1 mRNA in nestin$^+$ MSCs, but not osteoblasts, following phagocyte depletion. Moreover, phagocyte depletion did not reduce the numbers of bone marrow osteoblasts or nestin$^+$ MSCs. In contrast, Winkler et al. [114] observed reduced osteoblast numbers and decreased osteoblastic expression of CXCL12, Ang-1, and SCF following phagocyte depletion. Although more work is required to better define the mechanism(s) involved in phagocyte depletion-mediated HSPC mobilization, the data strongly suggest that bone marrow monocytes/macrophages produce trophic factors that are required for nestin$^+$ MSC and osteoblast maintenance and HSPC retention.

Stem cell mobilization

Clinically available mobilizing agents

Hematopoietic stem cell mobilization has been shown to be induced clinically or experimentally in animal models by a wide variety of molecules: cytokines such as G-CSF, granulocyte–macrophage colony-stimulating factor (GM-CSF), stem cell factor (SCF), and Flt3 ligand; chemokines like interleukin (IL)-8, Mip-1α, Groβ, or SDF-1; small molecule inhibitors and CXCR4/CXCL12 and VLA-4/VCAM-1 axes; and the chemotherapeutic agents cyclophosphamide (Cy) and paclitaxel. These molecules differ in their kinetics of mobilization, the type of cells mobilized, and efficiency. To date, only G-CSF (filgrastim, Neupogen®, Amgen, Thousand Oaks, CA, USA), GM-CSF (sargramostim, Leukine®, Bayer Healthcare Pharmaceuticals, Seattle, WA, USA), SCF (ancestim, Stemgen®, Amgen, Thousand Oaks, CA, USA available in Canada and New Zealand only) and plerixafor (Mozobil, AMD3100, Genzyme Corporation, Cambridge, MA, USA) are approved clinically for use in stem cell mobilization [115]. In general, these mobilizing agents function by disrupting HSPC adhesion to stromal cells and/or disrupting the chemotactic gradients responsible for maintaining stem cells in the bone marrow microenvironment. In the following sections we will discuss the mechanism of action of these mobilizing agents in greater detail.

Mobilization of HSPCs with G-CSF

G-CSF is a myeloid growth factor produced by monocytes, macrophages, fibroblasts, and endothelial cells and is the most commonly used mobilizing agent for HCT (clinical studies have been reviewed elsewhere in this text). Although the mechanisms by which G-CSF induces HSPC mobilization are not fully understood, there is evidence that at least three general mechanisms of mobilization are involved: activation of proteases, attenuation of adhesion molecule function, and disruption of CXCL12/CXCR4 signaling (reviewed in [116,117]).

CXCR4/CXCL12 axis and G-CSF mobilization

CXCR4/CXCL12-axis dysfunction in WHIM syndrome

Additional genetic evidence for the critical role that CXCR4 plays in leukocyte trafficking has been obtained from patients with the genetic immunodeficiency syndrome WHIM (warts, hypogammaglobulinemia, infections, myelokathexis). Although the majority of patients with WHIM syndrome have autosomal dominant C-terminal truncation mutations of 10-19 amino acids in CXCR4 [118–120], a single missense, non-truncating, gain-of-function mutation in the C-terminal tail of CXCR4 (E343K) was recently associated with the disease [121]. These mutations in the intracellular C-terminal tail of CXCR4 prevent internalization and desensitization in response to CXCL12 [118,119,121,122]. Some functional consequences of dysregulated CXCR4 signaling in WHIM syndrome is the failure of mature neutrophils to exit the bone marrow (myelokathexis) resulting in peripheral neutropenia, defects in B-cell development, reduced immune function, and other similarities to murine CXCL12 and CXCR4 knockout mice [121,123,124]. Interestingly, administration of the CXCR4 antagonist plerixafor appears to be a safe and effective strategy to correct this leukopenia, the major cellular mechanism of immunodeficiency in the disease [125].

Multiple lines of evidence suggest that G-CSF mediates mobilization through suppression of the CXCL12/CXCR4 axis. Although G-CSF administration results in an early, transient increase in CXCL12 production [126] prolonged treatment induces a progressive decrease in CXCL12 mRNA [68] in the BM with a concurrent drop in CXCL12 protein [68,126,127]. In fact, BM CXCL12 levels are inversely correlated with the magnitude of HSPC mobilization, with low levels of CXCL12 mRNA or protein correlating with maximal mobilization [51,68,126]. The expression of CXCR4 on HSPCs is dynamic and G-CSF treatment is associated with decreased cell-surface expression of CXCR4 on mobilized cells [127,128]. One potential mechanism for the decreased CXCL12 expression following G-CSF treatment, as discussed earlier, is that G-CSF treatment is associated with a marked suppression of osteoblasts [51,55,68,129], which are an important source of CXCL12 in the bone marrow. Importantly, no G-CSF-mediated mobilization is observed following neutralization of CXCR4 with monoclonal antibodies [126] or in CXCR4$^{-/-}$ BM chimeras [55]. These data indicate that disruption of the CXCR4/CXCL12 axis plays a dominant role in HSPC mobilization by G-CSF.

Degradation of CXCL12 may also be involved in the mobilization of HSPCs by G-CSF. Studies have shown that CXCL12 is rapidly processed in vivo at both the N- and C-termini [130]. The enzymes responsible for N-terminal truncation of CXCL12 include CD26 (DPP4, dipeptidylpeptidase IV), metalloproteases, leukocyte elastase and cathepsin G, and C-terminal truncation proceeds via carboxypetidase M and cathepsins B, K, L, and X [131–137]. These truncations at both the N- and the C-termini of CXCL12 have been shown to modify its affinity for CXCR4 and its biologic activity in in vitro assays. Interestingly, G-CSF-induced HSPC mobilization is defective in CD26-deficient mice or in wild-type mice treated with a specific CD26 inhibitor [138,139]. Furthermore, expression of CD26 on human CD34$^+$CD38$^-$ HSPCs is enhanced upon treatment with G-CSF, resulting in a decreased chemotactic response to CXCL12 [140]. These data suggest that CD26 is an essential component of G-CSF-induced HSPC mobilization.

G-CSF-mediated mobilization via activation of proteases

G-CSF is known to induce neutrophil proliferation, activation, and degranulation [141,142], resulting in the release of serine and metallo-proteases such as MMP-9, cathepsin G, and neutrophil elastase [143]. HSPCs themselves contribute to the development of a proteolytic microenvironment by secreting MMP-2 and MMP-9 [144], upregulating metalloproteinase membrane type 1-metalloprotease (MT1-MMP), and suppressing expression of the MT1-MMP inhibitor reversion-inducing cysteine-rich protein with Kazal motifs (RECK) after G-CSF treatment. G-CSF also directly stimulates BM MSCs to induce MMP-2 synthesis and its activation at the membrane. Since the accumulation of these proteases in the BM microenvironment and on HSPCs mirrors the kinetics of HSPC mobilization, it was suggested that these proteases promote mobilization by cleaving a variety of HSPC supporting molecules including SCF [76], VCAM-1 [145], CXCL12 [133,134,138,146,147], CXCR4 [127], and CD44 [148]. Moreover, expression of serpin1 and serpin3, naturally occurring inhibitors of these proteases, is markedly reduced after G-CSF treatment, thus making the marrow environment even more proteolytic [149,150]. Although the role these serpins play in G-CSF-mediated mobilization has not been evaluated, administration of serpin1a inhibits IL-8-mediated mobilization [150].

The role of proteases in G-CSF-mediated mobilization is controversial. Depletion of neutrophils in mice using an antibody against GR1 (Ly6C/G) results in reduced mobilization by G-CSF [151], IL-8 [77], and Groβ [151]. Furthermore, anti-MMP-9 antibodies inhibit mobilization by IL-8 or Groβ by 90% and mobilization by G-CSF by 40% in mice [78,151]. In agreement with these data, G-CSF-induced mobilization of HSPCs is impaired in MMP-9 [76] and MT1-MMP [148]-deficient mice and augmented by co-administration of poly-(1,6)-β-d-glucopyranosyl-(1,3)-β-d-glucopyranose (PGG), a compound that promotes MMP-9 release [152]. Similarly, inhibitors to MMP-9 or neutrophil elastase reduce G-CSF-mediated mobilization [126,151]. However, the role proteases play in G-CSF-mediated HSPC mobilization was brought into question when multiple groups were unable to reproduce the mobilization defect in MMP-9-deficient mice [153–156]. Furthermore, mice deficient in combinations of neutrophil elastase, cathepsin G, and MMP-9 display normal G-CSF-induced HSC mobilization even in the presence of a broad-spectrum metalloproteinase inhibitor [153]. Though the reason for the discrepancy between these studies is unknown, it is possible that the different genetic strains of mice used may account for the observed differences. Overall, the data suggest that while not absolutely required, proteases may augment HSC mobilization by G-CSF.

Disruption of VCAM-1/VLA-4 axis in G-CSF mobilization

The expression of VLA-4 on human CD34$^+$ HSPCs is known to be increased by IL-3 and SCF [157] and decreased by G-CSF [157–162]. Furthermore, G-CSF has been reported to induce proteases that cleave VCAM-1 [143,145]. In comparison to BM CD34$^+$ cells at steady-state, G-CSF-mobilized CD34$^+$ HSPCs exhibit reduced VLA-4 affinity and avidity for a soluble VCAM-1-Ig fusion protein [162]. Furthermore, Lichterfeld et al. [162] reported that the number of circulating CD34$^+$ cells following G-CSF administration is inversely related to the number of CD34$^+$/VCAM-1-Ig$^+$ cells in the periphery. These data suggest that a low activation state of VLA-4 on CD34$^+$ cells during G-CSF mobilization is associated with a higher number of circulating HSPCs. Therefore, both reduced VLA-4-expression and reduced VLA-4 avidity are associated with mobilization of human CD34$^+$ HSPCs by G-CSF.

c-Kit/SCF axis in G-CSF mobilization

The involvement of the c-kit/SCF axis in G-CSF-mediated mobilization is controversial. In support of a role, it is known that G-CSF promotes the production of proteases that cleave c-kit/SCF [76,146], releasing both as soluble forms. Consistent with this observation, G-CSF-mobilized HSPCs express lower levels of c-kit than BM resident HSPC [163] and serum-soluble c-kit levels exhibit a positive correlation with CD34$^+$ HSPC yield [164]. Furthermore, disruption of the c-kit/SCF axis with soluble c-kit [165] enhances mobilization by G-CSF. However, in conflict with a role in G-CSF-mediated mobilization, W/W^v mice with deficient c-kit signaling exhibit a 50% reduction in HSPC mobilization compared to controls following G-CSF treatment [166,167]. Furthermore, mice deficient in the protease matrix metalloproteinase 9 (MMP-9), which is believed to mediate SCF cleavage, mobilize normally in response to G-CSF in multiple strains of mice [153–155]. Finally, stimulation of c-kit signaling by administration of SCF during G-CSF treatment synergistically increases the number of mobilized HSPCs [168–172]. Despite the efficacy of SCF in enhancing G-CSF mobilization, its use is hindered by the infrequent occurrence of severe anaphylactoid reactions [173]. Although approved for use in Canada and New Zealand, SCF is not currently available in the United States, and it is seldom used in Europe because of the relatively high risk of side effects.

HGF/c-Met axis in G-CSF mobilization

Hepatocyte growth factor (HGF) is a heterodimeric molecule composed of an alpha and beta chain and has been reported to play critical roles in proliferation, migration, invasion, tumor angiogenesis, and lymphangiogenesis [174,175]. The various biologic activities of HGF are mediated via the c-met proto-oncogene receptor, c-Met tyrosine kinase, which is expressed on a variety of cell types and is upregulated by numerous inflammatory molecules [176,177]. Both HSPC and serum levels of HGF are elevated in G-CSF-mobilized donors [178–180] and G-CSF upregulates c-met expression in human CD34$^+$ and mouse HSPCs [179,180]. Direct injection of HGF to mice induces HSPC mobilization [72,180,181] and administration of c-Met neutralizing antibodies or the c-met inhibitor PHA-665752 inhibits G-CSF-mediated mobilization [180]. The main regulator of c-Met transcription, HIFIα, is upregulated by G-CSF treatment [182]. The HCF/c-Met axis may promote mobilization by activating mammalian target of rapamycin (mTOR) and increasing reactive oxygen species (ROS) production in HSPCs as well as stimulating the secretion of MMP-9 and surface expression of MT1-MMP on BM MNCs [179,180]. In agreement with these data, inhibition of mTOR with rapamycin [148,180] or ROS with *N*-acetyl-*L*-cysteine [180] reduced G-CSF-mediated mobilization. Furthermore, knockout of the thioredoxin-interacting protein, a mediator of oxidative stress, results in increased G-CSF-mediated mobilization, further suggesting a role for ROS in HSPC mobilization [183].

EGF/EGFR axis in G-CSF mobilization

Similar to humans, there is a wide variation in the magnitude of HSPC mobilization by different inbred strains of mice in response to G-CSF. Following G-CSF treatment for 5 days, Roberts et al. [167] observed a 10-fold range in the number of circulating progenitor cells between different inbred strains of mice, with the mobilization efficacy roughly aligning in the following order: DBA > 129Sv > BALB/c = SJL > C57Bl/6 = C3H/He. Using a forward genetic approach and a congenic mouse model, Geiger et al. [184] identified linkage between a locus on chromosome 11 and the magnitude of HSPC mobilization by G-CSF. Subsequent gene mapping studies by the same group identified that the gene for epidermal growth factor receptor (EGFR) on chromosome 11 negatively influences HSPC mobilization in response to GCSF [185]. Interestingly, G-CSF-induced HSPC mobilization was enhanced in EGFR signaling-deficient mice or in wild-type mice treated with the EGFR inhibitor erlotinib. Moreover, increased mobilization due to suppression of EGFR activity correlated with reduced activity of cell division control protein-42 (Cdc42), and mice deficient in Cdc42 exhibited enhanced G-CSF-induced mobilization [185]. These data suggest that EGF-EGFR signaling modulates G-CSF-mediated mobilization through modulation of Cdc42 activity.

Cellular mediators of G-CSF mobilization

G-CSF mobilizes HSPCs through a hematopoietic intermediate

G-CSF functions via the G-CSF receptor (G-CSFR), which is a member of the type I cytokine receptor family and expressed on both hematopoietic and non-hematopoietic cells. Mice lacking the G-CSFR (*Csf3r*$^{-/-}$) have reduced numbers of HSPCs in the bone marrow and are unresponsive to G-CSF stimulation [186]. Mobilization of HSPCs by G-CSF requires expression of G-CSFR in hematopoietic cells but not stromal cells [187]. Surprisingly, however, G-CSFR expression in HSPCs is not required for mobilization [187]. This observation suggests that G-CSF induces HSPC mobilization indirectly by acting on a hematopoietic intermediary that generates trans-acting signal(s) that lead to HSPC mobilization.

The specific hematopoietic cell type(s) that mediate HSPC mobilization by G-CSF is still under investigation. The G-CSFR is expressed on a broad range of hematopoietic cells, with high expression levels on mature and immature neutrophils, and intermediate levels on monocytes/macrophages, HSPCs, and a subset of B lymphocytes and NK cells [188]. Recent work by Christopher et al. [83] demonstrated that G-CSF signaling in a CD68$^+$ monocyte/macrophage lineage cell within the bone marrow initiates the HSPC mobilization cascade. In these studies, mice expressing the G-CSFR exclusively on CD68$^+$ monocytic cells (CD68:G-CSFR) restored HSPC to G-CSFR knockout mice, suggesting that activation of the G-CSFR in the monocytic lineage is sufficient to mediate HSPC mobilization. Moreover, G-CSF decreased the absolute numbers of monocytes in the bone marrow of both CD68:G-CSFR and wild-type mice. This observation agrees with the studies discussed earlier by Chow et al. [82] and Winkler et al. [114] showing that monocyte ablation induces HSPC mobilization. Taken together, these studies suggest that monocytic cells produce currently unidentified factors that support the retention of HSPCs in the bone marrow. Furthermore, G-CSF signaling in monocytes suppresses these signals and results in the mobilization of HSPCs.

The exact monocytic cell subset that mediates HSPC mobilization by G-CSF remains unknown. The CD68 transgene used by Christopher et al. [83] directs G-CSFR expression to at least four distinct monocytic cell populations: inflammatory monocytes/macrophages, resident monocytes/macrophages, myeloid dendritic cells, and osteoclasts. Therefore, all four of these cell populations are candidates to mediate HSPC mobilization by G-CSF. The role of osteoclasts in HSPC mobilization is controversial. Kollet et al. [72] found that activation of osteoclasts by administration of RANK ligand was associated with moderate HSPC mobilization and inhibition of osteoclasts, either genetically by deleting PTPε or pharmacologically via calcitonin administration, reduced G-CSF-mediated HSPC mobilization. In contrast, studies by others suggest that osteoclasts inhibit mobilization, as mice treated with pamidronate, an osteoclast-inhibiting bisphosphonate, promote mobilization by G-CSF [114,189]. Furthermore, Winkler et al. [114] reported no alterations in the number of osteoclasts on endosteal surfaces during G-CSF administration. Since ablation of CD169$^+$ macrophages resulted in HSPC mobilization [82], CD169$^+$ macrophages may be the monocytic cell population that mediates HSPC mobilization.

G-CSF suppresses osteoblast lineage cells in the bone marrow

Studies suggest that bone marrow macrophages are anatomically juxtaposed with endosteal osteoblasts and produce factors that support the growth and/or survival of osteoblasts [82,83,114,190]. Following initiation of G-CSF treatment, the number of inflammatory and resident monocytes/macrophages in the bone marrow decreases and reaches a nadir at 3 days of G-CSF treatment, a time point at which osteoblast suppression first becomes evident [83,129]. Indeed, G-CSF administration leads to a selective reduction in the number of mature endosteal and trabecular osteoblasts that is secondary to both an increase in osteoblast apoptosis and inhibition of osteoblast differentiation [51,55,68,114,129,191]. The fact that osteoblasts do not express G-CSFR supports the concept that G-CSF-mediated osteoblast suppression is at least partially mediated by cytokine-induced monocyte/macrophage depletion. Moreover, CXCL12, SCF, and Ang-1 expression in osteoblasts is decreased after G-CSF treatment. Osteoblast suppression is a common feature of cytokine-mediated mobilization, as both Flt3L and SCF induce similar alterations in osteoblast lineage cells [55]. However, the factors generated by bone marrow macrophages that regulate CXCL12 production by bone marrow osteoblasts or stromal cells have not, as yet, been identified.

G-CSF mobilizes HSPCs through the sympathetic nervous system

Since the magnitude of HSPC mobilization by monocyte/macrophage ablation is at least twofold less than that achieved after G-CSF administration [82,114], G-CSF must elicit HSPC mobilization via additional mechanisms. The sympathetic nervous system (SNS) is involved in both the physiologic and enforced egress of HSPC from the BM. Recent studies have shown that the G-CSFR is expressed by peripheral sympathetic neurons [58] as well as neurons in the central nervous system [192]. Although BM stroma do not express the G-CSFR, nestin$^+$ MSCs express both β_2- and β_3-adrenergic receptors and mediate HSPC release from the BM in response to variations in SNS tone [52]. In contrast, osteoblasts express only the β_2 receptor and regulate their own growth, as well as osteoclast activity, in response to SNS signaling [193].

Upon binding its receptor on neurons, G-CSF enhances the sympathetic tone by altering the ability of the sympathetic neurons to take up norepinephrine, thereby increasing catecholamines available to target cells within the BM niche [59]. Katayama et al. [51] showed that G-CSF-mediated HSPC mobilization is reduced in chemically sympathectomized mice, mice treated with the β-blocker propranolol, and in dopamine β-hydoxylase (Dbh; enzyme that converts dopamine into norepinephrine)-deficient mice. Treatment of Dbh knockout mice with the β_2-adrenergic agonist clenbuterol rescued in part the G-CSF mobilization defect in $Dbh^{-/-}$ mice and enhanced mobilization in $Dbh^{+/-}$ controls [51]. This rescue of HSPC mobilization by clenbuterol in $Dbh^{-/-}$ mice, as well as mobilization of wild-type mice with G-CSF, was associated with a flattening of the bone lining osteoblasts [51]. Since osteoblasts do not express G-CSFR [51,194], this atrophic appearance and stiffening of the osteoblasts was likely mediated by β_2-adrenergic signaling. Additional unrelated studies with G-CSF and clenbuterol showed that the vitamin D receptor (VDR) is a critical mediator of neuronal control in the BM niche. Here, Kawamori et al. [191] found that VDR-deficient mice exhibit a severe impairment in G-CSF-mediated osteoblast suppression and stem cell mobilization. Additional mechanistic studies revealed that the suppression of osteoblastic activity is dependent upon VDR upregulation in osteoblasts by SNS-mediated β_2-adrenergic signaling [191]. Taken together, these data suggest that G-CSF-mediated β_2-adrenergic signaling promotes HSPC mobilization through VDR-mediated suppression of bone lining osteoblasts and a decrease in BM CXCL12 levels.

There is also evidence supporting a role for β_3-adrenergic signaling in G-CSF-mediated HSPC mobilization. Treatment of $nestin^+$ MSCs with a β_3-adrenergic receptor agonist induces downregulation of CXCL12, SCF, angiopoietin-1 (Ang-1), and VCAM-1 in a manner similar to G-CSF. Related to this observation, Ferraro et al. [57] found that treatment of diabetic mice with G-CSF resulted in an expected loss of osteoblasts and downregulation of CXCL12 in osteoblasts, but no change in the expression of CXCL12 by $nestin^+$ MSCs, resulting in a dramatic reduction in G-CSF-mediated HSPC mobilization. Additional studies demonstrated that altered G-CSF-induced mobilization in diabetic mice was due to a defect in the activation of the sympathetic nervous system. This defect resulted in a functional blockade of G-CSF-induced downregulation of CXCL12 in $nestin^+$ MSCs and was abrogated through the administration of a β_3-adrenergic receptor antagonist [57]. Finally, Mendez-Ferrer et al. [195] recently examined G-CSF-induced HSPC mobilization in mice deficient in either β_2-adrenergic receptor, β_3-adrenergic receptor, or both β_2- and β_3-adrenergic receptors. Animals lacking the β_3-adrenergic receptor showed no defect in mobilization while mice lacking the β_2-adrenergic receptor showed a trend towards reduced HSPC egress. However, mice lacking both the β_2- and β_3-adrenergic receptors exhibited a significant (50%) reduction in HSPC mobilization, suggesting cooperation between the two signaling pathways following G-CSF administration. β_2- and β_3-adrenergic receptor deficiency does not abrogate HSPC mobilization induced by macrophage depletion with clodronate-containing liposomes. These data further illustrate that multiple pathways are involved in G-CSF-mediated mobilization.

G-CSF-mediated signaling through the sympathetic nervous system also has direct effects on HPSCs. Although β_3-adrenergic receptor expression is restricted to the stromal compartment, β_2-adrenergic receptors are expressed in both the stromal and hematopoietic compartments [196], including mouse [197] and human [198] HSPCs. β_2-adrenergic receptor expression is highest on the more primitive human $CD34^+CD38^-$ cell subset and is upregulated on all human $CD34^+$ HSPCs following G-CSF administration [198]. This increase in β_2-adrenergic receptor expression is associated with an increased response to neurotransmitters, leading to enhanced proliferation and motility of human $CD34^+$ progenitors, repopulation of the BM of immunodeficient mice and their egress to the circulation [198]. Since norephinephrine treatment of mice has also been shown to increase CXCR4 expression [199], adrenergic signaling may directly affect the CXCR4/CXCL12 axis in the BM niche.

In addition to contributing to HSPC mobilization following G-CSF administration, the sympathetic nervous system also regulates physiologic HSPC trafficking via a circadian rhythm. Studies by Frenette and colleagues demonstrated that under homeostasis, HSPC egress from the BM is orchestrated by rhythmic β_3-adrenergic signals delivered by the sympathetic nervous system that regulate norephinephrine release, CXCL12 production by stromal cells, and CXCR4 expression on HSPCs [50,52,200–202]. The lowest CXCL12 expression levels in the murine BM coincide with the peak of circulating HSPCs approximately 5 hours after the onset of light and the highest BM CXCL12 levels, which occur 8 hours later (1 hour after removal of light), match the lowest numbers of peripheral HSPCs. This sympathetic nerve regulation of HSPC egress is reportedly mediated indirectly by $nestin^+$ MSCs, which express less CXCL12, as well as SCF, Ang-1, and VCAM-1, following stimulation with a β_3-adrenergic receptor agonist [50]. Interestingly, the pattern of circadian HSPC mobilization in humans is opposite to that observed in mice, with maximal egress of human HSPCs occurring in the evening [203].

Mobilization by both G-CSF and AMD3100 is affected by the circadian oscillations of HSPC trafficking. In mice, significantly more leukocytes, CFU-C and LSK cells are recovered after G-CSF or AMD3100 administration when blood collection occurs at the peak circadian time [203,204]. This can be quantitated as a doubling of CFU-C and LSK in mice at steady state and a 1.5 to twofold increase in response to G-CSF or AMD3100, respectively. In healthy humans the difference in circulating CD34/mL blood can be as great as threefold (range 1.05–3) when examined at peak circadian time (evening) compared to morning [203]. Likewise, greater numbers of human $CD34^+$ HSPCs are collected after G-CSF treatment when apheresis procedures are started in the afternoon (closer to peak circadian time) compared to the morning [203]. These findings suggest that simple adjustments in timing of stem cell harvest may result in higher yields of $CD34^+$ HSPCs.

HSPC mobilization via pharmacologic disruption of the CXCR4/CXCL12 axis

The data obtained from CXCR4-deficient mice and WHIM patients support a model in which CXCL12 signaling through CXCR4 provides a key retention signal for HSPCs in the bone marrow. Furthermore, multiple preclinical and clinical studies have demonstrated that pharmacologic disruption of the CXCL12/CXCR4 axis using various CXCR4 modulators, including small molecule peptide and non-peptide antagonists, neutralizing antibodies to CXCR4, and modified agonists and antagonists for CXCL12 induce HSPC mobilization in a target-dependent manner [205,206]. In the following sections we will discuss some of these different types of CXCR inhibitors in greater detail.

Mobilization of HSPCs with the CXCR4 antagonist plerixafor

The observation that a single injection of plerixafor can synergize with multiple injections of G-CSF indicates that the mechanisms involved in G-CSF and plerixafor HSPC mobilization are not completely overlapping [207–209]. Given that many of the mechanisms of G-CSF-induced HSPC mobilization discussed earlier involve disruption of the CXCR4/CXCL12 axis, the synergism between G-CSF and plerixafor is surprising. One potential explanation is that pharmacologic inhibition of CXCR4 by plerixafor more effectively inhibits the CXCR4/CXCL12 axis and results in increased HSPC mobilization in vivo. In support of this explanation, Chow et al. [82] recently showed that mimicking one effect of G-CSF administration, in vivo phagocyte depletion, prior to plerixafor treatment, induced a twofold increase in the magnitude of HSPC mobilization by the drug. Furthermore, plerixafor mobilizes HSPCs expressing higher levels of CXCR4 both before and during G-CSF administration [210–214]. An alternative untested explanation is that G-CSF and plerixafor mobilize HSPCs from different niches within the bone marrow environment. In this regard, putative HSPCs have been found near the endosteum lined by osteoblasts (endosteal niche) and in association with sinusoidal endothelium (perivascular niche) [30,215].

Plerixafor and G-CSF mobilize phenotypically different CD34$^+$ cell subsets

HSPCs mobilized with plerixafor are intrinsically different compared to their counterparts mobilized with G-CSF [216]. For example, HSPCs mobilized with plerixafor contain a higher proportion of CD34$^+$ cells in G_1 phase of the cell cycle and a higher proportion of the more primitive CD34$^+$CD38$^-$ subset than G-CSF [211,212,217]. HSPCs mobilized with plerixafor and G-CSF exhibit increased surface expression of CXCR4 and VLA-4 (CD49d) which likely contribute to enhanced engraftment [210–212,218]. Furthermore, the gene expression profiles differ between CD34$^+$ HSPCs mobilized by plerixafor plus G-CSF and those mobilized by G-CSF alone, with plerixafor plus G-CSF associated with decreased expression of proapoptotic genes and increased expression of genes for CXCR4, cell cycle promotion, cell adhesion, cell motility, oxygen transport, DNA repair, and antiapoptosis [210]. Additional global gene and microRNA expression analysis on rhesus macaque CD34$^+$ HSPCs revealed that G-CSF mobilizes a greater proportion of HSPCs that are committed toward myeloid differentiation, whereas those mobilized by plerixafor are more likely to be committed toward B, T, and mast cells [219]. Taken together, these data clearly indicate that the composition of G-CSF and plerixafor-mobilized grafts are significantly different. What impact these different types of grafts have on the engraftment and function of HSPCs after HCT remains largely unknown.

Alternative agents to disrupt the CXCR4/CXCL12 axis

Despite the efficacy of current mobilization regimens, there is still a significant portion of patients who fail to mobilize an adequate number of HSPCs to proceed to transplantation [220]. For example, nearly one third of healthy donors mobilized with plerixafor alone require more than one apheresis to obtain the minimal number of CD34$^+$ HSPCs ($\geq 2.0 \times 10^6$ CD34$^+$ cells/kg) necessary for transplantation [221]. In addition, CXCR4 inhibitors may function as therapeutic agents in cancer therapy as antitumor, antimetastatic and/or chemosensitization agents [206,222–225], as well as in inflammation and tissue repair [226–233]. Therefore, efforts to discover and develop more potent and orally available inhibitors of the CXCR4/CXCL12 axis continue.

Mobilization of HSPCs using CXCR4 antagonists

A number of lead drug candidates that target the CXCR4/CXCL12 axis have recently been developed. These drugs fall into peptide and non-peptide CXCR4 antagonists and CXCR4 antibodies. The prototypical small peptide antagonist, T140 [234], was derived from naturally occurring peptides that had anti-HIV-1 activity. Stable derivatives based on T140 are in clinical development [235,238]. Furthest in development is POL6326 (Polyphor), a selective and reversible CXCR4 inhibitor belonging to a novel drug class based on β-hairpin protein epitope mimetics. A single injection of POL6326 to mice produces an 11–12-fold increase in circulating progenitor cells with a peak at 2–4 hours post-dosing [235]. POL6326 is currently being tested in phase I/II studies for HSPC mobilization in autologous and allogeneic HCT. Non-peptide molecules derived from the prototypical antagonist, plerixafor, have also been described and are in clinical development [206,236,237]. Finally, anti-CXCR4 antibodies [238,239] have been developed but fail to mobilize sufficient CD34$^+$ HSPCs but are in development to alter tumor stromal interactions [240].

Mobilization of HSPCs using CXCR4 agonists

CXCR4 pepducins

Pepducins are highly stable synthetic lipopeptides that are composed of the amino acid sequence of an intracellular loop of the target G-protein coupled receptor coupled to a lipid tether. The pepducins PZ-218, PX-305, and ATI-2341 (Anchor Therapeutics, Cambridge, MA, USA) are based on the first intracellular loop of CXCR4, while PZ-210 targets the third intracellular loop [241–243]. Like CXCL12, these pepducins activate CXCR4-dependent signaling pathways, induce receptor internalization, and promote both in vitro and in vivo chemotaxis. Interestingly, ATI-2341 also acts as a functional antagonist to mobilize HSPCs from the BM niche in both mice and non-human primates [242].

Small peptide analogs of SDF mobilize HSPCs

A large number of CXCL12 small peptide analogs have been designed as CXCR4 agonists and antagonists [244]. In general, CXCR4 agonists induce HSPC mobilization by downregulating cell surface expression of CXCR4 and/or collapsing the CXCL12 gradient between the BM niche and peripheral circulation. Studies have shown that disruption of the CXCL12 gradient between the bone marrow and the peripheral blood by the administration of sulfated polysaccharides [245] or adenovirus expressing CXCL12 [246] results in an increase in circulating CXCL12 and HSPC mobilization. Interestingly, plerixafor-mediated HSPC mobilization was recently shown to be associated with increased CXCL12 plasma levels and could be inhibited by anti-CXCL12 antibodies [247]. This observation suggests that in addition to acting as a CXCR4 antagonist, plerixafor may also promote HSPC mobilization by reversing the CXCL12 gradient between the BM niche and peripheral circulation. CTCE-0021 and CTCE-0214 are small cyclized CXCR4 agonist peptides that were developed by linking amino acids 1–14 and 55–67 of CXCL12 with four glycine residues [248,249]. CTCE-0214 differs from CTCE-0021 by the substitution of two amino acids to improve plasma stability. Both analogs rapidly mobilize HSPCs in mice through CXCR4 receptor desensitization and/or downregulation of surface CXCR4 expression [248,249].

NOX-A12 (NOXXON Pharma, Berlin, Germany) is an L-enantiomeric RNA oligonucleotide (Spiegelmer) that binds and neutralizes

CXCL12 [250]. Spiegelmers are mirror-image nuclease-resistant ribonucleotides that bind and inhibit target molecules in a manner conceptually similar to antibodies [251,252]. Preclinical studies in the mouse demonstrated that the 45 nucleotide long NOX-A12 increases the plasma level of CXCL12. Results from a clinical trial comparing the mobilization of HSPCs by NOX-A12 alone with that obtained in combination with G-CSF have not been reported.

HSPC mobilizing agents that target VLA-4

Mobilization of HSPCs by anti-α_4 integrin antibodies

Separate studies have shown that treatment of mice, primates, and humans with antibodies to VLA-4 increases the number of circulating HSPCs. Blockade of α_4 in mice [110,111,166,253–255] and non-human primates [253,255,256] induces rapid (<8 hours) and prolonged (>10 days) mobilization of HSPCs with long-term repopulating ability. Interestingly, a functional kit receptor is required for HSPC mobilization by an anti-α_4 integrin antibody [166] and either additive or synergistic HSPC mobilization was observed when the antibody was combined with G-CSF [255,256], plerixafor [253], SCF [255] and/or Flt3-ligand [255]. This enhanced mobilization of HSPCs following combination therapy is likely mediated by the disruption of the CXCR4/CXCL12 axis [55,254].

Elevated circulating levels of HSPCs are also observed following administration of an anti-VLA-4 antibody to patients with multiple sclerosis (MS). Natalizumab (Tysabri; Biogen Idec, Cambridge, MA) is a recombinant humanized neutralizing IgG4 monoclonal antibody against the α_4 subunit of VLA-4 that is approved for the treatment of relapsing–remitting multiple sclerosis. Similar to mice and non-human primates following anti-α_4 integrin antibody administration, MS patients treated with natalizumab display a rapid and sustained increase in circulating CD34$^+$ HSPCs [257–259]. The number of circulating CD34$^+$ cells/μL blood increased three- to fivefold during the first 72 hours after treatment and remained elevated at these levels (8–10 CD34$^+$ cells/μL) for at least a month after antibody treatment [257–259]. This number of circulating CD34$^+$ cells in natalizumab-treated MS patients is approximately one sixth and one third of those observed following treatment of healthy donors with G-CSF and plerixafor, respectively [220,221,257–259]. Functional and phenotyping studies on natalizumab-mobilized CD34$^+$ HSPCs indicated that they were primarily quiescent (>90% in G0), failed to transmigrate towards CXCL12, and belonged to the subset of more committed progenitors co-expressing CD38 [257–259].

Although natalizumab has shown clinical efficacy and provided validation for the involvement of the α_4 integrin pathway in autoimmune and inflammatory conditions, its use as a clinical stem cell mobilizing agent in normal healthy donors is not justifiable because of its prolonged immune-modulating effects and association with an increased risk for progressive multifocal leucoencephalopathy.

Clinical trials with small molecule α_4 integrin antagonists

Because natalizumab has shown clinical efficacy in the treatment of MS and Crohn's disease, the development of small molecule VLA-4 antagonists is seen as a promising pharmaceutical approach to a novel class of therapeutic agents [260]. Early studies with the α_4 integrin antagonists suffered from poor bioavailability and a short half-life. More recent trials with orally active new generation antagonists like SB-683699/Firategrast [261] and AJM-300 [262] suggest that these compounds exhibit more favorable pharmacologic properties. However, there is no published information on the effects of these α_4 integrin antagonists on CD34$^+$ HSPC mobilization.

Mobilization of murine HSPCs by small molecule antagonists of VLA-4

BIO5192 is a potent (K_d of <10 pM) and highly selective small molecule inhibitor of both the inactivated and activated forms of human, mouse, and rat $\alpha_4\beta_1$ integrin [263]. Blockade of VLA-4 with a single injection of BIO5192 alone resulted in a 30-fold increase in mobilization of murine CFU-Cs over basal levels [264]. Peripheral blood HSPC levels peaked within 1 hour after BIO5192 administration and returned to baseline by 6 hours. Interestingly, an additive effect on HSPC mobilization was observed when BIO5192 was combined with plerixafor (threefold compared to plerixafor alone), G-CSF (fivefold compared to G-CSF alone), or their combination (17-fold compared to G-CSF alone). These data provided the first evidence for the utility of small molecule inhibitors of VLA-4 either alone or in combination with G-CSF or plerixafor for mobilization of HSPCs.

To gain a better understand of the relationship between CXCR4 and VLA-4 in HSPC mobilization, Christopher et al. [55] generated CXCR4$^{-/-}$ bone marrow chimeras by transplanting CXCR4$^{-/-}$ fetal liver cells into lethally irradiated wild-type recipient mice. Following hematopoietic reconstitution, the CXCR4$^{-/-}$ chimeras were used to investigate the function of different mobilization agents in the absence of CXCR4/CXCL12 signaling. Surprisingly, mobilization by G-CSF or Groβ was completely abrogated in CXCR4$^{-/-}$ bone marrow chimeras [55]. In contrast, HSPC mobilization by BIO5192 was robust; exhibiting a nearly 3000-fold increase in the number of circulating CFU-Cs in the CXCR4$^{-/-}$ chimeras compared to untreated control mice. These observations suggest that α_4 integrin antagonist-induced mobilization of HSPCs occurs independently of CXCR4/CXCL12 signaling and further support the notion that CXCR4 and VLA-4 are the dominant receptors regulating HSPC migration from and retention within the bone marrow.

Other biologic factors involved in mobilization

Parathyroid hormone (PTH) and mobilization

Parathyroid hormone (PTH) is FDA-approved for treatment of osteoporosis in post-menopausal women. PTH increases the number of HSPCs by supporting the growth of osteoblasts [33]. In mice, some PTH regimens increase the number of HSPCs that can be mobilized by G-CSF [265], whereas others directly induce HSPC mobilization [266]. In a phase I trial, PTH combined with G-CSF successfully mobilized sufficient HSCs in 11 of 20 patients who failed a previous mobilization attempt [267].

Complement and mobilization

The complement system is part of the innate immune system and plays a role in mobilization of HSPCs in mice [268–273]. Complement is activated in the setting of inflammation and tissue injury such as occurs with strenuous exercise and hypoxia as well as a result of cytokine administration for stem cell mobilization. Defects in either the classical or alternative pathways of complement activation can abrogate mobilization by G-CSF, the polysaccharide zymosan, and AMD3100 [271]. G-CSF mobilization results in antibody generation that results in classical complement activation [271]. Zymosan activates complement in an antibody-independent manner, through the alternative pathway [271]. Complement C5 and its cleavage byproducts affect secretion of proteolytic enzymes that play a role in releasing HSPCs into the circulation [269,270,274]. AMD3100 directly affects neutrophil release of proteases that cleave and activate C5 [269]. This process is coupled with neutrophil egress from the marrow, which release proteases, exit the marrow first, and pave the way for HSPCs to egress from the marrow [269,274]. Recent evidence also suggests that egress of HSPCs is mediated by a sphygosine-1-phosphate (S1P) gradient that results from complement activation and interaction of the membrane attack complex with red blood cells (RBCs), a major cellular store of S1P [272]. This biology has been exploited in an attempt to improve mobilization by combining G-CSF with antagonism of the complement receptor C3aR, which results in increased mobilization of HSPCs [268]. Finally, complement C3a may directly interact with CXCL12 to enhance chemotaxis of human CD34$^+$ HSCs towards sources of CXCL12 [275].

Hypoxia, HIF-1α and mobilization

The diffusion of oxygen within the marrow is inversely related to the distance from vasculature: regions near bone have lower oxygen tension [182]. This molecular characteristic of the marrow microenvironment is altered during stem cell mobilization. Mobilization with G-CSF or cyclophosphamide results in increased regions of hypoxia in the central marrow [182]. Hypoxia can have pleotropic effects. One is induction of CXCL12 from stromal cells that under steady state maintain a gradient of increased levels near bone [89]. Another is increased HIF1α protein level by prevention of its degradation. HIF1α leads to increased vascular endothelial growth factor A (VEGF-A) production, which, as described previously, can result in protease production, increased vascular permeability and vasodilation [276]. As mentioned in the HGF/c-Met axis section above, the main regulator of c-Met transcription, HIF1α, is upregulated by G-CSF treatment [182]. In addition, the major cellular consumers of oxygen are megakaryocytes, granulocytes/monocytes, and adipocytes [182]. It is predicted that as little as three layers of monocytes/granulocyte progenitors may deplete subjacent oxygen levels. The change in oxygen tension during HSPC mobilization may account for some of the mobilization effects of cytokines such as G-CSF and GM-CSF that result in bone marrow expansion and increased progenitors but the CXCR4 antagonist plerixafor (AMD3100) seems to be less affected. Stabilizing HIF1α with FG-4497 and DMOG results in HSC quiescence and radio-protection [277] as well as synergistic mobilization when combined with G-CSF [90].

Bioactive sphingolipids and mobilization

Bioactive sphingolipids include S1P and ceramide-1-phosphate (C1P). These are ubiquitous cellular components with large stores of S1P found in RBCs. Gradients of S1P and C1P have been reported to be involved in HSC trafficking and mobilization [272,278–281]. Of the five G protein-coupled 7-transmembrane receptors for S1P, the principle receptor expressed on HSCs is S1P$_1$ [281]. S1P$_1$ receptor expression and an S1P gradient between blood and bone marrow is important for mobilization induced by CXCR4 antagonists such as plerixafor (AMD3100) [278]. Mice lacking S1P$_1$ show impaired mobilization to AMD3100 [278]. In addition, the small molecule inhibitor of S1P receptors, FYT-720, inhibits mobilization by AMD3100, while the S1P$_1$ agonist, SEW2871, enhances mobilization by AMD3100. The exact mechanism is not completely known but may be related to the gradient of S1P between peripheral blood and bone marrow that is directly correlated with CXCL12 levels [278,280]. However, plasma levels of S1P in normal human donors mobilized with AMD3100 do not correlate with mobilization of CD34$^+$ cells and levels of S1P actually decrease in plasma of mice treated with G-CSF or AMD3100 [278]. These conflicting data may be potentially reconciled by the fact that mobilization is a local event and general plasma levels of S1P may not reflect changes in the microenvironment. In fact this is a well-described phenomenon that occurs with the chemo-attractant, CXCL12, where plasma levels remain unchanged but bone marrow levels are reduced after G-CSF mobilization [127]. As mentioned earlier the interaction of complement and MAC may release S1P from intracellular stores in RBCs to facilitate mobilization by G-CSF [84] but the direct evidence for S1P/S1P$_1$ axis playing a role in G-CSF-mediated mobilization is not clear with two independent groups demonstrating different results in mice [278,280]. Potential ways that this pathway can be exploited using S1P receptor agonists may be explored in the future.

Conclusion

In conclusion, the biology of stem cell mobilization is a complex interplay between stem cell and the many bone marrow niche components. A number of cellular and non-cellular factors acting through direct structural or physical ways or indirect through humoral, paracrine, metabolic, or neurologic ways regulate the bone marrow environment and are targets for inducing HSPC mobilization. Novel agents targeting critical pathways of HSC retention in the niche are actively being developed for clinical applications.

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CHAPTER 6

Expansion of Human Hematopoietic Stem Cells

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Introduction

Hematopoietic stem cells (HSCs) are useful in a variety of clinical settings and are routinely available in sufficient numbers from a variety of sources, including bone marrow and mobilized peripheral blood. However, there are instances when the number of available HSCs is insufficient for adequate and/or timely engraftment of the host. This is particularly relevant for patients undergoing cord blood transplant (CBT) who receive approximately one log less of HSPC resulting in a significant delay in hematopoietic recovery, or in the case of gene therapy, where the frequency of transduced cells is too low. One potential solution to the problem of low stem cell numbers is ex vivo proliferation of the cells prior to transplantation. Extensive research has been done to define the optimal conditions necessary for ex vivo expansion of HSCs, and various expansion techniques have been developed for this purpose [1,2]. However, while there is still a lack of convincing data that pluripotent stem cells increase in number and retain an ability to engraft and sustain long-term multilineage hematopoiesis in a myeloablated recipient following ex vivo expansion, more recent advances in the field, particularly with respect to molecular mechanisms that control HSC amplification, make this goal more realistic.

To date, substantial effort has focused on the exogenous signals that may be used to favor stem cell self-renewal versus differentiation in order to develop optimal conditions for the ex vivo expansion of HSPCs. Earlier studies evaluated using combinations of cytokines and/or bone marrow stroma and met with limited success (for more details, see reviews [1,3]). The effect of cytokines that support hematopoietic cell survival, proliferation, and differentiation has been extensively studied in vitro, but a significant role for these cytokines in enhancing self-renewal has not been shown. Consequently, a stochastic model of cellular determination has been suggested in which the fate of hematopoietic precursors is not instructed by soluble cytokines, but rather by specific interactions between stem and other cells within a particular microenvironment or "stem cell niche." These interactions are mediated by extrinsic regulators of stem cell fate and are likely to play a key role in maintaining numbers of HSC by regulating their self-renewal and differentiation, now demonstrated by the work of several groups [4–6]. Thus, more recent studies are aimed at identifying intrinsic and extrinsic factors that target the molecular pathways involved in regulation of HSC fate. Initial clinical trials using expanded HSPCs, which also mainly evaluated cytokine-driven expansion systems, did not provide evidence for stem cell expansion, although did provide evidence for the feasibility and safety of ex vivo culturing of stem cells [1]. However, as discussed later, the last decade has witnessed an increase in the number of newer generation clinical trials, primarily with ex vivo expanded CB-derived HSPC that evaluate the use of extrinsic regulators of stem cell fate and co-culture systems utilizing non-hematopoietic components (mesenchymal stromal cells) of the stem cell niche with promising results. This chapter will focus on studies of human HSC expansion for clinical applications, including studies and novel approaches in the preclinical phase of development, a discussion of technologies and assessments required to translate promising in vitro systems to the clinic, and a review of current clinical trials using expanded HSPC (Figure 6.1).

Initial attempts at ex vivo stem cell expansion for clinical application using cytokine-based expansion systems

The first report of a clinical trial using in vitro cultured hematopoietic progenitor cells appeared in 1992. Since then, a number of trials have been carried out with the primary goal of enhancing hematopoietic recovery after high-dose chemotherapy/radiation with the infusion of expanded stem cell grafts derived from bone marrow (BM), mobilized peripheral blood, or CB.

Initial preclinical studies with human BM, mPBSC, or CB-derived HSC were cytokine-based expansion systems. These studies evaluated HSC culture in the presence of various cytokine combinations and were only moderately successful demonstrating significant expansions of committed myeloid progenitor cells but only two- to fivefold net increase in long-term repopulating cells after 4 to 21 days in culture [7–10]. With the expectation that these committed progenitor cells would enhance absolute neutrophil count (ANC) recovery, cytokine-mediated ex vivo expansion methods were translated to the clinic for autologous and allogeneic uses. Studies of expanded mobilized PBPCs were all done in the autologous setting, and included only those patients with sufficient stem cell harvests to allow the use of an aliquot of the apheresis product for expansion. This excluded "poor mobilizers" who clearly stand to benefit from expansion procedures allowing them to undergo treatment with high-dose therapy. However, it is not known whether HSCs derived from such patients are equivalent to those derived from patients with adequate mobilization of HSCs. These trials, primarily in breast cancer patients, assessed enhanced short-term repopulation as a function of decreased time to neutrophil and/or platelet engraftment. With one exception, these studies were unable

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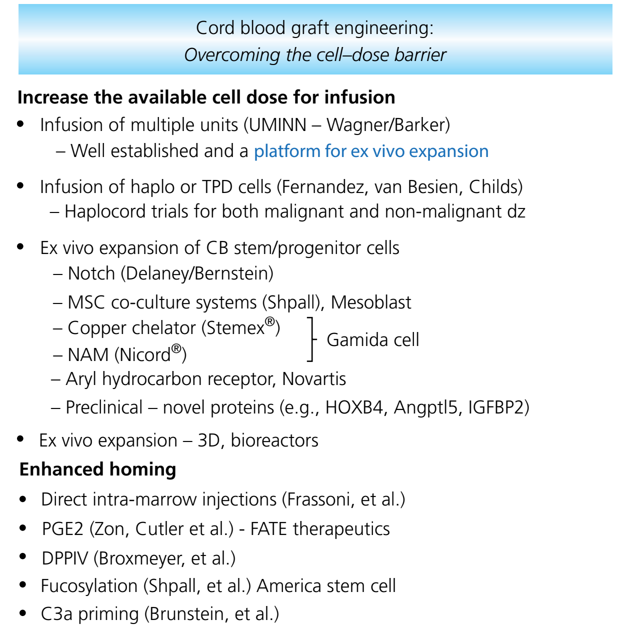

Cord blood graft engineering:
Overcoming the cell–dose barrier

Increase the available cell dose for infusion

- Infusion of multiple units (UMINN – Wagner/Barker)
    - – Well established and a platform for ex vivo expansion
- Infusion of haplo or TPD cells (Fernandez, van Besien, Childs)
    - – Haplocord trials for both malignant and non-malignant dz
- Ex vivo expansion of CB stem/progenitor cells
    - – Notch (Delaney/Bernstein)
    - – MSC co-culture systems (Shpall), Mesoblast
    - – Copper chelator (Stemex®)
    - – NAM (Nicord®)
      Gamida cell
    - – Aryl hydrocarbon receptor, Novartis
    - – Preclinical – novel proteins (e.g., HOXB4, Angptl5, IGFBP2)
- Ex vivo expansion – 3D, bioreactors

Enhanced homing

- Direct intra-marrow injections (Frassoni, et al.)
- PGE2 (Zon, Cutler et al.) - FATE therapeutics
- DPPIV (Broxmeyer, et al.)
- Fucosylation (Shpall, et al.) America stem cell
- C3a priming (Brunstein, et al.)

Figure 6.1 Examples of current approaches under preclinical and clinical development

to address the expansion of long-term repopulating HSCs because patients received non-myeloablative chemotherapy and/or infusion of non-cultured cells.

Overall, although the safety and feasibility of HSC expansion approaches were demonstrated, more rapid engraftment was not observed in patients receiving reduced-intensity preparative regimens. There have, however, been suggestions of ex vivo expansion of short-term repopulating cells in patients who have received myeloablative therapy. For example, in a study by Holyoake et al. [11], the gain of short-term repopulating cells at the expense of long-term repopulating cells was suggested. In that study, mobilized PBPCs cultured ex vivo with SCF, IL-1β, IL-3, IL-6, erythropoietin, and autologous plasma for 8 days were infused in the absence of non-cultured cells into patients who had received myeloablative chemotherapy. None of the four patients showed evidence of long-term hematopoietic recovery, and all required infusions of unmanipulated cryopreserved autologous back-up mobilized PBPCs, after which full hematopoietic recovery was seen. Three patients showed initial neutrophil engraftment that was not sustained, suggesting that short-term repopulating cells may have been generated ex vivo at the expense of long-term repopulating ones, possibly by inducing their differentiation.

A shortening of the neutropenic period following high-dose chemotherapy in 21 patients with breast cancer was also shown in a study in which autologous mobilized PBPCs were cultured with SCF, G-CSF, and megakaryocyte growth and development factor for 10 days. The patients in this study were divided into two cohorts: those who received expanded cells only, and those who received both expanded and unmanipulated cells in tandem [12]. Expanded PBPCs resulted in a more rapid neutrophil engraftment ($p = 0.02$ for cohorts 1 and 2 vs. the historical controls), the best predictor of time to neutrophil engraftment was the total number of cells harvested after expansion, and patients receiving more than 4×10^7 cells/kg engrafted by day 8. Reiffers et al. also reported a decreased time to neutrophil engraftment using the same culture conditions to expand autologous mobilized PBPCs for infusion with unmanipulated cells into patients with multiple myeloma after high-dose conditioning [13].

The use of continuous perfusion methods for the expansion of BM has been evaluated in three trials utilizing the automated perfusion bioreactor system Aastrom Replicell [14–16]. Notably, these are the only trials that used BM (unselected for CD34$^+$ cells) as the source of HSC for ex vivo expansion, thereby perhaps including accessory or stromal cells of unclear significance in expansion cultures, and perhaps enhancing the engraftment ability of this expanded cell population. In these studies, breast cancer patients underwent the same conditioning regimen, and cells derived from autologous BM were placed directly into the automated perfusion bioreactor system for expansion with Flt3L, erythropoietin, and PIXY321 cytokines, as well as fetal bovine serum and horse serum. In all trials, cultures were initiated with small amounts of harvested marrow (75–100 mL).

The trials by Stiff et al. and Engelhardt et al. used expanded cells only, while Pecora et al. also infused mobilized PBPCs in low doses. Engraftment was not enhanced but did occur, suggesting the maintenance of HSCs in the bone marrow cultures, and all trials reported a correlation between the time to neutrophil and platelet engraftment with CD34$^+$Lin$^-$ cell dose/kg. Stiff et al. reported that only 2×10^5 CD34$^+$ cells/kg of the expanded cells were needed to produce optimal platelet engraftment. This cell dose was lower than the number of cells required for predictable platelet engraftment by day 28 in breast cancer patients undergoing PBPC transplantation with unmanipulated PBPCs, in which the minimal number of CD34$^+$ cells required was reported to be $2–5 \times 10^6$/kg [17].

In a trial of CBT in individuals with hematologic malignancies undergoing high-dose preparative regimens, Shpall and colleagues used a 3-cytokine cocktail (stem cell factor (SCF), thrombopoietin (TPO), G-CSF) to expand a portion of single CB units where the non-manipulated fraction was infused on day 0 and the remaining fraction was infused on day 10 of expansion [18]. While modest progenitor cell expansion (fourfold median) was achieved and no toxicities were observed, the time to neutrophil engraftment was not reduced as compared to historic controls [18]. Based on the favorable safety and feasibility data demonstrated in this trial, a double CBT randomized trial is currently underway in which patients are randomized to receive either two non-manipulated CB units or one non-manipulated unit and one unit expanded ex vivo [19]. Although interim analysis shows no significant difference in time to neutrophil engraftment overall, an effect was seen in a subset of patients who received reduced intensity conditioning (median time to neutrophil engraftment of 7 days vs. 14 days). Similarly, Kurtzberg and colleagues cultured a portion of a single CB unit for 12 days using the Aastrom Replicell bioreactor in the presence of cytokines but failed to achieve an improvement in neutrophil or platelet engraftment [20].

The above-mentioned studies demonstrate that cytokine-mediated expansion methods are safe but generate only moderate increases in progenitor cell numbers with at best modest improvements in clinically relevant outcomes such as time to neutrophil recovery. This lack of clinical success has led to a paradigm shift in current approaches to ex vivo expansion to target molecular pathways involved in stem cell self-renewal including those that play fundamental roles in governing cell-fate decisions throughout development. Our work with the Notch signaling system is an example of one such approach.

Pre-clinical approaches for ex vivo HSPC expansion: intrinsic and extrinsic regulators of cell fate

Optimization of cytokine-driven expansion systems has not led to clinically significant HSC expansion, perhaps due to a predominantly permissive, rather than directed, role in determining stem cell fate. The search for extrinsic and intrinsic regulators that act directly on human HSCs in regulating cell fate and self-renewal, homing/retention, and mobilization has suggested a role for regulatory molecules active in early development that are important in HSC maintenance and regulation. More recent studies have focused on the regulation of intrinsic signaling pathways by retrovirus-mediated transduction of HSCs, for example expression of homeobox genes, which have been shown to be involved in hematopoietic cell fate determination. However, culture of cells for clinical application requires the use of extrinsic regulators of cell development including bone morphogenic proteins (BMPs), angiopoietin-like proteins, IGFBP2, pleiotrophin, Sonic hedgehog (Shh), Wnt, and Notch ligands, or a combination of such factors.

For example, addition of Angptl5 and/or IGFBP2 to CD133$^+$ CB cells in culture has resulted in significantly improved in vivo reconstitution in NOD/SCID mice at 2 months post-transplant (0.8% vs. 11.3, 17.3, and 39.5% human engraftment for standard cytokines vs. standard cytokines plus IGFBP2, Angptl5, or both, respectively). Importantly, enhanced secondary transplantation was also observed [21]. Pleiotrophin, which has been found essential for maintenance of murine HSC, has recently been shown to modestly enhance ex vivo human CB HSPC expansion (twofold greater expansion of CD34$^+$38$^-$ cells compared to control) as well as in vivo reconstitution of immunodeficient mice with threefold improved human engraftment at 4 weeks and a sevenfold increase at 8 weeks post-transplant compared to uncultured control [22].

Retrovirally mediated overexpression of Hox transcription factors, in particular HoxB4, has led to extensive ex vivo HSC expansion in vitro (up to 3 logs over control cultures) with murine, human, and non-human primate HSC. Importantly, these transduced cells maintained full in vivo lymphomyeloid repopulating ability as well as the ability for serial transplantation [23–26]. However, clinical application of cells that have been genetically altered by virally mediated gene transfer is not preferred secondary to risk of insertional mutagenesis. Thus, stromal cell layers expressing a HoxB4 fusion protein have been used in the ex vivo expansion of human CB CD34$^+$ cells. The HoxB4 fusion protein is passively taken up by the CD34$^+$ cells, and the expanded cells resulted in a 2.5-fold increase in long-term repopulating cells compared to uncultured control [27]. The self-renewal induced by HoxB4 has resulted in further exploration of a variety of other extrinsic regulators of cell fate, particularly those known to play roles in stem-cell self-renewal, developmental and cell-cycle regulation, such as BMP-4, a member of the TGF-β superfamily, Shh of the Hedgehog family of proteins, and Notch ligands.

Through the use of transgenic mouse model systems, especially with respect to deconstructing the bone marrow microenvironment, and the extensive knowledge that has been gained over the last decade toward better understanding of the HSC niche, significant advances have been achieved toward ex vivo manipulation of HSC and its progeny. A variety of such identified molecules known to play roles in stem cell self-renewal, developmental and cell-cycle regulation, are currently being extensively evaluated, although to date they have not been manipulated for significant ex vivo expansion. In ex vivo expansion of human CB, soluble human BMP-4 has been shown to increase the survival of repopulating blood cells in ex vivo culture, while Sonic hedgehog has been shown to induce a few-fold increase in repopulating cells via mechanisms that are dependent on downstream BMP signals [28–31]. Likewise, the transcription factor SALL4, which has been implicated in the regulation of HSC self-renewal properties, when overexpressed in human hematopoietic stem/progenitor cells resulted in ex vivo expansion of cells capable of engraftment in an immunodeficient mouse model [32].

Wnt proteins, which are involved in the growth and differentiation of a variety of primitive tissues, have also been implicated in the regulation of hematopoiesis, possibly exerting their effects through stromal cells [33]; however, the role of the Wnt pathway in stem cell self-renewal based on gain- and loss-of function studies of Wnt pathway components remains controversial [34,35]. In studies with human CB progenitors, manipulation of the Wnt pathway through overexpression of Wnt5a or with glycogen synthase kinase-3B (GSK-3B) inhibitors did not augment expansion when added ex vivo to cultures but did enhance engraftment when infused in vivo post-transplant in irradiated mice [36–38]. There are also data supporting the presence of an interactive relationship between the various signaling pathways. For example, Notch-1 receptors have been shown to be upregulated in response to Wnt signaling in HSCs [39].

Additional potential targets have been identified that could result in expansion of HSCs ex vivo. For example, PI3-kinase-AKT pathway activation as a result of inactivation of the negative regulator PTEN has been shown to result in short-term HSC expansion but with long-term exhaustion of the stem cell pool [40]. Augmented stem cell self-renewal has been demonstrated in knockout murine models which result in inactivation of the cyclin-dependent kinase inhibitors p16Ink4a and p19Arf [41,42]. Finally, p21$^{-/-}$ mice demonstrate significantly impaired stem cell self-renewal, implicating p21, a downstream target of p53, in stem cell quiescence and a potential target [43]. Thus there is compelling murine data that these molecular pathways play an important role in the regulation of HSC self-renewal and thus may be applicable in human studies. However, the direct applicability to clinical translation is not yet known, especially as manipulation of these pathways, particularly those with tumor-suppressor function, may permit accumulation of mutations during stem cell culture, generating potentially oncogenic cells. Furthermore, as we begin to better understand more about the biologically distinct types of human hematopoietic cells that are responsible for early and long-term sustained hematopoietic recovery and the individual factors that are critical for these distinct subtypes, it is highly likely that the most effective approaches for clinical translation of expanded HSPC will require combinations of individually optimized approaches. Perhaps the most extensively studied and successfully utilized extrinsic regulators have been ligands that activate the Notch pathway, with now extensive studies indicating a role for the Notch family of receptors in mediating cell-fate decisions during hematopoietic development. We and others have shown that the Notch pathway enables hematopoietic stem and progenitor cells to sense the surrounding microenvironment and assume one of several cell fates, including myeloid and B-cell differentiation, self-renewal, or T-cell development. Furthermore, as described in the following section, our group has developed novel and clinically feasible methods utilizing engineered Notch ligands for the ex vivo expansion of human HSPCs for clinical application.

Notch signaling in hematopoiesis

Notch signaling was initially implicated as a critical pathway in hematopoiesis after our group's detection of the human *Notch-1* gene in CD34$^+$ or CD34$^+$Lin$^-$ human hematopoietic precursors in the early 1990s. As in multiple other developmental systems, the decisions that determine the fate of stem cells and their progeny result from intercellular interactions with adjacent cells within a given microenvironment and are modulated by several molecular families, including the *Notch* gene family [44–46]. The *Notch* gene encodes for a transmembrane receptor that is activated by binding of ligand to its extracellular domain. Four highly conserved Notch receptor paralogs (Notch-1–4) and five ligands (Jagged-1 and 2, and Delta-1, 3, and 4) have now been identified in vertebrates (for review, see [47,48]), with all four Notch receptors also detected in hematopoietic cells and the Notch ligands Delta-1 and Jagged-1 detected in human bone marrow stromal cells and human hematopoietic precursors [49–52].

Additional earlier studies by our group further demonstrated that use of a retrovirus to overexpress the intracellular domain of Notch-1 in primary murine HSC led to emergence of enhanced self-renewal and an immortalized pluripotent cytokine-dependent cell line capable of multilineage in vivo repopulation, demonstrating a role for Notch in HSPC self-renewal [53]. In addition, several investigators have now demonstrated that differential activation of Notch target genes results from selective activation of the different Notch receptors as a result of specific ligand interactions leading to different cellular outcomes. For example, activation of Notch-1 by Delta ligands 1 and 4 is required for inducing T-cell and inhibiting B-cell differentiation, while Notch-2 activation by Jagged-1 and possibly Delta-1 is sufficient to induce effects on HSPC [54–56].

These findings further suggested that manipulation of the Notch signaling pathway ex vivo in primary HSC could prove to be a novel approach for expanding HSPC. To this end and to avoid the potential safety concerns of retroviral transduction, we engineered a Notch ligand consisting of the extracellular domain of the Notch ligand Delta-1 in order to activate endogenous Notch signaling in HSPC during ex vivo expansion [57]. Initial use of engineered ligand immobilized to the tissue culture surface revealed profound effects on the growth and differentiation of isolated murine marrow precursors with a multilog increase in the number of Sca-1$^+$Gr-1$^-$ cells with short-term lymphoid and myeloid repopulating ability [58]. Similarly, studies with human CB CD34$^+$CD38$^-$ precursors cultured in serum-free conditions with immobilized ligand and cytokines resulted in an approximately 100–200-fold increase in the number of CD34$^+$ cells generated compared with control cultures, and included cells capable of repopulating immunodeficient mice [59,60]. Moreover, more recent studies with both murine and human hematopoietic progenitors have demonstrated density-dependent effects of Delta-1 on fate decisions of hematopoietic progenitors, and suggest that optimal ligand densities are required to promote expansion of cells that retain repopulating ability [59,61]. Other investigators have shown similar outcomes. Kertesz et al. demonstrated that culture of murine Lin$^-$ hematopoietic progenitor cells with immobilized Jagged-1 resulted in expansion of serially transplantable HSCs, with superior expansion dependent on the combinatorial effect of Notch and cytokine-induced signaling pathways [62]. Taken together, these data indicate that manipulation of Notch signaling can enhance stem cell self-renewal ex vivo and thereby increase HSC numbers for transplantation.

A key aspect of these studies was determination of whether the magnitude of Notch signaling played a role in the optimal generation of repopulating cells. Murine studies found that the relatively lower amount of Notch signaling induced in cells cultured with lower densities of Delta-1 led to self-renewal of progenitors with primarily B-lymphoid and myeloid potential whereas higher amounts of Notch signaling inhibited B-cell differentiation and promoted differentiation towards the T-cell lineage [61]. Concurrent studies with human CB also revealed important ligand dose-dependent effects whereby relatively lower densities of immobilized ligand substantially enhanced generation of NOD/SCID repopulating cells, whereas higher ligand densities promoted differentiation towards the T-cell lineage at the expense of repopulating cells [59]. An in vitro requirement for distinct Notch signal strengths presumably reflects selective in vivo usage of specific Notch receptor homologs with differing transcriptional activating strengths thus activating gene programs that are associated with stem/progenitor cell self-renewal versus those that promote early T-cell development. For example, Notch-2, which promotes HSPC self-renewal, may be a less potent transcriptional activator than Notch-1, which is required for T-cell differentiation [56].

Based on the above promising preclinical studies, methods utilizing an engineered Notch ligand for the ex vivo generation of increased numbers of CD34$^+$ cells is now under clinical investigation. The ability to generate increased numbers of HSPC is particularly important for patients undergoing CBT where both total nucleated and CD34 cell doses are limiting, resulting in delayed hematopoietic recovery and a higher risk of early transplant-related mortality. The development of methodologies for clinically feasible ex vivo expansion of CB-derived HSPC has generally been more successful than those using adult HSC derived from PBSC and BM. In the following section, our studies investigating the use of ex vivo expanded CB HSPC to augment CBT are discussed, along with other recent approaches under clinical evaluation and those under development.

Clinical trials using ex vivo expanded/manipulated cord blood HSPC

Rationale

With more than 30,000 CBT performed to date since 1988, CB has emerged as a source of HSC for hematopoietic cell transplantation. This is especially important for minority patients and patients of mixed ethnicity, for whom CB is a particularly attractive alternative donor stem cell source as it is readily available with no donor attrition and allows reduced stringency in human leukocyte antigen (HLA) matching without an increase in graft-versus-host disease (GVHD) [63,64]. Numerous clinical studies have consistently shown that the total nucleated cell and CD34$^+$ cell doses in CB grafts are highly correlated with the rate of neutrophil and platelet engraftment, as well as the incidence of graft failure and early transplant-related complication [65–70]. In fact, a recent analysis of adult single CBT (sCBT) recipients demonstrated that infused CD34$^+$ cell dose is the most important predictor of myeloid engraftment [71]. Furthermore, a recent publication from the groups at the Fred Hutchinson Cancer Research Center and University of Minnesota have shown that non-relapse mortality (NRM) is highest in double CBT (dCBT) recipients when compared to matched and mismatched unrelated donor recipients [72]. The majority of the NRM occurred within the first 100 days post-transplant with infection being the most common cause of death. Importantly, an analysis of the risk factors for NRM among dCBT recipients revealed a higher risk in patients with delayed myeloid recovery (time to ANC >500/μL) if the recovery was ≥26 days, the median time to engraftment in dCBT recipients. However, when the analysis of risk factors for NRM was restricted to include only those dCBT recipients engrafting before day 26, no difference could be found between the donor sources, emphasizing the important contribution of delayed engraftment to increased risk of NRM. Efforts to overcome the obstacle of small cell numbers in CB grafts by ex vivo cytokine-mediated expansion have not yet demonstrated improved time to engraftment. Infusion of multiple CB units as an alternative approach to increase cell numbers is now routinely used in adults and larger children; however, the significant delay in engraftment has not been abrogated in these patients [72,73]. Thus, the collective data point to improved outcome following CB transplantation in patients receiving a higher cell dose, in terms of both $CD34^+$ and total nucleated cell doses, an observation leading to the hypotheses that ex vivo expansion of $CD34^+$ cell numbers or the infusion of more than one unit of CB may improve the rate of CB engraftment and overall survival.

Several lines of evidence suggest that CB contains a higher frequency of primitive hematopoietic progenitor cells and early committed progenitors than adult bone marrow or peripheral blood with higher proliferative potential of CB HSPC in vitro in response to cytokine stimulation [74,75]. There is also increasing evidence that the CB-derived HSCs are less mature and have greater proliferative capacity [76,77]. A potential contribution to the differences observed between CB and adult HSCs may arise from the differential response of HSCs from different sources in cytokine-driven expansion systems, leading to variations in cell-cycle status and homing ability of the expanded cells. Other possible sources of stem cells may have even greater proliferative potential. For example, murine fetal liver cells have greater proliferation and repopulation potential than HSCs isolated from adult murine bone marrow or peripheral blood [78,79]. Overall, these results suggest that CB progenitor cells are functionally superior to adult bone marrow, with greater proliferative potential and possibly greater self-renewal capacity. Thus, CB may represent a more viable target for ex vivo stem cell expansion, a possibility that has led to several clinical studies on the ex vivo expansion of CB cells to augment conventional CBT.

Notch-mediated ex vivo expansion systems for clinical application

At the Fred Hutchinson Cancer Research Center, a phase I trial assessing the safety and ability of CB HSPCs cultured in the presence of an engineered form of the Notch ligand Delta-1 to contribute to rapid early engraftment in patients undergoing a myeloablative TBI-based CBT has just recently completed accrual. Final analyses are not yet available due to continued clinical follow-up in this small study of 23 patients in total, of whom 21 were evaluable for engraftment. One patient did not receive the expanded cell product as it did not meet release criteria and one patient experienced primary graft failure. The expanded cell product provided an average of 12.5×10^6 (median 8.3×10^6, range 0.9 to 49) $CD34^+$ cells/kg resulting from an average 178-fold expansion of $CD34^+$ for infusion. These cells were safely infused and led to a significant reduction in the time to neutrophil engraftment. The median time to neutrophil recovery (500/μL) was just 12 days compared to a median time of 25 days ($p < 0.0001$) in a concurrent cohort of 40 patients undergoing identical treatment but with two non-manipulated CB units (updated, unpublished data) [80]. Importantly, we have identified a cell dose relationship between $CD34^+$ cell dose/kg and time to rapid neutrophil recovery in recipients of partially HLA-matched expanded CB products, where 8 out of 11 patients who received greater than 8×10^6 $CD34^+$ cells/kg achieved an ANC ≥ 500/μL within 10 days. Median time to platelet engraftment was also encouraging at a median of 35 days (range 19–56) for platelets >20,000 (unpublished data).

Contribution to donor engraftment as derived from the expanded or non-manipulated grafts was determined weekly in the first month beginning at day 7 post-transplant on peripheral blood sorted cell fractions. Of the 21 evaluable patients, there were sufficient numbers of peripheral blood sorted myeloid cells for evaluation in all but one patient and in each of these patients revealed a predominance of donor cell engraftment derived from the expanded cell graft in both the CD33 and CD14 cell fractions. The one patient that was not evaluable at day 7 due to an insufficient quantity of cells had concurrent reactivation of HHV6, a virus that has been shown to inhibit progenitor cell proliferation [81]. To date, the expanded cell graft persisted beyond day 21 post-transplant in half of the patients (with three patients still too early to evaluate in vivo persistence) and 19 out of the 21 patients evaluable for engraftment experiencing engraftment prior to the median of 26 days seen in conventional dCBT recipients, suggesting a potential facilitating effect exerted by the ex vivo expanded cells on the non-manipulated CB graft. In addition, longer term in vivo persistence (defined as at least 6 months post-transplant) of the expanded cell graft has been observed in seven patients.

The observed in vivo persistence of the expanded graft to at least 6 months post-transplant suggests maintenance of some longer term repopulating progenitor cells despite ex vivo expansion. It remains unclear whether the lack of long-term engraftment from the expanded unit in the other patients represents loss of stem cell self-renewal capacity after in vitro culture or is indicative of immune-mediated rejection of the expanded unit (which is devoid of T cells) in vivo. In fact, our studies have also demonstrated T-cell-mediated effects to be responsible for rejection of one of two infused CB units in conventional dCBT [82]. Thus, it was surprising that in vivo persistence of cells derived from the expanded unit survived longer term in any patients as we anticipated eventual rejection of the expanded cell graft by the T-cell-containing, non-manipulated unit.

Although these preliminary studies of Notch-mediated expansion of CB repopulating cells points to the promise of expanding stem cells to achieve clinically relevant effects, phase II and III trials will be necessary to evaluate whether co-infusion of this expanded cell product decreases the occurrence of serious infection, improves survival, or affects duration of hospital stay in recipients of CB grafts. However, the 3–4 weeks required to identify and perform real-time, on-demand expansions of partially matched (at least 4–6 HLA-matched) CB units reduces the usefulness of this approach for treating patients at high risk of recurrent disease or to extend this therapy beyond CBT, for example to treat chemotherapy-induced neutropenia. To address this challenge, we developed a universal, "off-the-shelf," pre-expanded cellular therapy product which obviates the requirement for real-time production and HLA-matching due to the fact that this product is derived from enriched $CD34^+$ cells obtained from a single CB unit and is, therefore, devoid of T cells. We have now developed an optimized Notch-mediated expansion methodology specifically designed to expand HSPC isolated from fresh CB units. Following the 2–3 week expansion, the Notch-expanded cells are cryopreserved for future use as a universal donor product. Preliminary safety studies are currently underway to assess the safety and clinical feasibility of using this product to provide a transient myeloid bridge in a myeloablative CBT setting and in the non-transplant setting in adult patients with AML undergoing induction chemotherapy.

Other emerging approaches to ex vivo expansion

In addition to our trials using Notch-mediated expanded CB progenitor cells, there are currently several other approaches for the ex vivo expansion of CB HSPC under investigation, including ones based on culture with stromal elements to provide a "niche" for stem cell self-renewal or that have targeted defined molecules to achieve ex vivo expansion. A selection of clinical trials using ex vivo expanded CB progenitor cells is presented in the following, as are methods currently in the preclinical stage of development.

Stromal-cell based culture: Ex vivo expansion of HSPC using stromal-cell based co-culture systems have been explored as a means of providing an environment more analogous to the in vivo hematopoietic microenvironment which provides many of the molecular signals necessary for directing HSC self-renewal and proliferation as well as regulating their differentiation. To date, mesenchymal stem cells (MSC) have shown the most promise for use ex vivo as they are plastic adherent and seem to restore stromal interactions seen in the BM. In pre-clinical work using either CB or adult HSPC, it was demonstrated that modest expansion of the total nucleated and progenitor cell populations (up to 20- and 37-fold increases, respectively) could be achieved [83–85]. Based on these studies, Shpall and colleagues have recently published their initial results in 31 adult patients with hematologic malignancies utilizing the dCBT platform and a myeloablative conditioning regimen, in which they combine a non-manipulated CB unit with a unit that has been expanded for 14 days in the presence of cytokines (SCF, Flt3 ligand, G-CSF, and TPO) on either family member donor-derived MSCs or "off-the-shelf" MSCs (mesoblast) [86]. On day 14 the cells from the bags and the flasks are combined, washed and infused following the infusion of the non-manipulated CB unit. In this trial, they demonstrated a median 30-fold expansion of $CD34^+$ cells and a median time to neutrophil and platelet engraftment of 15 (range 9–42) and 42 (range 15–62) days, respectively. Importantly, no adverse events were attributed to the expanded cells and ultimately the unmanipulated unit was responsible for sustained donor engraftment. The cumulative incidence of neutrophil engraftment was 88% on day 26 which compared favorably to 53% in their control cohort of patients from the CIBMTR. Likewise, cumulative incidence of platelet engraftment at day 60 compared favorably to the CIBMTR control cohort at 71% and 31%, respectively. These studies confirm a potential role for cell-based HSPC expansion methodologies and suggest the need for further investigation to define the molecules responsible for these effects.

Copper chelator: Tetraethylenepentamine (TEPA): Based on the findings that cellular copper concentrations influence cell differentiation, Peled et al. evaluated the use of the copper chelator TEPA and cytokines (*TPO*, *Flt3 ligand*, *IL-6*, and *SCF)* in ex vivo culture of CB HSPC and demonstrated enhanced expansion of $CD34^+$ CB progenitors with improved SCID-repopulating ability [87,88]. Clinical trials have been conducted evaluating use of these expanded cells. In contrast to the other trials utilizing ex vivo expanded CB progenitors which have been based primarily on a double CBT model, in the initial phase I trial, Shpall and colleagues cultured a portion of a single CB unit with TEPA and cytokines for 21 days and co-infused these cells with the remainder of the untreated cell fraction. This approach was found to be safe and did result in sustained donor engraftment; however, there was no improvement in the kinetics of hematopoietic recovery [89]. Subsequently, a phase II/III, multicenter, multinational historical cohort-controlled study was conducted to evaluate the safety and efficacy of this approach ("StemEx®"). This trial, sponsored by Gamida Cell, was conducted at 25 bone marrow transplant centers worldwide in 101 patients with advanced hematologic malignancies and has completed accrual. Results have not been published at this time.

Aryl hydrocarbon receptor (AhR) antagonist: With the goal of identifying novel reagents that might be useful in HSPC ex vivo expansion, Boitano et al. used a 5-day culture assay with primary human $CD34^+$ cells and performed an unbiased screen of a library containing 100,000 heterocycles. Through this approach they identified a novel class of aryl hydrocarbon receptor antagonist (StemRegenin1 or SR1) found to be capable of enhancing $CD34^+$ cell generation from the blood of mobilized donors [90]. Similarly, SR1 was also found to significantly enhance the ex vivo generation of $CD34^+$ cells fivefold as compared to DMSO control after 3 weeks in cytokine-containing cultures and 50-fold after 5 weeks in culture. SR1-treated cells were found to promote retention of CD34 expression during the culture period. Of note, when in vivo limiting dilution transplants were performed in an immunodeficient mouse model, a 17-fold improvement in SCID-repopulating cells was observed as compared to uncultured cells. In addition, SR1-treated cells demonstrated enhanced long-term engraftment with 12-fold improvement in repopulation capacity following secondary transplant. These encouraging results have led to a phase I clinical trial using this technology, which has just recently initiated accrual. Similar to the previously reported studies, this study is being conducted in the myeloablative double CBT setting in which one unit is ex vivo expanded with SR1.

Alternative strategies to overcome the limiting cell dose in CB grafts: ex vivo modulation to enhance HSC homing

In addition to the ex vivo expansion strategies aimed at overcoming the limiting cell doses available in single and double CB grafts, significant efforts are underway to develop methods that enhance the efficiency of CB HSPC homing to the marrow microenvironment. Although not the focus of this chapter, this strategy deserves mention, as it is highly likely that overcoming the cell dose limitation associated, the use of CB grafts will depend on combination approaches where ex vivo expansion strategies are performed and the final product enhanced via ex vivo modulation to improve efficiency of marrow homing.

One example of ex vivo modulation to enhance homing is the pulse treatment of CB units prior to infusion with a prostaglandin derivative. Using a bench-to-fishtank-to-bedside approach, North et al. identified novel regulators of HSC self-renewal and proliferation in a high throughput identification screen and then demonstrated prostaglandin E2 (PGE2) as capable of enhancing HSC formation in zebrafish [91]. Likewise, pulse treatment of murine cells for 1–2 hours ex vivo with PGE2 prior to infusion also resulted in significantly higher numbers of repopulating cells being generated in a limiting dilution transplant model compared to uncultured control cells (3.3-fold at 6 weeks, 2.3-fold at 24 weeks). While its precise mechanism of action is unknown, PGE2 has been shown to interact with the Wnt pathway by increasing β-catenin expression levels [92]. In addition, ex vivo treatment of CB with dmPGE2 results in upregulation of genes involved in homing to the bone marrow (e.g., CXCR4), proliferation (e.g., CyclinD1), and survival (e.g., Survivin) [93,94]. Fate Therapeutics recently completed a phase Ib trial demonstrating that ex vivo modulation by pulse-treating CB units with a prostaglandin derivative for 2 hours prior to infusion is safe and accelerated neutrophil recovery by several days (Pratik Multani, personal communication). Based on these results, Fate Therapeutics are now evaluating this enhanced CB product (ProHema) in a multi-center phase II clinical trial.

Several additional pilot clinical trials have been conducted or are underway using various methods of ex vivo "priming" to enhance homing efficiency and sustained donor engraftment of CB grafts, including those targeting inhibition of CD26/dipeptidylpeptidase IV (DPPIV), fucosylation, and C3a priming [95–99]. All of these separate approaches are based on preclinical studies demonstrating enhanced homing and engraftment HSPC in murine models, with clinical trials that are currently underway or recently completed (results not published). The clinical impact of these approaches in improving outcomes following CBT is not yet determined. Moreover, the ability of ex vivo modulation to potentially impact multilineage engraftment, such as T-cell recovery, is also under investigation.

Conclusion

Although optimal conditions for expanding HSC numbers remain undefined, the collective results of studies outlined above suggest that enhanced short-term and long-term repopulating ability may be achievable with cytokine-induced expansion systems. However, these studies further suggest that cytokine-induced effects on HSC self-renewal and expansion remain limited, and clinically significant expansion has not been achieved with this approach. It is likely that we have not yet identified/optimized the critical factors and combinations required to induce symmetric HSC self-renewal. Thus current efforts are now focused on identifying and exploiting factors previously shown to regulate stem cell fate in other developing organ systems or during embryogenesis. This approach is beginning to yield promising results, and improved methods for HSC expansion are emerging. Identification of genes and regulatory elements that are responsible for preventing stem cell senescence and driving symmetric divisions of the HSC, and a better understanding of how culturing affects the homing ability of stem cells and of the complex cellular interactions in the bone marrow microenvironment, are all essential to the ultimate success of ex vivo expansion. These areas are discussed in more detail in other chapters.

Clinically, ex vivo expansion studies of primitive cells especially those using CB-derived HSPC have demonstrated the clinical feasibility of ex vivo expansion, and have suggested the enhancement of short-term repopulating cells perhaps at the expense of long-term repopulating cells. This is particularly relevant in patients undergoing myeloablative CBT where delayed myeloid engraftment is a known risk factor that is associated with the low CD34$^+$ cell doses provided in a single or double CB graft. Thus, with the goal of enhancing the time to myeloid recovery, sustained donor engraftment and decreasing the risk of early transplant-related mortality, extensive recent efforts have been aimed at expanding the available numbers of HSPC derived from individual CB units. The ultimate goal of this work would be the generation of short-term repopulating cells that meet an as of yet undefined threshold to abolish post-transplant neutropenia while providing long-term repopulating cells for sustained donor hematopoiesis. There are now several clinical trials underway using novel ex vivo expansion approaches that demonstrate great promise towards the generation of clinically meaningful absolute numbers of repopulating cells.

As discussed earlier, other investigators have focused on improving homing efficiency of the limiting numbers of HSPC in CB grafts by ex vivo modulation. While this approach may improve the incidence of sustained donor engraftment, it is not likely to result in a significant reduction in the duration of post-transplant neutropenia due to overall lower numbers of both HSPCs and more committed progeny in the primary graft. The achievement of reliable reduction of post-transplant cytopenias using ex vivo expanded cells is likely dependent on a combination of approaches that are aimed at enhancing the absolute number of HSPCs for transplant and increasing the efficiency of these expanded cells to home to the marrow microenvironment.

Despite the many promising approaches highlighted above, challenges clearly remain. A key issue is the expansion of the long-term repopulating HSC for indications such as gene therapy versus abrogation of the prolonged neutropenia that occurs following high-dose chemotherapy and radiation as in myeloablative CBT which has been successfully demonstrated with the infusion of short-term repopulating progenitors. The cost of these approaches will also ultimately affect the application of stem cell expansion methodologies. Currently, these therapies are not economically feasible and will require clear demonstration of improved patient outcomes such as more rapid hematopoietic recovery and therefore decreased transfusion requirements and decreased infections as well as a reduction in the number of days in the hospital, to be considered. Development of biologically based culture systems, such as that developed by Csaszar et al. represent important steps in cost reduction and improved expansion [2]. In their "fed-batch" system, the inhibitory factors that accumulate in culture and prevent ongoing expansion of HSPCs over time are addressed real time through computational approaches to design an optimal "feeding" schedule. Furthermore, bioprocesses involving cell selection, investigation of novel bioreactors that may recreate an artificial stem cell niche, effectively utilizing or re-utilizing media and cytokines, and three-dimensional lattices allowing for maximal expansion will certainly contribute to better optimized, cost-effective approaches [100–102].

Another aspect of ex vivo expansion that cannot be overlooked is the need to ensure safety of the expanded cell products, particularly when pathways that maintain genomic integrity may be perturbed allowing for aberrant differentiation and/or oncogenesis. New techniques for global assessment of the genome and epigenome will enable assessment of whether the genome and chromatin landscape of the expanded cell populations accurately reflect their non-manipulated counterparts and thus are predictive of appropriate cell behavior. During the next few years, we can expect that further development of the promising methods described herein will result in substantially enhanced generation of HSPCs for clinical application, and importantly, the effectiveness of such approaches will be tested in randomized clinical trials.

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CHAPTER 7

Mesenchymal Stromal Cells and Hematopoietic Cell Transplantation

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Introduction

Hematopoietic cell transplantation (HCT) is a well-established therapy for hematologic malignancies as well as many non-malignant disorders. Over the last several years, the spectrum of diseases that may be treated with HCT has dramatically expanded increasing the importance of this therapeutic modality and extending HCT beyond the traditional bounds of hematology and oncology.

HCT is founded on the principle that hematopoietic stem cells (HSCs) can be intravenously infused, will home to and engraft in the stem cell niche within the bone marrow microenvironment, and then proliferate and differentiate to repopulate all lineages of the blood. The cells that comprise the microenvironment support, and regulate hematopoiesis. However, one cellular constituent of the microenvironment, the marrow mesenchymal stromal cells (MSCs), may have quite complex biologic functions that can be exploited in the development of primary or adjunct cell therapy with HCT or in regenerative medicine.

MSCs are spindle-shaped plastic-adherent cells isolated from bone marrow as well as other tissue sources [1]. First recognized nearly 50 years ago [2], MSCs have emerged as one of the most rapidly evolving areas of research in HCT. The trilineage differentiation of MSCs, capable of giving rise to osteoblasts, chondrocytes, and adipocytes in vitro, led investigators to hypothesize a vast differentiation potential and focus research on the regenerative capacity of MSCs in an effort to repair diseased or damaged tissue. More recently, MSCs have been recognized to possess striking immunomodulatory properties and it is this characteristic of MSCs that has generated the greatest interest among physicians and investigators in the field of HCT [3,4]. Indeed, MSCs produce an extensive array of immunomodulatory cytokines as well as a variety of other, known and likely undiscovered, biochemical mediators. The secretion of these biologic response modifiers has proven to be the most biologically and clinically important property of MSCs [5].

While MSC research continues along many avenues, this chapter will focus on the biology and clinical applications of MSCs in HCT.

Brief history of MSCs

The notion that a mesenchymal progenitor cell with osteogenic differentiation potential resided within bone marrow was considered in the latter nineteenth century. However, it was the pioneering work of Alexander Friedenstein that first identified the cells which now bear the designation "MSC" [2]. He showed that these cells were precursors for osteoblasts, comprised the hematopoietic microenvironment, and that a fraction of the population, cells he termed CFU-F, possessed a high proliferative potential demonstrated by forming clonal colonies when plated at low density. Friedenstein went on to demonstrate the extensive expansion potential showing that single cells could regenerate an entire population of cells, suggesting that individual cells were precursors for fibroblasts in vivo [6]. However, Friedenstein did not suggest that the marrow stromal fibroblasts that he had identified were stem cells.

The first formal presentation of the concept that a non-hematopoietic stem cell may reside within the bone marrow microenvironment was by Maureen Owen [7]. She proposed that the marrow stroma consisted of a lineage of cells analogous to the hematopoietic system. She then expanded this theory and proposed a stromal stem cell with fibroblastic, osteogenic, and adipocytic cells being the terminally differentiated phenotypes. The term mesenchymal stem cell was proposed by Arnold Caplan hypothesizing that this cell could differentiate to a wide variety of mesenchymal tissues and proposed a broad lineage "tree" suggesting that the mesenchymal stem cell could give rise to bone, cartilage, muscle, hematopoietic-supportive stroma, tendon, ligament, adipose, and other connective tissues [8]. Data demonstrating the identification of MSCs by reactivity with the monoclonal antibodies SH2 and SH3, the considerable self-renewal capacity, and the multilineage differentiation potential in vitro supported the notion that these cells were a homogeneous population of stem cells [9]. However, more recent data demonstrated that the adherent cells isolated from marrow are a heterogeneous population of cells [10,11], although a non-hematopoietic stem cell may exist within this cell population [12]. Many other investigators, too numerous to recount here, have made important contributions to the scientific development of MSCs.

At present, MSCs are generally accepted to be a heterogeneous population of cells with diverse biologic properties suggesting a remarkable potential as cell therapy. The cytokine-generating and immunomodulatory properties portend a substantial impact in HCT.

Nomenclature

The nomenclature of these cells merits clarification. MSCs have been designated by a wide variety of terms including marrow stromal cells, marrow stromal fibroblasts, and most often mesenchymal stem cells, which was the basis for the acronym, MSC. While the specific properties of MSCs studied in the laboratory may vary

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depending on the methods of isolation and culture expansion, the assortment of terms, in general, refer to the same heterogeneous population of cells.

The term "stem cell," however, implies specific biologic properties. Our general concept of a stem cell evolved from our understanding of the hematopoietic stem cell. Till and McCullough suggested that the stem cell could be defined as a cell with extensive self-renewal capacity and the potential to terminally differentiate into two or more lineages [13]. While MSCs can, indeed, undergo extensive self-renewal and trilineage differentiation to osteoblasts, chondrocytes, and adipocytes in vitro, true "stemness" is certainly much more complex and may be best defined operationally. As this idea became increasingly recognized, many investigators suggested that convincing data were lacking to support the notion that the unfractionated population of plastic adherent cells from bone marrow designated as MSCs are properly considered as *stem* cells [14].

While a legitimate mesenchymal stem cell most assuredly exists [15] the entire unfractionated population of cells isolated by plastic adherence that we designate as MSCs does not meet the criteria of a homogeneous population of stem cells. Therefore, in 2005, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) recommended that the term *mesenchymal stromal cell* is a more appropriate designation for this heterogeneous population of cells, maintaining the acronym "MSC" while reserving the term, "mesenchymal stem cell," for a unique cell that demonstrates stem cell activity in vivo by clearly stated criteria [14]. Nearly 10 years since this nomenclature was proposed, the terminology is now largely used by the MSC community.

Biologic role of MSCs in situ

The search for the physiologic mesenchymal stem cell has generated great interest among investigators. Over the last several years, important data have emerged which revealed much biology but also generated much controversy.

Sacchetti et al. identified a CD146-expressing stromal cell from human bone marrow [16]. In situ, these CD146 cells reside in the subendothelial layer in sinusoidal walls as adventitial reticular cells. CD146 expression is found on all CFU-Fs and their clonal progeny, but on only about 30% of the heterogeneous populations of stromal cells. After isolation and ex vivo expansion, the CD146-expressing cells were capable of forming the hematopoietic microenvironment, including bone and marrow space, in heterotopic locations after subcutaneous transplantation into immune-deficient mice. Bone was evident at 4 weeks after transplantation. Then, capillaries formed and developed into a system of sinusoids similar to human bone marrow. The endothelium of the sinusoids was murine, but the adventitial cells were human indicating that the transplanted human adventitial cells were capable of self-renewal. Adipocytes were also observed in the developing marrow space. By 8 weeks, murine hematopoietic cells had migrated into and colonized the marrow space of the regenerated bone. Thus, the CD146-expressing cells isolated from human bone marrow and accounting for all CFU-Fs, seemed to be able to give rise to bone and the entire bone marrow microenvironment sufficient to attract primitive hematopoietic cells and support the ensuing hematopoiesis.

Crisan et al. reported that CD146-expressing cells exist as pericytes in multiple human organs [17]. These cells expressed typical MSC markers, lacked hematopoietic markers, and differentiated to osteoblasts, adipocytes, and chondrocytes thereby meeting the criteria for MSCs. Interestingly, the cells exhibited myogenic potential in vitro and in vivo. These studies bear resemblance to prior reports suggesting MSCs isolated from different tissue sources show different gene expression profiles and the expression patterns, in some cases, seem to reflect the tissue from which they were derived [10]. Moreover, these two studies are consistent with the evolving paradigm of MSCs that ostensibly similar cells isolated from different tissues may have markedly different biologic – and therapeutic – potential. Furthermore, it underscores the need to consider the source of MSCs in the development of MSC-based cell therapies.

While murine MSCs share some characteristics with their human counterparts they also seem to differ in many ways [18]; hence, murine studies of MSC biology must be interpreted with the understanding of potential interspecies differences. Such caution notwithstanding, murine studies have also shed light on the physiologic role of MSCs. Morikawa et al. identified a subset of cells from the heterogeneous population of MSCs of murine bone marrow characterized by platelet-derived growth factor receptor α (PDGFRα) and stem cell antigen 1 (Sca-1), termed PαS cells. The population was highly enriched for CFU-Fs, expressed common MSC markers, was capable of self-renewal in vitro, and underwent differentiation to osteoblasts, adipocytes, and chondrocytes. Unexpectedly, these cells also seemed to differentiate to endothelial cells. In healthy mice, the cells were found in the perivascular space, similar to the human CD146-expressing cells which we presume are human MSCs [16,17]. After intravenous transplantation of fresh cells (without ex vivo expansion), the PαS cells gave rise to osteoblasts and adipocytes as well as perivascular cells, consistent with stem cell-like behavior.

Mendez-Ferrer et al. [19] have identified a nestin-expressing MSC within murine bone marrow. While the cells were found exclusively in the perivascular region adjacent to bone or within the bone marrow, they were distinct from typical vascular endothelial cells. The nestin-expressing MSCs contained all CFU-Fs and exhibited the differentiation characteristics attributed to MSCs. Interestingly, the cells were able to undergo self-renewal in serial transplantation, although they were propagated in vitro as non-adherent aggregates termed mesenspheres. In vivo, these cells were intimately associated with HSCs and seemed to be critically important for HSC homeostasis and marrow homing of transplanted HSCs.

Park et al. [15] identified an *Mx1+* stromal cell within murine bone marrow that migrates to injured tissue and gives rise to osteoblasts at fracture sites. Importantly, single cell transplantation studies revealed progenitor self-renewal as well as progeny differentiation to osteoblasts. Moreover, these cells were capable serial transplantation, one of the hallmarks of "stemness." The cell identified by Park et al. seems to most closely correspond to the prevailing view of clinically applied MSCs; however, the differentiation potential of this cell was restricted to the osteolineage.

Bianco et al. [20] have recently offered a perspective on the translation of MSC biology into clinical trials. While several important issues were brought to light, prominent laboratory-based and clinical investigators with a vast collective experience in this field rebutted the perspective emphasizing the most basic principles of experimental medicine as well as the incalculable potential differences between a mesenchymal (stem) cell in situ and the ex vivo expanded cells [21,22].

Taken together, these studies illustrate the important, evolving biologic paradigm of mesenchymal stem cells and underscore that MSCs as agents of cell therapy after ex vivo expansion may bear little biologic resemblance to their physiologic counterparts in situ. Thus, understanding the engineered biology of ex vivo expanded MSCs, utilizing the irreplaceable data derived from clinical trials, is critically important to the development of effective cell therapy.

Working definition of ex vivo-expanded MSCs for clinical cell therapy

MSCs are a heterogeneous population of cells that defy simple definition because, presently, no specific marker or combination of markers is accepted to uniquely define the cells. Moreover, we lack a single functional assay that distinguishes MSCs, analogous to the repopulation assay for HSCs.

To begin to address this issue, the Mesenchymal and Tissue Stem Cell Committee of ISCT, in 2006, sought to standardize a current working definition of MSCs by proposing a set of minimal criteria to denote MSCs [23]. First, MSCs must be plastic-adherent when maintained in standard culture conditions. Second, MSCs must express CD105, CD73, and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79 or CD19, and HLA-DR surface molecules. Third, MSCs must differentiate to osteoblasts, adipocytes, and chondroblasts in vitro (Figure 7.1, Table 7.1). These criteria are by design very general and do not indicate any specific biologic activity. While they will certainly require modification as new knowledge unfolds, despite the description of several novel surface markers of MSCs [24], a more functionally relevant surface phenotype has not yet been described.

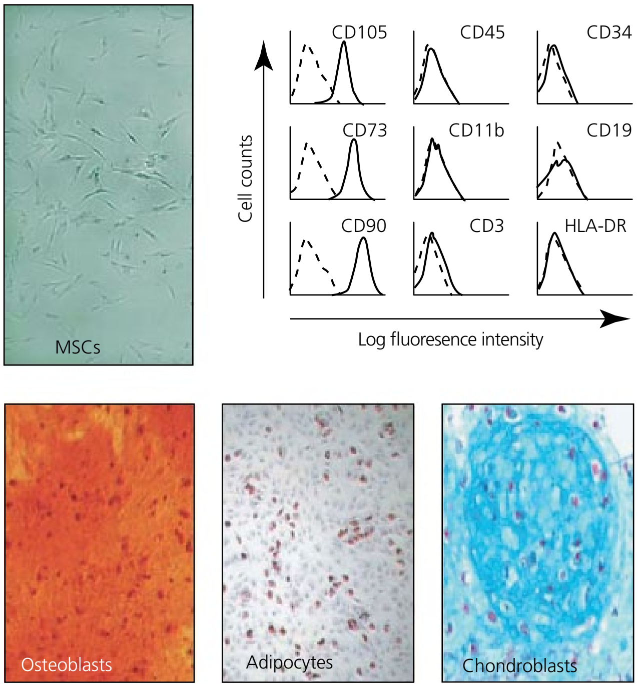

Figure 7.1 A panel of data demonstrating the defining characteristics of MSCs: adherence, immunophenotype, and in vitro differentiation. (Top, left) Photomicrograph of undifferentiated MSCs showing the characteristic spindle shape and adherent properties of the cells. Original magnification, ××40. (Top, right) Flow cytometry histograms demonstrating the typical expression pattern of surface antigens (—) and isotype control (- - -), as indicated. (Bottom) Immunocytochemical staining demonstrating the differentiation of MSCs into osteoblasts (Alizarin Red stain), adipocytes (Oil Red O stain), and chondroblasts (Alcian Blue stain). Source: Martinez C et al. Human bone marrow mesenchymal stromal cells express the neural ganglioside GD2: a novel surface marker for the identification of MSCs. *Blood* 2007; **109**(10): 4245–4248. Reproduced with permission of American Society of Hematology. (From Martinez et al. *Blood* **109**: 4245–4248, 2007, with permission) (For a color version of this figure, please see the color plate section.)

Table 7.1 Summary of criteria to identify mesenchymal stromal cells

1. Adherence to plastic in standard culture conditions		
2. Phenotype	Positive (≥95% +)	Negative (<2% +)
	CD105	CD45
	CD73	CD34
	CD90	CD14 or CD11b
		CD79α or CD19
		HLA-DR
3. Differentiation: (by staining of *in vitro* cell culture)	Bone, Adipose, Cartilage	

HLA, human leukocyte antigen.
Source: Dominici et al. 2006 [23]. Reproduced with permission of Elsevier.

Cell biology of ex vivo expanded MSCs

Tissue sources

MSCs are most commonly isolated from bone marrow [25] or adipose tissue [26], but have also been obtained from placenta [27], and umbilical cord blood [28]. Mobilized peripheral blood cells have been reported as a source of MSCs [29]; however, current consensus is that MSCs do not circulate in cytokine-mobilized or steady-state peripheral blood. Reports of MSCs in umbilical cord blood have also been conflicting; however, we now recognize that cord blood unit storage time, volume, and cell count are critical parameters affecting the efficiency of MSC isolation [28] and there is general agreement that MSCs do reside within cord blood.

Isolation

MSCs are most often isolated by "adherence selection." For bone marrow, mononuclear cells are placed in tissue culture and the MSCs will adhere to the plastic surface of the tissue culture vessel. Non-adherent cells are easily removed by changing the tissue culture media. Similar protocols are used when MSCs are isolated from other tissue sources. Prospective isolation of MSCs using surface markers has been pursued; however, in all cases, culture expansion of the selected cells was required to obtain a sufficient sample for further study. If prospective isolation of MSCs is to become a common practice, a substantial advantage must be demonstrated.

Phenotype

The expanded MSC product is a heterogeneous population of cells most easily demonstrated by in vitro adipocyte differentiation where only a subset of cells develops cytoplasmic fat globules and a CFU-F assay where a small fraction of cells exhibit a high proliferative colony-forming potential. However, this assorted population does have some common characteristics. The uniform surface phenotype of MSCs includes expression of CD105, CD73, and CD90 which can be used to define the cells, as well as CD49b, CD49e, CD166. STRO-1, an IgM monoclonal antibody generated by inoculating mice with human CD34^{+} cells, binds to human MSCs (or perhaps MSC precursors) in freshly isolated bone marrow, but does not bind to murine MSCs or human MSCs in tissue culture [30]. The antigen recognized by STRO-1 has not been identified. The low affinity nerve growth factor receptor, LNGFR or CD271, is also expressed on human MSCs in freshly harvested bone marrow but not culture-expanded cells [31]. Ex vivo expanded MSCs lack expression of hematopoietic and endothelial antigens, CD34, CD45, CD3, CD19, CD14, CD11b, and CD31.

MSCs demonstrate a surface antigen profile predicted to be hypoimmunogenic. At baseline, MSCs express moderate levels of HLA class I molecules but lack expression of HLA class II molecules on the cell surface. Upon stimulation with interferon γ in vitro, Class II antigens are found on the cell surface. MSCs do not express co-stimulatory molecules CD80, CD86, CD40, and CD40L [32].

One of the most distinguishing characteristics of MSCs, in contrast to other spindle-shaped, plastic-adherent cells, is the capacity for trilineage differentiation in vitro. MSCs differentiate to osteoblasts, chondrocytes, or adipoctyes when cultured under linage-specific inductive conditions and the cells can be identified by either tissue-specific immunohistochemical staining, for example, Oil Red O to identify the cytoplasmic fat globules of adipocytes, or gene expression.

Ex vivo expansion

MSCs have a remarkable capacity for ex vivo expansion. In standard culture conditions, MSCs isolated by adherence selection demonstrate a population doubling time of about 2 to 5 days [33]; however, plating cell density can impact the expansion potential [34]. Most MSC preparations can be maintained in continuous culture for 4–6 months before senescence. MSCs isolated from different species exhibit differing growth potentials. While human MSCs rapidly expand in culture, murine MSCs expand more slowly. Moreover, different mouse strains show a marked difference in growth and differentiation [18].

MSC cultures require some serum supplementation although serum-free media is under development. Fetal bovine serum has been the most commonly used supplement and continues to be the standard in the laboratory. The rapidly increasing clinical applications of MSCs in addition to the report of a clinically significant immune response against fetal bovine serum proteins in one patient who received MSCs [35], has led to the development of human serum/platelet lysate [36] supplementation which is rapidly becoming the clinical standard.

Secretome

MSCs have an enormous capacity for secretion of soluble mediators, and indeed, it is these mediators that comprise the principle mechanism of biologic/therapeutic activity. MSCs secrete stromal derived factor-1 (SDF-1) [37], which plays a critical role in the homing of HSCs to the marrow niche [38]. In vitro, MSCs constitutively secrete interleukin (IL)-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15, macrophage colony-stimulating factor (M-CSF), Flt3 ligand, and stem cell factor (SCF). Upon IL-1α stimulation, MSCs are induced to further express IL-1α, leukemia inhibitory factor (LIF), granulocyte colony-stimulating factor (G-CSF), and granulocyte–macrophage colony-stimulating factor (GM-CSF) [32]. Finally, MSCs can secrete several chemokine ligands including CCL2, CCL4, CCL5, CCL20, CX_3CL1, and CXCL8 [39].

Importantly, the list of cytokines secreted by MSCs is rapidly growing. The secretome of an MSC product is, in part, dependent on the tissue source and the conditions of ex vivo expansion [10,11]. In theory, then, specific tissue sources can be identified and specific expansion protocols can be developed to generate a secretome that is especially well suited for specific clinical indications. Indeed, the search for as yet undescribed cytokines generated by MSCs and processing conditions to induce expression of specific cytokines or other mediators are two areas of exceptionally active investigation.

Homing and migration

Homing refers to a specific biologic process in which cells localize to a defined tissue typically mediated by chemotactic factors or ligand–receptor interactions. MSCs do not seem to home to the bone marrow space; however, the cells can be engineered to home to the bone endosteum [40].

Lifespan of MSCs

The lifespan of ex vivo expanded MSCs after intravenous infusion is difficult to conclusively prove; however, three lines of evidence suggest MSCs persist about 6–9 months in the recipient. First, Nolta et al. infused human IL-3 secreting-MSCs into NOD-SCID mice to support human hematopoiesis and were able to detect human IL-3 in the murine serum for up to 9 months [41]. Second, Horwitz et al. infused human MSCs into children with osteogenesis imperfecta and observed a striking acceleration of growth velocity over the first 6 months with a subsequent slowing of growth [35]. Most recently, von Bahr et al. reported DNA evidence of donor MSCs in autopsies of patients who died within 50 days of the MSC infusion, but not long term [42].

Immunobiology

While the detailed mechanisms of the MSC immune modulation remain to be fully elucidated, MSCs unquestionably have a profound impact on immunity and considerable progress over the past several years has brought some clarity to this arena [43,44]. While the field is rapidly advancing, several overriding themes emerge. First, all arms of the immune system interact with MSCs. Second, there seems to be a dynamic cross-talk between the immune effector cells. Third, substantial differences between murine and human MSCs as well as the immune systems, suggest caution when extrapolating murine findings to humans and translating laboratory discoveries to the clinics. Indeed, resolution of a major scientific controversy came from the recognition that immune suppression by murine MSCs is principally mediated by nitric oxide (expression of inducible nitric oxide synthase, iNOS) while indoleamine 2,3-dioxygenase (IDO) and heme-oxygenase-1 (HO-1) primarily underlie human MSC activity [45].

The immunomodulatory properties of MSCs may be exploited therapeutically to suppress graft rejection, treat severe graft-versus-host disease (GVHD), and possibly prevent clinical GVHD. Indeed, the recognition that MSCs can suppress the immune response is what attracted the collective attention of the HCT.

T lymphocytes

The first studies to suggest that MSCs may possess inherent immunomodulatory properties were based on the recognition that ex vivo expanded third party human MSCs inhibit the proliferation of allogeneic lymphocytes in a mixed lymphocyte reaction (Figure 7.2) [46]. The effect is non-specific as MSCs inhibit the proliferation of T cells in response to alloantigens [47–49] and mitogens [46], and it is MHC-unrestricted as both allogeneic and autologous MSCs

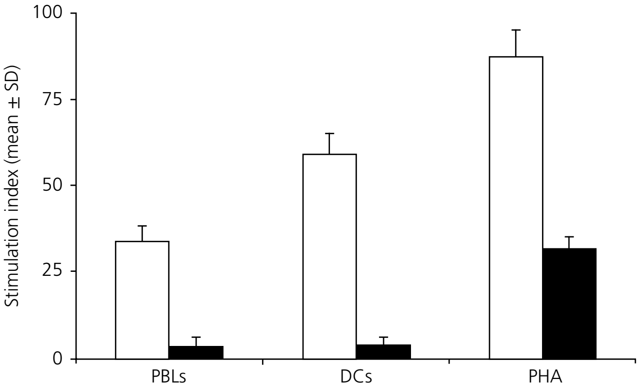

Figure 7.2 Mesenchymal stromal cell (MSC)-induced suppression of T-cell proliferation in response to allogeneic peripheral blood lymphocytes (PBLs), dendritic cells (DCs) or phytohemagglutinin (PHA) in a mixed lymphocyte reaction. Seven-day mixed cultures were performed with (black bars) or without (white bars) MSCs. Data are expressed as mean ± standard deviation (SD) of triplicates of six separate experiments. *Statistically significant (minimum $p = 0.004$). Source: Di Nicola et al. 2002 [46]. Reproduced with permission of American Society of Hematology.

generate a similar suppression [47–50]. The antiproliferative effect of MSCs has been reported to resemble divisional arrest anergy of activated T cells based on evidence that MSCs induce a cell cycle arrest in the G_1 phase which persisted after removal of the MSCs from the in vitro culture [51]. In contrast to T cell anergy in general, MSC-induced T cell anergy can only be partially overcome by IL-2. MSCs also inhibit the cytotoxicity of T lymphocytes [48], increase the proportion of $CD4^+CD25^+FoxP3^+$ regulatory T cells, which possess inherent potent immune suppressor activity [52], and inhibit the differentiation of $CD4^+$ T cells into Th17 cells and suppress cytokine production [53]. Finally, MSCs decrease interferon γ production by Th-1 cells, and increase interleukin-4 by Th-2 cells, suggesting a shift from a pro-inflammatory to an anti-inflammatory state [54].

B lymphocytes

MSCs also interact with B lymphocytes, although the effects are unclear. MSCs seem to inhibit B-lymphocyte proliferation in vitro via divisional arrest anergy, impair B-lymphocyte maturation and antibody secretion, and decrease the chemotactic properties [55–57]. By contrast, some studies have suggested that MSCs support the survival, proliferation, and differentiation of B lymphocytes to mature, antibody-secreting cells [58,59].

NK cells

MSCs inhibit IL-2- and IL-15-induced proliferation of freshly isolated, resting NK cells and prevent the induction of cytotoxic activity and cytokine production; however, proliferation of NK cells preactivated with IL-2 was only partially inhibited [60–62]. Interestingly, MSCs seem to be highly susceptible to NK cell-mediated lysis due to expression of surface ligands involved in NK cell activation, such as NKG2D and DNAX accessory molecule-1 ligands [60] as well as low level MHC class I expression. However, ligand expression varied considerably among different MSC preparations which translated to widely discrepant results of NK cytotoxicity assays and may suggest strategies to generate MSCs for specific clinical applications. Importantly, MSCs exposed to interferon γ in vitro which upregulates MHC class I expression on MSCs led to reduced susceptibility to measured NK cytotoxicity, which may impact the in vivo MSC-immune interactions and therapeutic applications.

Dendritic cells (DCs)

MSCs can inhibit the differentiation of $CD14^+$ monocytes and $CD34^+$ cells into DCs as well as the maturation and function of DCs in vitro [63,64]. MSC co-culture inhibited the GM-CSF-, IL-4-induced differentiation of monocytes to DCs, but the inhibition was reversible. MSC-DC co-culture also induced mature DCs to downregulate surface expression of CD83, CD1a, CD80, CD86, and HLA-DR as well as IL-12 secretion. Moreover, MSC-treated DCs showed an impaired capacity to prime allogeneic T cells, consistent with the observation that MSCs direct DCs to acquire a tolerogenic phenotype.

General mechanisms of immune modulation

The molecular mechanisms of MSC-mediated immune suppression are the central focus of much research in this field leading to an increasingly clear understanding. In general, immune modulation is currently thought to be mediated by secreted factors in contrast to cell-to-cell contact. Factors considered to be essential for MSC immune suppressive activity include galectins, prostaglandin E2, transforming growth factor β, human leukocyte antigen G5, and TNFα-stimulated gene 6 protein. Additionally, three metabolic enzymes are central: IDO, HO-1, and iNOS. Interestingly, the adhesion molecules intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) have been reported to correlate with the immune suppressive activity of MSCs suggesting the possibility of a contact-dependent mechanism as well [65].

Immune privilege

MSCs are considered to be immune privileged by many investigators [66]. Indeed, MSCs exhibit a hypoimmunogenic phenotype and do not elicit a T-cell proliferative response in vitro. Moreover, HLA-disparate MSCs can be safely infused into patients and often demonstrate a therapeutic effect, for example in the treatment of GVHD [67,68]. However, it is not strictly correct to infer immune privilege from such data. Immune suppressive activity and immune privilege are distinct biologic properties and immune suppression does not a priori indicate immune privilege. Murine studies and in vitro studies of human MSCs do not necessarily reflect clinical (in vivo) immune activity and infusion of allogeneic MSCs into immunosuppressed hosts does not provide data to support (or refute) the immunogenicity of the cells.

An early observation to persuasively suggest human MSCs can be recognized by the immune system and may function as an antigen-presenting cell in patients, came from a clinical trial of intravenous infusions of gene-marked MSCs in (immune-competent) children with osteogenesis imperfecta [35]. In this first trial of allogeneic MSC infusions in humans, the MSCs were ex vivo expanded in fetal bovine serum (10%)-containing media, divided into two fractions for retroviral transduction with either a vector expressing the neomycin phosphotransferase gene (neo^R), a bacterial protein, or a non-expressing vector and then both cell populations were infused into the patients without an immunosuppressive preparatory regimen. In all evaluable patients, cells containing the non-expressing vector were identified while cells expressing the bacterial protein, neo^R, were never identified (Figure 7.3). This observation suggests the possibility that the MSCs expressing neo^R were immunologically recognized in the immunocompetent hosts. Additionally, an in vitro cytotoxicity assay using mononuclear cells obtained from patients after the cell infusions revealed lysis of autologous MSCs transduced with the neo^R-expressing retroviral vector but not of MSCs transduced with the silent vector (Figure 7.4) supporting the notion that the neo^R-expressing cells were subject to immune recognition and elimination. Finally, neither fraction of cells was identified in one patient. This child developed hives and urticaria promptly after the second MSC infusion. Assay of antifetal bovine serum antibodies in the patients revealed that this patient had a 160-fold increase in antibody titer in the post-MSC infusion serum compared with the pre-infusion serum and he was the only child to exhibit a rise in titer (Figure 7.5). Together with the laboratory data of Spees et al. who showed that MSCs internalize fetal bovine serum proteins from the culture media [69], these clinical data suggest that fetal bovine serum proteins were taken up from media and the processed peptide antigens were presented by the MSCs to elicit a systemic immune response in this patient.

The preponderance of current data suggests that MSCs are not intrinsically immune privileged, and, in fact, may stimulate an immune response in some conditions. However, MSCs seem to be hypoimmunogenic due to their low-level expression of MHC molecules and complete lack of co-stimulatory molecule expression and this property may be exploited in a variety of clinical settings. To be sure, the use of allogeneic HLA-unmatched MSCs appears to be safe and elicits a therapeutic response in some patients.

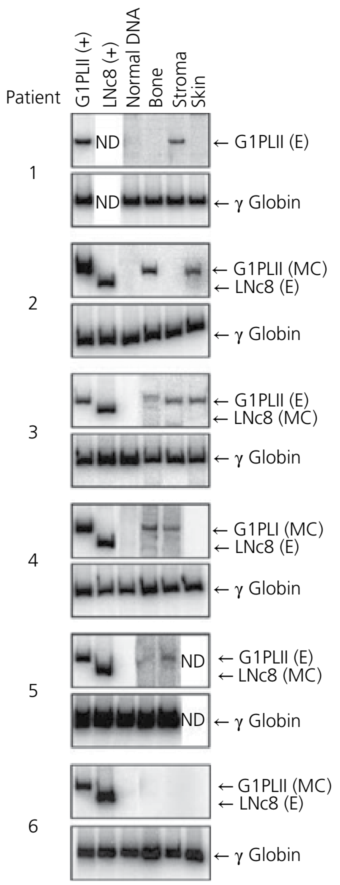

Figure 7.3 A polymerase chain reaction analysis for the gene-marking vector sequences in DNA isolated from osteoblasts, stromal cells, and skin fibroblasts of each patient, at 4–6 weeks after two infusions of gene-marked mesenchymal stromal cells (MSCs). DNA isolated from human cells transduced with either the G1PLII or LNc8 vector served as a positive control; normal human DNA was the negative control. Analysis of the gamma-globin (γ Globin) gene controlled for the quality and quantity of DNA. MC and E designate whether the signal represents minimally cultured cells or expanded cells, respectively. The vectors were alternated among the patients to avoid vector bias. ND, Not determined. Source: Horwitz et al. 2002 [35]. Reproduced with permission of National Academy of Sciences.

Risks of ex vivo expanded MSCs as cell therapy

Malignant transformation

MSCs invariably undergo ex vivo expansion for all clinical applications. This extensive cell replication raises the possibility of spontaneous transformation to a malignant phenotype, the most serious potential adverse event in a clinical trial. Murine MSCs seem to more readily undergo malignant transformation than human MSCs [70] possibly due to their significantly greater susceptibility to chromosomal aberrations and spontaneous mutations with prolonged culture [71] suggesting that such models are not good predictors of transformation of human MSCs in clinical trials.

Although there are several reports of malignant transformation of MSCs in vitro, the two most often cited studies [72,73] have been retracted [74,75] as the corresponding authors found their results were due to contamination of the MSC cultures by malignant cell lines. Further studies have confirmed the lack of spontaneous transformation [76]. During ex vivo expansion, MSCs may acquire chromosomal aberrations which have been suggested to predict the likelihood of malignant transformation [77]. However, such theoretical predictions have not been validated and substantial data to the contrary has been presented [78,79].

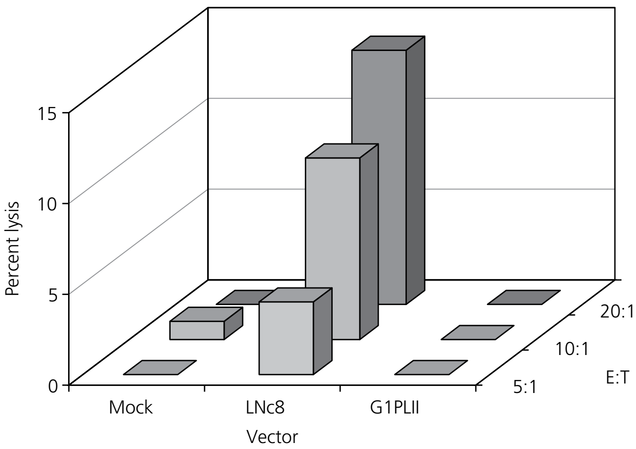

Figure 7.4 The cytotoxic response of peripheral blood mononuclear cells against transduced mesenchymal stromal cells (MSCs) is depicted. The immune cell-mediated lysis of donor MSCs transduced with the LNc8 retroviral vector (expressing neoR) in contrast to MSCs transduced with the G1PLII vector (no transgene expression) or mock supernatant. E : T designates the effector-to-target cell ratio. Each bar represents the mean of triplicate determinations. Source: Horwitz et al. 2002 [35]. Reproduced with permission of National Academy of Sciences.

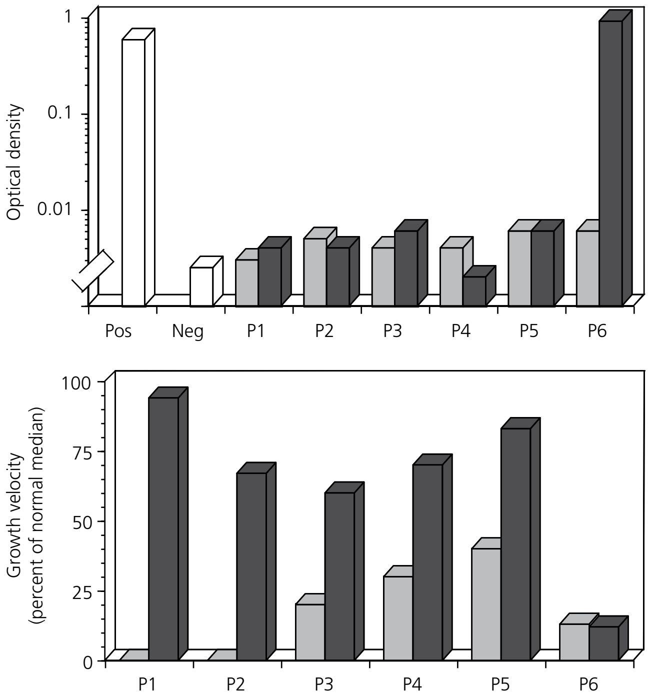

Figure 7.5 (Upper panel) Enzyme-linked immunosorbent assay measuring antifetal bovine serum antibodies in the sera of patients before (□) and after (■) both MSC infusions. Each bar represents the mean of triplicate determinations. Pos, positive control; Neg, negative control. (Lower panel) Growth velocity of the patients during the 6 months immediately before (□) and after (■) the first MSC infusion. The values are percentages of the median growth of age- and sex-matched unaffected children. Source: Horwitz et al. 2002 [35]. Reproduced with permission of National Academy of Sciences.

Malignant transformation of MSCs after infusion into human subjects in a clinical trial has never been reported. This may be because it is an exceedingly rare event and there have not yet been sufficient numbers of patients for such an event to occur. Alternatively, the latency for such an event could be quite long. Since MSCs do not seem to survive in the patients for more than 1 year, the likelihood of malignant transformation of ex vivo processed, transplanted MSCs in patients may be significantly less than envisioned according to the experience with hematopoietic stem cell transplantation [80], gene therapy [81], or predicted from karyotype analysis [79].

We emphasize that the field is relatively young, and definitive conclusions are not yet available; however, current option favors the notion that it is an unlikely outcome and should not impede clinical investigation.

Ectopic tissue formation after systemic infusion

MSCs readily differentiate to osteoblasts, chondrocytes, and adipocytes in vitro which raises the possibility that MSCs may inappropriately differentiate to a mesenchymal tissue, such as bone, after intravenous infusion. A single report of murine MSCs injected into experimentally infarcted myocardium demonstrated calcifications at the site of the cell injection [82]. However, similar findings of ectopic tissue formation have not been reported in countless experimental clinical studies.

Opportunistic infections

Given the remarkable immune modulatory capacity of MSCs, there is concern that MSCs may sufficiently suppress patients' immunity rendering them susceptible to opportunistic infections. Such an outcome has never been reported and MSCs seem to exert differential effects on alloantigen and virus-specific T-cell responses [83] suggesting MSC therapy is unlikely to induce clinically significant increased risk of opportunistic infections.

Clinical applications

MSCs to foster engraftment of HSCs following HCT

MSCs were originally considered to be stem cells comprising the hematopoietic supportive element of the marrow space and were studied in an effort to rebuild the microenvironment to foster HSC engraftment after allogeneic transplantation. The marrow microenvironment is, in fact, damaged by the chemoradiotherapy preparatory regimen which likely disrupts the HSC niche [84] thereby contributing to the delay of hematopoietic reconstitution after both autologous and allogeneic HCT [85,86]. Although, we now recognize that MSCs do not appreciably engraft in the host marrow microenvironment after intravenous infusion; the cells may foster engraftment by immune suppressive mechanisms which could reduce the risk of graft rejection, or facilitate hematopoietic reconstitution by secreting cytokines into the circulation that would promote hematopoiesis reducing the risk of primary graft failure and hasten the time to neutrophil, erythrocyte, and platelet recovery.

The first effort to determine whether MSCs can promote engraftment and hematopoietic reconstitution in patients was reported by Koc et al. in 2000 [87]. Twenty-eight women undergoing autologous HCT with mobilized peripheral blood cells for advance breast cancer were infused with 1 to 2.2 × 10^6 ex vivo expanded MSCs/kg 4 hours before the hematopoietic graft. The MSCs were harvested about a month prior to infusion, before the patients underwent mobilization for stem cell collection, and expanded for 2 to 6 passages. The median time to attain a neutrophil count great than 500/μL was 8 days (range, 6 to 11 days) and a platelet count greater than 20,000/μL was 8.5 days (range, 4 to 19 days). Definitive clinical conclusions cannot be drawn from this single-arm uncontrolled trial, but the rapid hematopoietic recovery demonstrated the potential of MSCs to enhance reconstitution in patients after autologous HCT.

Lazarus et al. assessed the capacity of ex vivo expanded MSCs obtained from HLA-identical sibling donors to facilitate hematopoietic recovery after co-transplantation with either bone marrow or peripheral blood stem cells in patients with hematologic malignancies [88]. MSCs were isolated, expanded, and cryopreserved prior to the initiation of the conditioning regimen. The MSC units were thawed at the bedside and intravenously infused into the patients 4 hours before the hematopoietic cell graft. The median hematopoietic cell doses were 3.6 × 10^8 mononuclear cells/kg for bone marrow and 5.0 × 10^6 CD34$^+$ cells/kg for peripheral blood grafts, while the MSC dose ranged from 1 to 5 × 10^6 cell/kg for both patient groups. Overall, the median time to attain a neutrophil count of 500/μL was 14 days (range, 11 to 26 days) and to a platelet count of 20,000/μL was 20 days (range, 15–36 days). The patients receiving peripheral blood grafts demonstrated a more rapid neutrophil recovery (median, 13.5 days) compared to those receiving bone marrow (15.5 days). While this study also lacked a control cohort of patients, the time to neutrophil recovery of bone marrow appears to be somewhat faster than what is often experienced. On the other hand, it is less clear whether the time to engraftment of PBPC represents a hastening of hematopoietic recovery.

Haploidentical HCT in children typically results in rapid hematopoietic reconstitution, often in as little as 10 days [89]. However, primary graft failure/rejection occurs frequently with these highly T-cell depleted grafts and a second hematopoietic cell infusion is often required. Ball et al. sought to determine whether MSCs could positively impact engraftment in haploidentical children. Fourteen children underwent transplantation with HLA-disparate CD34$^+$ cells obtained from mobilized peripheral blood. A median of 21.5 × 10^6 CD34$^+$ cells/kg were infused. MSCs were isolated from bone marrow about 5 weeks before HCT and expanded up to three passages. The ex vivo expanded cells were infused, either cryopreserved or fresh, at a dose of 1 to 5 × 10^6 cells/kg 4 hours before the hematopoietic stem cell graft. Compared to 47 age-, sex-, and diagnosis-matched historical controls, there was no difference in the time to neutrophil recovery (12 vs. 13 days) or the time to platelet (20,000/μL) recovery (10 vs. 13 days). However, the time to attain a total leukocyte count of 1 × 10^6/μL was shorter in the MSC group (11.5 days) compared to controls (14.9 days, $p = 0.009$). More importantly, none of the children receiving MSCs ($n = 14$) experienced graft failure. In contrast, the graft failure rate among the controls was 15% consistent with the experience of others.

One of the consistent themes of the preclinical studies is that MSCs are most beneficial at limiting doses of HSCs. This observation suggests that umbilical cord blood transplantation may be the most useful setting for MSC co-transplantation. Umbilical cord units must have a threshold cell count for clinical use, as discussed elsewhere in this text, since low cell doses increase the risk of graft failure. Moreover, the time to neutrophil and platelet reconstitution tends to be longer when umbilical cord grafts are used as compared with other HSC sources.

Based on this rationale, Macmillan et al. [90] have co-transplanted parental (haploidentical) MSCs with unrelated umbilical cord blood grafts. Eight patients underwent transplantation with an HLA-matched unrelated umbilical cord graft for a hematologic malignancy. Haploidentical MSCs were isolated from parental bone marrow obtained at the time the patient was referred for transplant and the ex vivo expanded cells were cryopreserved for storage. MSCs, thawed immediately prior to intravenous infusion, were transplanted at a median dose of 2.1×10^6 cells/kg 4 hours prior to the umbilical cord blood graft. The median umbilical cord blood total nucleated cell dose was 3.1×10^7 cell/kg. All patients attained a neutrophil count of 500/μL at a median of 19 days (range, 8 to 28 days) as compared to 86% of historical control patients at a median of 30 days (range, 10 to 59 days). Six of the 8 patients attached a platelet count of 20,000/μL at a median of 1.7 months (range, 1.1 to 3.2 months) compared to 79% of historical controls at a median of 2.7 months (range, 1.5 to 6.6).

Two additional studies of co-transplantation of MSCs with umbilical cord blood cells have been reported [91,92]. However, none were able to show a statistically significant benefit of engraftment or hematopoietic reconstitution; however, this may be due to the small sample size in each study.

Interestingly, Macmillan et al. [90] showed a striking, but not significant reduction of regimen-related toxicity and a corresponding improvement of overall survival. Thus, MSCs may find their greatest application in HCT as a cell therapy to reduce regimen-related toxicity and improve overall survival without directly impacting hematopoietic recovery, consistent with the recent reports that MSCs may confer tissue protection against radiation injury [93,94].

Three studies have used MSCs to rescue HCT patients after graft failure. Le Blanc et al. used MSC therapy to induce hematopoietic recovery in 3 patients with either primary or secondary graft failure [95]. Meuleman et al. rescued 2 of 6 patients [96] and Fouillard et al. reported a single patient in which haploidentical MSCs stimulated autologous hematopoietic recovery after primary failure of a mafosfamide-purged autograft [97]. Collectively, these case reports suggest that MSCs may most significantly foster hematopoietic engraftment/reconstitution under “failure” conditions. Thus, MSCs may find greater value as an agent to rescue graft failure in contrast to up-front use to promote primary engraftment.

Hematopoietic stem cell expansion

Ex vivo expansion of hematopoietic stem cells has been a long sought, but elusive goal. Stem cell expansion would obviate the problem of low stem cell doses which can be problematic in all graft sources; however, most notable in umbilical cord blood transplantation where low cell doses are often limiting and the overall quantity and HLA spectrum of available units is far less than what is currently available among unrelated volunteer donors.

Before MSCs became a cell therapeutic agent, “stromal cells” were used to generate long-term bone marrow cell cultures, which, in principle can be applied clinically to ex vivo expanded hematopoietic progenitors. De Lima et al. recently reported a remarkable clinical outcome employing this strategy [98]. Thirty-one patients underwent HCT using two cord-blood units, one of which contained cord blood that was expanded in ex vivo culture with allogeneic MSCs resulting in a 12.2-fold expansion of total nucleated cells and 30.1-fold expansion of $CD34^+$ cells. Comparing the study patients to patients who underwent standard double-cord blood transplantation, improvements in the time to neutrophil engraftment (15 vs. 24 days, $p < 0.001$), platelet engraftment (42 vs. 49 days, $p = 0.03$), and neutrophil graft failure (12% vs. 47%, $p < 0.001$) were observed. However, these striking results are thought to be due to an expansion of hematopoietic progenitors, but not long-term stem cells. Nonetheless, these data are encouraging and this strategy holds promise to overcome one of the major barriers to umbilical cord transplantation.

MSCs to treat GVHD

The immunomodulatory capacity of MSCs suggests that these cells may be effective agents for the treatment of GVHD. Based on limited preclinical in vitro data, and without animal models, a 9-year-old boy with severe refractory gut and liver GVHD after a matched unrelated donor peripheral blood stem cell transplant was treated with ex vivo expanded MSCs derived from his haploidentical mother [67]. While continuing to receive prednisolone and cyclosporine, an infusion of 2×10^6 MSCs/kg led to a prompt reduction of his serum bilirubin concentration and stool output. His cyclosporine was then discontinued to enhance a graft-versus-leukemia effect, but led to an acute exacerbation of his gut and liver GVHD. A second infusion of MSCs (1×10^6 cells/kg) again resulted in rapid reduction of his serum bilirubin and stool output. About 18 months after the MSC infusions, the patient had recurrent GVHD, developed interstitial pneumonia and died. Although the final outcome of the patient was regimen-related death, this landmark report indicated the potential of MSCs to effectively treat GVHD.

Subsequently, many small studies (case series) have been published which mostly support the notion that MSCs may be effective therapy for GVHD; however, the outcomes are highly varied, possibly due to the different MSC expansion protocols as well as the diversity of patients and treatment schedules. Notably, the growth media supplementation with fetal bovine serum, or human serum platelet lysate, or growth factors with or without serum, may alter the immune modulatory capacity of the processed MSC product.

The largest published trial is a European Group for Blood and Marrow Transplantation phase II multicenter study to treat steroid-resistant acute GVHD [68]. Fifty-five patients (30 adults, 25 children) were enrolled with Grade II ($n = 5$), III ($n = 25$), or IV ($n = 25$) disease whom had previously failed one to five prior therapies. Patients received a median total dose of ex vivo expanded bone marrow MSCs of 1.4×10^6 cells/kg patient body weight (range, 0.4 to 9.0 $\times 10^6$) derived from HLA-identical sibling donor ($n = 5$), haploidentical donors ($n = 18$), or third party HLA-disparate donor ($n = 69$). Twenty-seven patients received one infusion of MSCs, 22 received two infusions, and 6 received three to five infusions, all without MSC-related toxicity. Thirty patients (55%) had a complete response and 9, a partial response (16%) yielding an overall response rate of 71%. HLA-matching had no effect on the response. Importantly, complete responders had a lower 1-year transplant-related mortality compared to partial and non-responders (37% vs.72%, $p = 0.002$) and a higher overall survival (53% vs. 16%, $p = 0.018$). These data portend great utility of MSCs for the treatment of GVHD; however, such remarkable results have not yet been reproduced.

The only large, randomized, double blind, placebo-controlled phase III trial of MSC therapy of steroid-refractory acute GVHD to date was conducted by Osiris Therapeutics, Inc. The study outcome has been released to the public but not published in the peer-reviewed literature. Briefly, patients ($n = 260$, 28 children) received 2×10^6 MSCs/kg or placebo twice per week for 4 weeks. The durable complete response (primary endpoint) was not statistically different between the treatment and placebo groups (35% vs. 30%) and overall survival was not improved; however, the response specifically in the liver (76% vs. 47%, $p = 0.26$ and gut (88% vs. 64%, $p = 0.018$) were significant improved. Although disappointing to the MSC community, our increasing understanding of the effect of prolific expansion and cryopreservation on the immune modulatory capacity of MSCs [99,100] suggests that the cell product tested in this trial was likely not optimal for GVHD therapy. Further biologic studies of ex vivo expanded cells that comprise the clinical MSC product are required to devise superior production protocols yielding MSCs better suited for GVHD therapy.

Future considerations

We have made considerable progress in understanding the highly complex biology of ex vivo expanded MSCs that will undoubtedly foster clinical applications. MSCs hold great promise to reduce the risk of graft rejection and potentially facilitate hematopoietic engraftment reconstitution, especially when the HSC dose is limiting. Additionally, MSCs clearly can modulate the immune response and more definitive evidence of efficacy in the treatment and/or prophylaxis of GVHD is likely to emerge in the not-too-distant future. With our ever increasing understanding of the MSC-immune system interaction, MSCs will likely play a role as adjunct therapy with HCT and possibly as a single cell agent for the treatment of genetic or rheumatologic disorders.

Three great challenges lie ahead. First, we must identify surface markers of MSCs that will and define subsets of cells for specific clinical applications. Second, we must more clearly understand the mechanisms of MSC immune modulation and how to most effectively apply MSCs clinically. Finally, we must conduct numerous pilot clinical trials followed by large, multicenter clinical trials to first demonstrate and then validate the clinical utility of MSCs.

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CHAPTER 8

Genetic Manipulation of Hematopoietic Stem Cells

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Introduction and history

Hematopoietic stem cells (HSCs) are particularly amenable to *ex vivo* manipulation and have long been considered an attractive target for gene therapy. There are many potential therapeutic applications, including curing genetic diseases, making the hematopoietic system resistant to alkylating agents to allow more effective chemotherapy, and protecting the hematopoietic system from human immunodeficiency virus (HIV) infection. Methods for the genetic manipulation of HSCs evolved from the pioneering efforts of investigators working to establish means for efficient gene transfer to mammalian cells. Initial studies of gene transfer into murine hematopoietic repopulating cells utilized calcium phosphate-mediated transfection of mouse DNA encoding drug-resistance genes into mouse bone marrow (BM) cells [1,2]. The gene transfer efficiency was low in these studies, but they established the potential for HSC gene transfer and the potential for chemoprotection of HSCs.

The development of retroviral vectors allowed for efficient *ex vivo* gene transfer to murine hematopoietic progenitor cells [3] and pluripotent repopulating cells [4–6]. Transduction with retroviruses results in unique, well-defined proviral vector integrants that can be used to track the progeny of individual stem or progenitor cells. Retroviral proviruses were used as markers to demonstrate that self-renewing clones capable of both myeloid and lymphoid repopulation potential could be transduced [4–6]. Two additional prescient predictions were made in these early studies. First, the culture conditions that alter stem cell kinetics may also affect transduction efficiency [5]. Second, retroviral integrants near genes involved in hematopoietic proliferation could alter the phenotype of these stem cells, and retroviral markers might be used to identify nearby genes involved in hematopoiesis, analogous to the use of viruses to identify oncogenes [4]. These studies established the feasibility of gene therapy for hematopoietic disorders by demonstrating gene transfer to HSCs capable of expansion into multiple hematopoietic lineages with persistent transgene expression.

Improvements were made to the retroviral vector systems to reduce the possibility of generating contaminating replication-competent retrovirus in vector preparations, and *ex vivo* gene transfer to large-animal HSCs was attempted. Relatively efficient gene transfer to canine hematopoietic progenitor cells in vitro was achieved [7], but the transduction of repopulating HSCs in large-animal models was inefficient in early studies [8,9]. The first clinical trials evaluated gene transfer to autologous BM collected from acute myeloid leukemia patients or neuroblastoma patients after intensive chemotherapy [10,11]. Long-term gene marking was significantly higher than observed in previous large animal studies, with G418-resistant colonies detected up to 18 months post-transplant at frequencies of approximately 5%. The presence of the retroviral marker in resurgent blast cells of two patients with acute myeloid leukemia demonstrated that infused autologous marrow could be a source of leukemic cells. These studies also demonstrated for the first time in humans that infused gene-modified autologous cells could contribute to long-term hematopoiesis. Subsequent clinical gene transfer studies resulted in lower transduction frequencies, but transduction protocols were refined and the first clearly curative HSC gene therapy was reported 7 years later by Cavazzana-Calvo et al. for X-linked severe combined immunodeficiency (SCID-X1) [12]. This success was then marred by the development of leukemia as a result of insertional mutagenesis and clonal dominance in five out of twenty patients treated in this trial; however, the therapeutic benefits provided by gene therapy still outweighed the risk of leukemia in this patient population [13,14]. Still, myelodysplasia and leukemia have subsequently occurred as the result of insertional mutagenesis in two additional gene therapy trials, one to treat chronic granulomatous disease and the other to treat Wiskott–Aldrich syndrome, both of which used gammaretrovirus vectors to accomplish gene transfer into HSCs[15–17]. These adverse side effects have underscored the need to refine gene transfer protocols for increased safety, resulting in the development of new, safety-modified vector systems and bringing attention to strategies for targeted gene integration and precise gene editing.

Gene transfer vectors

Retroviral vectors

For most HSC gene transfer applications, the goal is to transduce multipotential HSCs and obtain transgene expression in mature hematopoietic cells following expansion and differentiation. Retroviral vectors have been by far the most widely used vectors for *ex vivo* gene transfer to HSCs, due in large part to their ability to integrate efficiently into target cell chromosomes. This allows the integrated vector provirus containing the transgene to be transmitted to all HSC progeny via mitosis during the extensive expansion of HSCs to form the entire hematopoietic system. All retroviruses contain *gag*, *pol*, and *env* genes, replicate using an RNA intermediate, and integrate into the host genome forming a defined provirus structure with two long terminal repeats (LTRs) (Figure 8.1). Integration occurs throughout the host genome but is not random;

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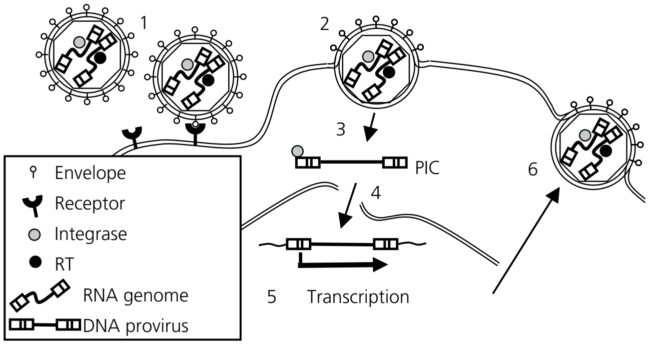

Figure 8.1 Retroviral life cycle. The retroviral virion is composed of matrix, nucleocapsid, and capsid proteins from the *gag* gene that form the virion core containing two viral genomes, and is surrounded by a plasma membrane with incorporated envelope proteins produced from the *env* gene. (1) The envelope protein mediates attachment to the cellular receptor. (2) Fusion of the viral and cellular membranes allows entry of the retroviral core into the cytoplasm. (3) The RNA genome is converted into a double-stranded DNA genome by the viral reverse transcriptase (RT). For foamy retroviruses, this occurs before virus entry. (4) The viral genome and associated proteins, referred to as the pre-integration complex (PIC), must transit to and enter the nucleus. (5) Once in the nucleus, the viral integrase mediates integration of the reverse-transcribed double-stranded DNA viral genome into the host genome. This produces a proviral vector structure with LTRs at each end of the vector genome that form a junction with the host chromosome. The LTRs contain enhancer and promoter sequences to drive viral gene expression. (6) After production of viral proteins and RNA genomes from the integrated provirus, viral cores bud through host cell membranes containing viral envelope to produce new virions.

gammaretroviruses, lentiviruses, and spumaretroviruses each have distinct integration site preferences.

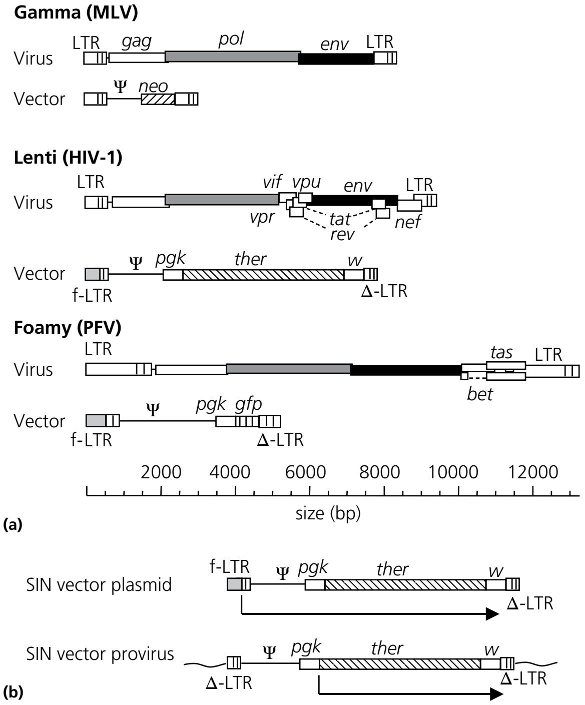

Figure 8.2 Retroviral vectors. (a) Gammaretrovirus, lentivirus, and foamy virus vectors. Schematics for Moloney murine leukemia virus (MLV), human immunodeficiency virus (HIV-1), and primate foamy virus (PFV) are shown, with an example vector schematic below. For each vector, only *cis*-acting regions required for efficient gene transfer are present in the vector, which include the long-terminal repeats (LTRs) and the packaging signal, Ψ. The vectors do not express viral proteins; the structural proteins *gag*, *pol*, and *env* are provided *in trans* (not shown) so the vectors are replication-incompetent. The MLV vector shown expresses a neomycin phosphotransferase reporter gene (*neo*) from the vector LTR. The HIV-1 vector shown expresses a therapeutic transgene (*ther*) from an internal phosphoglycerate kinase (pgk) promoter. Vector transcripts are driven by a fusion LTR promoter (f-LTR) that contains a Rous sarcoma virus promoter with HIV-1 LTR elements. This vector also contains a woodchuck post-transcriptional regulatory element (w) to enhance transgene expression. The foamy virus vector expresses a green fluorescent protein from an internal pgk promoter, and vector transcripts are expressed from a fusion LTR promoter that includes cytomegalovirus promoter elements. (b) Self-inactivating vector design. For the lentiviral vector shown, vector transcripts made during viral vector production are driven by an f-LTR promoter and the 3′ LTR contains a deletion in the U3 region. During reverse transcription, the deleted U3 region of the 3′ LTR is copied to the 5′ LTR of the vector provirus, resulting in an integrated vector with LTRs that are essentially inactive. In the integrated vector provirus, transcription is driven by the internal pgk promoter.

Gammaretroviral vectors

Gammaretroviruses, formerly a subset of the *Oncovirinae* or oncoretrovirus subfamily, are "simple" retroviruses that do not contain accessory genes. Gammaretroviral vectors were the first vectors used for HSC gene transfer and continue to be widely used for many gene transfer applications. To generate vectors, most of the viral structural gene sequences are replaced by the desired transgene(s). LTR sequences and viral *cis*-acting sequences required for efficient incorporation of vector genomes into virions referred to as the Ψ region are retained (Figure 8.2(a)). This results in a replication-incompetent vector where the internal transgene can be expressed either from the viral LTR promoter or from an internal promoter. Vector virions are produced by supplying the viral structural genes *in trans*. Gammaretroviral vectors have evolved from early vectors that contained complete LTR sequences, and could generate contaminating replication-competent viruses by recombination with helper plasmids during preparation, to highly engineered vectors with LTR fusion promoters and "self-inactivating" (SIN) deletions in the LTR (Figure 8.2(b)). Gammaretroviral vector preparations are commonly produced using a packaging cell line where the helper proteins are produced from stably integrated *gag*, *pol*, and *env* genes. This allows facile production of vector-containing supernatants by introducing the desired vector into a packaging cell line (Figure 8.3(a)). An important disadvantage of gammaretroviral vectors for gene transfer to HSCs is that the target cell must be actively dividing for efficient transduction [18]. This is presumably because the viral pre-integration complex requires breakdown of the nuclear membrane during mitosis in order to enter the nucleus [19]. HSCs are quiescent, residing primarily in G_0, so efficient transduction by gammaretroviral vectors requires stimulation into the cell cycle. For *ex vivo* gene transfer, cell cycle stimulation lengthens the culture time and promotes some differentiation, which compromises multipotency, and thus long-term engraftment potential [20].

Lentiviral vectors

Lentiviruses such as HIV-1 are "complex" retroviruses that encode accessory genes in addition to *gag*, *pol*, and *env*. Lentiviral vectors have several advantages over gammaretroviral vectors, including the ability to efficiently transduce quiescent cells [21]. The ability of lentiviral vectors to enter the nucleus independently of mitosis is

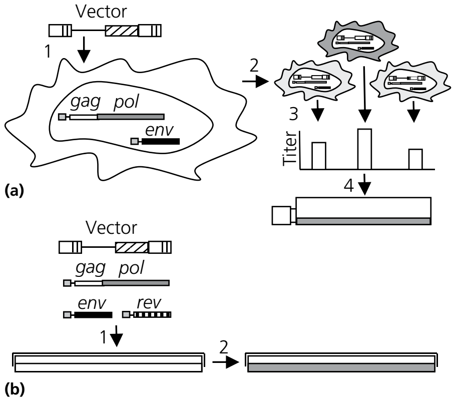

Figure 8.3 Retroviral vector production. (a) Isolating high-titer gammaretroviral producer clones using packaging cell lines. Established packaging cell lines express the vector structural proteins Gag, Pol, and Env. (1) The vector of interest is introduced into the packaging cell line to generate cells with stably integrated vector. The vector can be introduced into the packaging cell lines by transfection of a vector plasmid, or alternatively, if the vector contains a functional LTR, it can be introduced by infection of the packaging cell line with vector virions. (2) Individual "producer" clones are isolated using cloning rings or by limiting dilution. (3) Producer clones are tested to identify a clone that produces vector at high titer. (4) The producer clone is expanded and the producer cell culture medium containing vector virions is collected. (b) Lentiviral vector production by transient transfection. (1) Typically, human embryonic kidney 293 cells are transfected with a plasmid that produces the vector transcript and plasmids that express helper proteins required to form vector virions including Gag, Pol, and an Env pseudotype. (2) Following transfection, the cells are washed and incubated for 24–72 hours, and culture medium containing the vector virions is harvested up to three times during this period.

thought to be responsible for efficient transduction of quiescent cells, but other differences may also be involved [22]. Lentiviral vectors efficiently transduce aphidicolin-arrested cells, demonstrating that they do not require mitosis, but transduction of serum-starved G_0 cells is less efficient. It is now generally accepted that lentiviral vectors require progression of the cell cycle to G_{1b}, likely because reverse transcription is inefficient in G_0 cells [23]. Several groups have shown that efficient lentiviral transduction of repopulating HSCs requires cytokine stimulation supporting this conclusion. SIN lentivirus vectors can be produced at high titers and can efficiently deliver large complex transgenes that reduce the titers of gammaretroviral vectors, such as locus control region-driven globin genes. Most lentiviral vector preparations used for HSC gene transfer have been produced by transient transfection of human embryonic kidney 293 cells. A typical four-plasmid transient vector production scheme is shown in Figure 8.3(b). High titer producer lines have also been developed for lentiviral vectors [24,25].

A disadvantage of lentiviral vectors has been the concern that recombination between vector genomes and viral helper genes may lead to a contaminating replication-competent lentivirus that has some pathogenic properties of the parent virus. Most current lentiviral vector systems have deletions in the viral *tat*, *vpr*, *vpu*, and *nef* accessory genes and have extensive deletion of the viral *env* gene, but do require *rev*. They also have SIN deletions in the LTR including the TATA box. Additional safety measures have been undertaken to reduce the homology between helper and vector sequences by codon optimization, and to reduce the potential for replication-competent recombinants by splitting the Gag and Pol helper functions onto different plasmids [26]. It is thus impossible to generate the wild-type parent virus from the vector and helper plasmids, but concerns remain that a novel replication-competent virus could be generated. To address this, assays have been developed to detect a potential replication-competent lentivirus in vector preparations to be used for clinical applications [27]. Following the development of insertional mutagenesis resulting in leukemia associated with gammaretrovirus-mediated gene transfer in HSCs, lentivirus constructs became the most common gene transfer modality for human HSC gene therapy applications.

Foamy viral vectors

Foamy or spumaretrovirus-based vectors have been developed more recently and have several properties that make them well suited for HSC gene therapy. Foamy viruses have never been shown to cause disease in any host, including humans. They are the largest of the retroviruses at approximately 13 kilobases (kb) and can carry large (at least 9 kb) transgene cassettes efficiently. Foamy viruses are "complex" retroviruses that contain the accessory genes *tas* (formerly called *bel-1*) and *bet*. They infect many host species but are not endemic in human populations, although zoonotic infections can occur. In current foamy virus vectors, there are deletions of the *tas* and *bet* accessory genes and deletions in the LTR, making these vectors SIN. Foamy virus vectors require mitosis for transduction, but they form a stable transduction intermediate capable of being maintained in quiescent cells [28]. This may explain their ability to efficiently transduce hematopoietic repopulating cells with minimal *ex vivo* stimulation [29,30]. Foamy virus vectors are typically produced by transient transfection of human embryonic kidney 293 cells.

Retroviral pseudotypes for HSC gene transfer

Retroviral vectors can be "pseudotyped" at varying efficiencies by substituting a heterologous envelope protein from another enveloped virus for their envelope. This can alter the tropism of the vector to allow transduction of the desired target cell type. The choice of pseudotype is critical for HSC gene transfer as the availability of the receptor on HSCs can affect the transduction efficiency. Early studies showed that the ecotropic envelope of murine leukemia virus-based gammaretroviral vectors allowed for highly efficient gene transfer to murine repopulating cells. However, the human homolog of the mouse ecotropic receptor does not allow for entry via the ecotropic envelope, and the amphotropic envelope receptor is not highly expressed on human progenitor cells [31]. Receptor expression levels can influence the efficiency of gene transfer [32,33] so alternative pseudotypes have been evaluated. The envelope glycoprotein from the vesicular stomatitis rhabdovirus (VSV-G) has been commonly used due to its broad tropism and its ability to allow for efficient concentration by ultracentrifugation. Lentiviral vectors pseudotyped with VSV-G can be concentrated 1000-fold to titers of over 10^9 transducing units/mL and retain most of their infectivity after freezing. Additionally, lentiviral vectors pseudotyped with VSV-G can mediate efficient gene transfer into large-animal long-term repopulating cells. However, there are drawbacks to using VSV-G. VSV-G is toxic, which has limited its use in packaging cells, and human sera can inactivate VSV-G-pseudotyped vectors, which limits *in vivo* delivery. The envelope glycoprotein from cocal virus, a vesiculovirus that causes vesicular stomatitis in animals and is serologically distinct from VSV, efficiently pseudotypes lentiviral vectors. Cocal pseudotyped lentiviral vectors are more resistant to human serum inactivation than VSV-G pseudotyped vectors and efficiently transduce human CD34$^+$ cells that are able to repopulate immunodeficient mice [34].

The feline endogenous virus RD114 envelope has been used for pseudotyping both gammaretroviruses and lentiviral vectors and appears to be more efficient for the transduction of hematopoietic cells than VSV-G when compared at similar multiplicities of infection. The RD114 allows for efficient concentration of both lentiviral vectors and gammaretroviral vectors by centrifugation. A lentivirus that displays a stem cell factor-influenza hemagglutinin fusion in addition to a modified RD114 envelope has shown promise for transduction of human CD34$^+$ cells and allows *in vivo* delivery [35]. This pseudotype is designed to selectively target c-kit$^+$CD34$^+$ cells. The gibbon ape leukemia virus (GALV) envelope has allowed for efficient transduction of long-term repopulating cells in primates [36]. In some cases, pseudotyping can be inefficient, for example when pseudotyping HIV-derived vectors with the RD114 or GALV envelopes. Here, creating chimeric envelopes by substituting the transmembrane or cytoplasmic domain with the corresponding domain(s) from the amphotropic envelope, which does efficiently pseudotype HIV vectors, increases titers. Foamy virus vectors do not normally pseudotype efficiently with VSV-G or with gammaretroviral envelopes due to a specific requirement for interaction between the foamy Gag and Env proteins. Luckily the foamy virus Env allows efficient transduction of large-animal HSCs and also allows concentration by ultracentrifugation. A system has been developed to allow efficient pseudotyping of foamy virus vectors with VSV-G by creating an interaction between the VSV-G and foamy Gag protein using small-molecule controlled dimerization [37].

Envelope glycoproteins from many other viruses such as rhabdoviruses (Chandipura, rabies, and Mokola), filoviruses (Ebola, Marburg, and Lassa), and alphaviruses (Ross River virus and Semliki forest virus), have been used to pseudotype retroviral and lentiviral vectors, but efficient transduction of repopulating HSCs has not been demonstrated using these pseudotypes (reviewed in [38]).

Other vector systems

Adenoviruses are non-enveloped icosahedral viruses that have double-stranded DNA genomes of approximately 30–38 kb pairs and contain fiber projections that mediate attachment and entry. Adenoviral vectors can be produced at very high titers ($\geq 10^{12}$/mL), have a large transgene capacity, and are able to mediate highly efficient gene transfer and gene expression in non-dividing cells. However, most adenoviral vectors have been developed from the Ad5 serotype, which utilizes a fiber that binds the Coxsackie-adenovirus receptor and requires alpha integrins, which are not abundant on hematopoietic cells. Additionally, adenoviruses do not integrate efficiently into the host cell genome, thus gene expression is lost as transduced cells divide and expand. Modified adenoviral vectors have been developed for HSC gene therapy which contain fibers that allow transduction of hematopoietic cells, and contain inverted terminal repeats (ITRs) from adeno-associated viruses (AAVs), which increase the integration frequency [39]. Adenoviral vectors with AAV ITRs that have erythroid-specific transgene cassettes can deliver genes to human cord blood-derived CD34$^+$ cells with transduction frequencies of 5% in erythroid colonies [40].

AAV vectors are derived from helper virus-dependent parvoviruses and can mediate efficient gene transfer to several tissues, particularly liver and muscle. AAV vectors require S phase for efficient transduction, and their transgene capacity is approximately 5 kb, but very high titers can be produced and several different capsid proteins are available to modify the tropism of these vectors. As discussed earlier, the AAV ITRs can mediate integration, but following transduction a large percentage of AAV vector genomes remain in complex episomal forms. There have been reports of transduction of hematopoietic cells with AAV2 vectors, the first AAV vectors developed and the most commonly used, but it has been generally accepted that transduction of hematopoietic cells with AAV2 vectors is inefficient (reviewed in [41,42]). The frequency of gene transfer to rhesus monkey repopulating cells with AAV2 vectors was estimated to be approximately 0.001% [43]. More recently, better AAV2 transduction efficiencies and stable integration has been reported in human hematopoietic cells with long-term repopulating phenotypes in vitro, but has not been demonstrated in large animal models [44]. Improved transduction of mouse HSCs has been reported using AAV1 vectors [45], but large-animal studies have also not yet been reported using this serotype. For both the AAV2 and AAV1 serotypes, second-strand DNA synthesis has been implicated as the rate-limiting step in efficient transduction [45]. Additional AAV serotypes have been evaluated for the ability to efficiently transduce HSCs including serotypes 1, 3, 4, 5, 7, 8, and 10. In the most recent report, serotypes 1, 7, 8, and 10 were all found to be more efficient at transducing murine HSCs in a serial transplant model [45].

Herpesvirus vectors have large genomes (120–230 kb pairs) that can be engineered to carry large transgene cassettes and also have the ability to transduce non-dividing cells. Herpesvirus genomes do not normally integrate but can be maintained episomally or can be engineered to integrate by introducing AAV ITRs. Replication-competent, incompetent, and herpes virus "amplicon" vectors, which are bacterial plasmids that contain only the necessary *cis*-acting regions necessary for replication and packaging, have been developed for various gene therapy applications. Transduction of CD34$^+$ cells has been demonstrated [46].

Non-viral vectors are appealing for HSC gene transfer from the perspective of facile large-scale production and characterization for clinical use. There are many non-viral approaches (reviewed in [47]), but plasmid DNA is typically introduced by electroporation (or more recently nucleofection), by lipid formulation designed to effect delivery into the cytoplasm or by particle bombardment. However, HSCs are difficult to transfect. The main challenges for plasmid gene delivery are achieving efficient delivery of DNA into the nucleus in quiescent cells without significant toxicity from the procedure, and also achieving efficient integration or replication and segregation of episomes during the extensive expansion of HSCs. Efficient non-viral gene delivery to cord blood-derived CD34$^+$ cells has been reported using nucleofection, and electroporation of plasmids that allow for integration via a transposon have been used to mark CD34$^+$ cells, but gene transfer to human CD34$^+$ cells capable of repopulating immunodeficient mice was inefficient [48]. Both the Sleeping Beauty and piggyBac transposon systems have been reported to efficiently transduce human CD34$^+$ hematopoietic cells, allowing for stable, long-term gene expression in a subset of disease models, most notably thalassemia and sickle cell disease, but large animal model utility has yet to be demonstrated (reviewed in [49,50]).

Gene editing and targeted gene integration

Within the past decade, engineered nucleases with designer capacity to alter the DNA sequence cleaved have been developed which make specific genome editing possible. Designer nucleases include transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and homing endonucleases also referred to as meganucleases. TALENs and ZFNs consist of a nuclease domain fused with a DNA-binding domain to confer sequence specificity, while meganucleases consist of a dual-function recognition and cleavage domain (reviewed in [51,52]). The effect of each of these types of proteins is a double-strand break in the DNA sequence specified, which can result in genetic disruption, reconstitution or editing depending on the DNA repair process, non-homologous end-joining (NHEJ) or homology-directed repair (HDR), which mends the breakpoint. In order to utilize HDR for gene editing and reconstitution, a homologous ectopic template must be provided in addition to the sequence-specific nuclease proteins. One example of targeted disruption in clinical application is a current clinical trial utilizing ZFNs which can target the C-C chemokine receptor 5 (CCR5) locus, a co-receptor for HIV viral entry, resulting in gene disruption and resistance to HIV infection [51]. This strategy recapitulates the functional cure achieved in the "Berlin patient," an HIV$^+$ leukemia patient who received an allogeneic bone marrow transplant from a donor who carried a homozygous mutation in the CCR5 gene following myeloablative chemotherapy [53]. This patient maintained an undetectable HIV viral load after transplant even in the absence of highly active antiretroviral therapy (HAART). While preclinical data suggested this strategy could be applied to HSCs, the current clinical study is testing targeted CCR5 disruption in T cells.

Preliminary data for targeted gene insertion into genomic "safe harbors" exists (reviewed in [51,52]), but has not yet reached clinical study in any cell type (reviewed in [51,52]). Some of the challenges to these approaches include delivery of both nucleases and homologous template sequences to align the break and repair process for the most efficient gene insertion, HDR permissiveness in primary cell types, such as HSCs, and the off-target effects of these nucleases, which could compromise the safety of specific gene targeting.

Gene transfer to HSCs

Source of HSCs for gene transfer

Presently, it is accepted that long-term repopulating, self-renewing HSCs are CD34$^+$ and that isolation of these cells from either mobilized peripheral blood or bone marrow based on CD34 expression allows for efficient *ex vivo* gene transfer of some true HSCs. Furthermore, it is accepted that engraftment of a sufficient number of these cells in an appropriately conditioned host will result in long-term, multilineage hematopoiesis (reviewed in [51]). More refined HSC phenotypes have been identified; however, it remains to be proven whether these populations are able to effectively repopulate and reconstitute hematopoiesis in patients. These populations can include CD34$^+$/CD38$^-$/Rho^{lo} cells [54] or the more complex CD34$^+$/CD38$^-$/CD90$^+$/CD45RA$^-$/CD49f$^+$ cells [55]. One of the largest barriers to clinical implementation of these refined cell subsets for transplantation is the very low numbers of these cells that can be identified and then subsequently purified. Improvements in cell sorting technology may be refined to increase the yield of these true HSCs, but will likely require *ex vivo* expansion techniques to provide cell numbers that are clinically effective in transplantation.

Manipulating a mouse donor using chemotherapy with 5-fluoruracil can improve the transduction efficiency of murine BM-derived HSCs [4]. BM recovering from 5-fluoruracil treatment is enriched for cycling early progenitors and stem cells, which likely explains the efficient transduction with gammaretroviral vectors. In large-animal models, the cytokine granulocyte colony-stimulating factor (G-CSF) alone or in combination with stem cell factor (SCF) is commonly used to prime BM, or to mobilize primitive repopulating stem cells into the peripheral blood prior to collecting target cells for gene transfer. However, SCF is unavailable for clinical use in the United States due to anaphylactic reactions in some patients, so G-CSF alone has also been evaluated as a priming/mobilizing cytokine.

Hematti et al. observed low transduction frequencies in rhesus macaques with gammaretroviral vectors using G-CSF-mobilized peripheral blood cells [56]; however, efficient gene transfer was observed in the baboon model using G-CSF-mobilized cells when gammaretroviral vectors were produced with a GALV pseudotype in human 293 packaging cells [36]. More recently, AMD3100, a CXCR4 antagonist, has been used for highly efficient mobilization. AMD3100 reversibly blocks the interaction between the chemokine receptor CXCR4 and its ligand SDF-1α which plays an important role in regulating stem cell mobilization. In the rhesus macaque, cell-cycle analysis of AMD3100-mobilized CD34$^+$ peripheral blood cells shows that these cells have significantly fewer cells in G_0 (31%) compared with G-CSF-mobilized cells (79%), and significantly higher numbers in the G_1 phase [57]. Additionally, a higher percentage of AMD3100-mobilized cells expressed CXCR4 and were able to migrate towards the CXCR4 ligand SDF-1α in vitro, suggesting they may have improved homing ability. In this study, *in vivo*-gene-marking levels with a gammaretroviral vector were higher for AMD3100-mobilized cells than for G-CSF-mobilized cells. A comparison of the ability of AMD3100 and G-CSF-mobilized patient CD34$^+$ cells to repopulate immunodeficient mice also showed improved engraftment. These studies serve to illustrate the importance in selecting the source of cells and the vector pseudotype for efficient HSC gene transfer, as the phenotypes of target cells can vary significantly.

Cord blood is another promising source of HSCs for gene transfer, although the low number of repopulating cells that can be harvested has limited its utility for application in adults. Most gene transfer experiments in large animals and humans use CD34$^+$ cells isolated using magnetic beads coupled to an anti-CD34 antibody. While more refined cell surface marker expression has been reported to elucidate the true long-term repopulating HSC [55], separation techniques are not yet available to isolate cells with these complex phenotypes in scale for preclinical transplantation beyond small animal models.

Ex vivo culture conditions

During *ex vivo* culture, the objective is to stimulate the target cells into the cell cycle to increase gene transfer efficiency while maintaining their engraftment and long-term repopulating ability. Conditions for efficient transduction of murine HSCs were established over 20 years ago, but these conditions were not effective for large-animal studies or for clinical trials. *Ex vivo* conditions for efficient gene transfer to large-animal repopulating cells have more recently been developed by experimentation with different cytokine cocktails and by employing CH-296 fibronectin fragment [58]. CH-296 (RetroNectin®) is a modified recombinant 639 kDa human fibronectin fragment that contains the fibronectin cell-binding and heparin-binding domains, and enhances transduction of HSCs with gammaretroviral, lentiviral, and foamy virus vectors. The enhancement is believed to occur as a result of co-localization of vectors and target cells, at least for gammaretroviral vectors, as well as from inhibiting apoptosis of repopulating cells during *ex vivo* culture [59] and increasing engraftment [60]. Many cytokine cocktails have been used (reviewed in [61]), and for large-animal studies SCF, G-CSF, megakaryocyte growth and development factor (MGDF), fms-like tyrosine kinase ligand (Flt3-ligand), interleukin

(IL)-3, and IL-6 are commonly used with CH-296. The use of IL-3 has been shown to reduce the engraftment capability of murine HSCs [62], but IL-3 has been used in cytokine cocktails for high-level gene transfer in primate models and in clinical studies. For lentiviral and foamy virus vectors, efficient gene transfer to long-term large-animal repopulating cells has been demonstrated with minimal *ex vivo* culture, as short as 18 hours. For gammaretroviral vectors, efficient transduction of large-animal repopulating cells typically requires a prestimulation period of at least 24 hours, and a total *ex vivo* culture time of 72–96 hours, presumably due to the fact that gammaretroviral vectors require mitosis and are more labile than lentiviral or foamy virus vectors in quiescent cells [28]. The media in which CD34$^+$ cells are cultured for clinical applications requires minimal animal-derived components. There are several commercial media available which are marketed to preserve HSC viability and even promote HSC expansion, as well as meet good manufacturing practice requirements for use in the gene transfer process for clinical application. Varied degrees of success in clinical trials have been reported with each media formulation and completely animal-product-free media are currently being investigated for preclinical utility.

In vitro assays for HSC gene transfer

In vitro assays have limited use for predicting gene transfer efficiency to true long-term repopulating cells but are essential tools for vector development. Hematopoietic cell lines are useful for establishing the tropism of novel vectors and pseudotypes, and also for establishing the specificity of lineage-restricted promoters. When combined with methods such as flow cytometry to elucidate specific cell lineages, the composition of the gene modified population can be better elucidated, but there is currently no evidence to support that this information provides valuable insight into engraftment within specific hematopoietic compartments. Long-term culture initiating cell assays can be used as a surrogate assay for gene transfer to repopulating cells, but more commonly gene transfer into hematopoietic progenitors is determined using colony-forming unit assays. Cells enriched for hematopoietic progenitor cells are isolated and exposed to vector before being plated in semi-solid media with cytokines that allow for the development of myeloid and erythroid hematopoietic colonies. Colony-forming unit assays allow evaluation of the transduction efficiency of immature hematopoietic cells, but also indicate whether gene expression has been maintained during expansion of the individual progenitor cells into a colony. In some cases, mosaic transgene expression is observed, which can indicate delayed integration. Low-level gene expression in colonies, with loss of transgene expression over time in parallel liquid cultures, indicates transient gene expression, commonly observed when using non-integrating vectors, or as a result of pseudotransduction. Pseudotransduction is the transfer of protein present in the vector preparation to target cells [63], which can be a problem, particularly when using concentrated vector preparations. Furthermore, individual colony assessment for number of retrovirus integrations can yield informative data on the average number of vector insertions (copy number) in repopulating cells. This information is useful in determining the optimal conditions to balance efficient gene transfer with safety, as multiple integration events increase the risk of insertional mutagenesis as discussed further later.

Gene transfer to mouse HSCs

Mice continue to be useful for developing methods for efficient gene transfer to HSCs due to their relatively low cost. Typically, BM cells are isolated from the femurs of donor mice, enriched for HSCs by depletion of cells with defined lineage markers expressed on the cell surface, exposed to vector, and reinfused by tail vein or intrafemoral injection into lethally irradiated congenic recipients. Mouse repopulating cells that give rise to long-term multilineage reconstitution and self-renewal can be highly purified by fluorescence-activated cell sorter by removing cells with lineage (Lin) markers (CD3, CD4, CD5, CD8, Ter119, B220, Gr-1, and Mac-1 can be used) and collecting cells that express Sca-1 and c-kit markers. Purified mouse Lin$^-$Sca-1$^+$c-kit$^+$ cells, or “LSK” cells, contain virtually all the repopulating ability, but selecting Thy1.1^{lo} and Flk2$^-$ cells can be used to further enrich for stem cells. After transplantation, donor cells are commonly detected using the Ly5 allele, which is expressed on the cell surface (also known as CD45 or protein-tyrosine phosphatase receptor, type c) and can be detected by flow cytometry. Stable gene transfer to pluripotent HSCs with self-renewal capability can be demonstrated by analyzing integration sites in secondary recipients [5]. Mouse models are commonly used to develop improved methods for HSC purification and *ex vivo* transduction [64], to study methods for *ex vivo* expansion of HSCs [65], and more recently to compare the genotoxicity of viral vectors due to insertional mutagenesis [66]. Some disease candidates for gene therapy, including Fanconi anemia, lack large animal models in which to assess the efficacy of gene transfer strategies, thus mouse models provide the best therapeutic analysis for gene therapy in these settings. With the development of xenotransplantation mouse models to assay gene transfer into human hematopoietic cells, an increasing number of studies are now performed in immunodeficient mouse models. For diseases such as SCID, where large animal models exist, the use of immunodeficient mouse models has provided an inexpensive and more rapid means to evaluate safer gene transfer modalities before proceeding with large animal preclinical testing [67]. Furthermore, the recent development of humanized mice by reconstitution of immunocompromised mouse model with human CD34$^+$ cells has allowed for additional disease modeling, specifically for HIV infection, allowing *in vivo* gene therapy strategies to be evaluated [68,69].

Immunodeficient xenotransplant mouse models

The SCID-hu mouse [70], the SCID mouse [71], and the immunodeficient BNX (B is beige or reduced natural killer (NK) cell number, N is nude or athymic, and X is X^{id}, which reduces lymphokine-activated killer cells) mouse [72] xenograft models set the stage for studies aimed at improving gene transfer to the human hematopoietic cells. The human cells that repopulate immunodeficient mice, now commonly referred to as SCID repopulating cells or SRCs, were found to be distinct from hematopoietic progenitors assayed in vitro and were relatively refractory to transduction with gammaretroviral vectors [73]. Efficient transduction of SRCs with gammaretroviral vectors was obtained by stimulating human CD34$^+$ cells with cytokines and extending the *ex vivo* culture time [74]. Conversely, efficient transduction of SRCs with lentiviral and foamy virus vectors can be obtained with minimal *ex vivo* culture [29,75]. An early study by Ferrari et al.[76] used the BNX mouse to demonstrate functional correction of human adenosine deaminase (ADA)-deficient patient cells using a retroviral vector. Further refinements to immunodeficient mouse models, such as deletion of the IL-2 common γ chain (NOG and NSG mice, (reviewed in [77]) have allowed for robust engraftment of both myeloid and lymphoid cells using low numbers of transplanted human cells [78] and have more recently resulted in a “humanized” mouse, recapitulating a functional immune system capable of modeling human immunodeficiency virus infection (reviewed in [79]). However, all mouse models are restricted by a short life span and a limited proliferative-demand placed on the transplanted cells. Long-term hematopoietic potential can be evaluated with the use of serial transplantation into secondary recipients, but as this requires removal of the cells from the primary host environment and re-engraftment into a subsequent niche, it is not an accurate representation of long-term engraftment and hematopoiesis.

A comparison of baboon repopulating cells with baboon SRCs using the same source of retrovirally marked CD34$^+$ cells showed that SRC engraftment did not correlate with long-term engraftment in the baboon [80]. In this study, a comparison of the individual clones that repopulated the baboons and the SRCs revealed common proviral vector integrants at 6 weeks but not at 6 months after transplantation. These results suggest that distinct hematopoietic stem/progenitor cells are responsiblefor hematopoietic reconstitution in immunodeficient mice compared with non-human primates. Thus, while immunodeficient mouse models are useful for improving gene transfer techniques to HSCs, transduction frequencies in SRCs may not correlate to frequencies observed in human and large-animal long-term repopulating cells.

Gene transfer to large-animal repopulating cells

Long-term marking frequencies obtained in gene transfer experiments in large-animal models such as canines and monkeys have more accurately predicted gene transfer frequencies obtained in clinical trials than have mouse studies. This is likely due to similar stem cell kinetics, as discussed earlier, and the fact that the cytokines used in these models during *ex vivo* transduction protocols have higher identity to human cytokines and thus also more similar function. Gene transfer to long-term repopulating cells in large animals models was initially difficult to achieve (reviewed in [81]), but more recently efficient gene transfer has been observed using improved protocols with CH-296 fibronectin fragment [82], typically used with a cytokine cocktail that includes SCF, MGDF, and Flt3 ligand, and may also include G-CSF, IL-3, and IL-6. With sufficient *ex vivo* prestimulation and culture time, gammaretroviruses can now be used for stable polyclonal gene transfer to primate long-term repopulating cells at frequencies of 10–25% and in one case 60% in the peripheral blood.

Gene transfer into baboons and rhesus macaques using HIV-derived lentiviral vectors has been inefficient, presumably due to post-entry restriction by Trim5α [83]. Hanawa et al. have achieved high levels of multilineage gene marking (average 18%) into rhesus macaques using simian immunodeficiency virus-derived lentiviral vectors [84], which are not restricted by rhesus Trim5α. Similar gene transfer frequencies have been obtained using HIV-derived lentiviral vectors into pigtailed macaques (*Macaca nemestrina*), which are permissive for HIV-based lentiviral transduction [85,86]. In these studies, amphotropic (simian immunodeficiency virus)- and VSV-G (HIV)-pseudotyped lentiviral vectors gave similar marking efficiencies with *ex vivo* culture times of 72 hours and 48 hours, respectively.

The limiting factor(s) for HSC transduction is unclear in these large-animal experiments, but it is interesting to note the similar transduction efficiencies using lentiviral vectors pseudotyped with the retroviral amphotropic envelope or with VSV-G. Transduction in the presence of CH-296 allows saturation of the amphotropic vector in human CD34$^+$ cells in vitro [87], so it will be of interest to determine whether transduction efficiencies can be further increased by combining vectors with different pseudotypes. Efficient polyclonal multilineage gene transfer to canine repopulating cells has been achieved using lentiviral vectors [88] and foamy virus vectors [30] using an even shorter 18-hour *ex vivo* culture protocol. In summary, efficient gene transfer to large animal repopulating cells can be achieved with gammaretroviral, lentiviral, or foamy retroviral vectors. However, for lentiviral and foamy virus vectors, rapid *ex vivo* transduction protocols can be employed, which may enhance engraftment.

Most notably, non-human primate models can now be used to model HIV treatment by use of a hybrid HIV/simian (S)IV virus referred to as “SHIV.” The ability to study HSC biology and transplantation in this model with an intact immune system has enabled pioneering work in HIV/AIDS gene therapy [89,90]. The use of this specific large-animal model is particularly important for these studies because the level of gene-modified HSC engraftment and lymphoid reconstitution significantly influences therapeutic benefit when anti-HIV infection strategies are employed.

In vivo delivery of transgenes to HSCs

The ability of hematopoietic cells to efficiently home to the BM and provide lifelong hematopoiesis has helped to establish *ex vivo* transduction as the most common method for gene transfer to HSCs. However, direct injection of vector preparations into recipients may be particularly advantageous for diseases such as Fanconi anemia, in which *ex vivo* manipulation of HSCs is problematic due to their fragility, as well as infectious diseases such as HIV, since expensive and complex mobilization, collection and *ex vivo* transduction approaches are costlyand not readily mobile. Additional advantages are that enrichment and *ex vivo* culture of HSCs, which are expensive for large-animal and clinical studies, are not required, and loss of engraftment ability due to *ex vivo* culture may be avoided. Direct intrafemoral injection of lentiviral vectors pseudotyped with VSV-G results in low but persistent transduction levels in mouse HSCs [91], but VSV-G pseudotyped vectors are inactivated by human sera, so for clinical *in vivo* applications, alternate pseudotypes, such as Cocal, or alternate vector types, such as foamy virus, will be important.

Ravin et al. evaluated the intravenous delivery of RD114-pseudotyped gammaretroviral vectors into 3-day-old SCID-X1 dogs [92]. In this model, gene-modified repopulating T cells have a selective advantage, and therapeutic levels of corrected T cells were achieved in three of four dogs. Therapeutic levels of T cells were not achieved in the dog that was administered the lowest dose of vector; the level of corrected T cells was very low and a limited number of clones was present. Surprisingly, in this study, a significant number of gene-modified B lymphoid and myeloid cells were observed. Since these cells are not expected to have a selective advantage, this suggests that gene transfer was relatively efficient. It will be interesting to determine whether *in vivo* delivery can be used to efficiently transduce HSCs in large-animal models and to determine whether lentiviral vectors have an advantage for *in vivo* delivery to HSCs, which are considered to be quiescent.

Expansion of gene-modified cells prior to infusion

Ex vivo expansion of long-term repopulating cells prior to or during transduction could increase the number of infused cells and thus increase the engraftment of gene-modified cells. Infusion of a higher number of stem cells would more efficiently compete with endogenous stem cells so less conditioning and potentially less toxicity would be required. This would be especially important for genetic and infectious diseases, in which only a reduced-intensity conditioning may be possible. Although self-renewal has been demonstrated *ex vivo*, attempts to expand long-term repopulating cells with cytokine cocktails have only allowed for modest increases in HSCs.

Another approach is to more directly manipulate pathways involved in stem cell maintenance or expansion such as homeobox proteins, or Notch and Wnt signaling proteins. Homeobox, or Hox, genes regulate differentiation during embryogenesis; *HoxB3* and *HoxB4* are preferentially expressed in BM cells that are highly enriched for primitive cells. Expression of *HoxB4* from a gammaretroviral vector resulted in a 40-fold increase in mouse HSCs [93], and a Tat–HoxB4 fusion protein has been developed for *ex vivo* applications to avoid potential genotoxicity from permanent *HoxB4* expression. The Wnt and Notch pathways are involved in stem cell maintenance and self-renewal. Wnt proteins and Notch ligands have also been used to expand repopulating cells, but the level of expansion observed was less dramatic.

More recent efforts to expand HSCs have included factors such as pleiotrophin [94,95], prostaglandin [96,97], and the combination of angiopoietin-like 5 and insulin-like growth factor [98]; however, the most notable *ex vivo* expansion of CD34$^+$ hematopoietic cells was supported by addition of the aryl hydrocarbon receptor antagonist, StemRegenin 1 (SR1) [99]. However, the engraftment of HSCs expanded by any of these methods in patients is currently unknown. Furthermore, it is believed that a combination of methods will most likely produce the most therapeutically effective *ex vivo* HSC expansion. The combination of Notch pathway modulation and SR1 is currently being clinically investigated, but likely will better define effects on short-term reconstitution rather than long-term hematopoiesis (reviewed in [51]).

Expansion of gene-marked cells *in vivo*

Increasing the percentage of gene-modified repopulating cells post-transplant using *in vivo* selection could expand the potential of HSC gene therapy to hematopoietic diseases, such as thalassemias, and infectious diseases, such as HIV, which require relatively high levels of gene transfer [100]. Positive selection of cells post-transplantation has been demonstrated using a method in which drugs that bind to and dimerize a modified receptor molecule transgene transmit a growth signal to the cell [101]. The small-molecule drugs are called chemical inducers of dimerization, and the receptors are modified to remove their extracellular domain and to contain binding regions for their small-molecule ligands. To date, this approach has been limited in that receptors capable of expanding HSCs have not been identified.

Negative selection using alkylating agents that are toxic to stem cells such as 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) or temozolomide can be performed to expand gene-modified stem cells that are protected by a mutant O6-methylguanine-DNA methyltransferase (*MGMT*) transgene [102]. In this approach, the drug O6-benzylguanine inactivates endogenous MGMT protein but not the mutant MGMT protein. Following *ex vivo* gene transfer of the mutant *MGMT* transgene to HSCs, treatment with BCNU and O6-benzylguanine is toxic to cells that did not receive the *MGMT* transgene and results in expansion of gene-modified cells. This approach allows for the durable expansion of repopulating cells in large-animal models (Figure 8.4), demonstrating that selection occurs in very primitive cells (reviewed in [103]). *MGMT*-mediated selection might be used for gene therapy of hematopoietic diseases, such as thalassemias, by including therapeutic globin and *MGMT* transgenes together in a bicistronic vector. Gene transfer of *MGMT* to HSCs can also be used to protect the hematopoietic system from high-dose chemotherapy during treatment for solid tumors or leukemias. Early clinical studies explored the use of the multidrug resistance gene *MDR-1* for this purpose, but the marking was very low and *in vivo* selection was not observed. More recently, a clinical study to evaluate chemoprotection of the hematopoietic system using *MGMT*-mediated selection in poor-prognosis glioblastoma patients reported increased survival and hematopoietic chemotherapy resistance after combination chemotherapy including O6-benzylguanine and temozolomide [104]. In this study, a gammaretroviral vector was used to transfer a mutant *MGMT* to G-CSF-mobilized patient CD34$^+$ cells *ex vivo* and single agent BCNU was administered as a chemotherapy for treatment of the brain tumor and as conditioning prior to infusion of the gene-modified cells after a <96-hour culture and transduction period. Following recovery from BCNU-induced myelosuppression and engraftment of the mutant *MGMT*-expressing CD34$^+$ cells, patients began receiving 28-day cycles of temozolomide, the standard chemotherapy treatment for glioblastoma, in combination with O6-benzylguanine. Transient increases in gene-modified cells in the peripheral blood of these patients were observed after each chemotherapy cycle, in concert with mild myelosuppression as a result of chemotherapy. However, the number of gene-modified colony-forming cells in each patient increased over time and with chemotherapy, suggesting that selection at the stem/progenitor cell level was achieved with this regimen *in vivo*. While two of three patients retained gene-modified cells at levels greater than 35% of peripheral blood white blood cells for the duration of the study, a third patient with relatively low levels of gene-modified cells lost gene marking after 9 cycles of combination chemotherapy approximately 10 months after transplantation. This suggests that chemoselection may induce clonal exhaustion if a small number of HSCs are available to contribute to hematopoiesis. However, large-animal studies of the same mutant *MGMT* have not resulted in loss of gene-modified cells over time and have also demonstrated long-term *in vivo* selection of gene-modified colony-forming and peripheral blood white blood cells, to

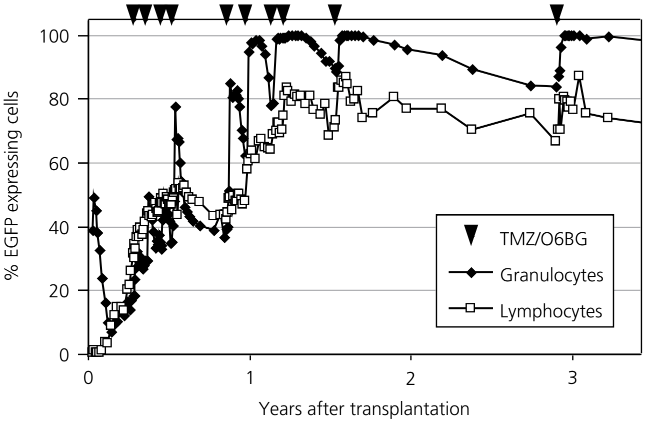

Figure 8.4 *MGMT*-mediated *in vivo* selection. A dog was transplanted with autologous CD34$^+$ cells transduced with a gammaretroviral vector expressing the P140K mutant MGMT and enhanced green fluorescent protein (EGFP). After hematopoietic reconstitution, *in vivo* selection was achieved using a series of chemotherapy treatments with the alkylating agent temozolomide (TMZ) and the MGMT inhibitor O6-benzylguanine (O6BG). Stable increases in gene-modified granulocytes and lymphocytes indicate selection of primitive long-term repopulating cells.

high levels (>80%), even when initial gene marking was low (<5%) [86]. Most of these animals received O6-benzylguanine in combination with BCNU, rather than temozolomide. It is postulated that this difference in selection kinetics between BCNU and temozolomide results from the type of cytotoxic DNA damage induced by each individual agent, and the number of cell divisions required to produce a cytotoxic effect. This suggests that BCNU-induced DNA damage is more cytotoxic to HSCs when given in combination with O6-benzylguanine.

Another strategy to mediate *in vivo* selection of gene-modified cells is through knockdown of the hypoxanthine ribosyl transferase (HPRT) gene with expression of an interfering RNA [105]. Purine analogs such as 6-thioguanine (6TG) and 6-mercaptopurine (6MP), which have been used in the treatment of leukemia, are converted to fraudulent purine nucleotides which can be incorporated into DNA during cell division, causing mismatch repair pathway activation, cell-cycle arrest, and initiation of apoptosis. Conversion of these purine analogs to their bioactive, cell-death-initiating forms requires HPRT function. Thus, by including an HPRT-specific interfering RNA within a transgene cassette, cells expressing the gene modification will be resistant to the effects of 6TG and/or 6MP. The primary toxicity of these purines is myelosuppression, although some patients have also experienced hepatotoxicity from 6TG treatment [106]. Extensive clinical use of these oral drugs has demonstrated that they are tolerated for long periods of time and can be titrated to achieve optimum hematologic toxicity. Preclinical studies in murine HSCs have demonstrated that lentivirus-mediated delivery of an interfering RNA-mediated purine analog (iPAR) to knockdown HPRT expression resulted in 6TG resistance in transplanted mice [105]. When treated with 6TG, these mice demonstrated increases in iPAR gene-modified cells in both peripheral blood and bone marrow; however, prolonged treatment of 6TG was required to elicit the selection (2 cycles consisting of daily administration over 15 of 21 days with a 2–3 week break in between cycles), and stability of HSC selection was monitored to only 5 weeks after the second cycle. Furthermore, the level of gene-modified HSC maintenance was wildly variable in the mice treated (range 0–44.8% of HSCs) and no clonal analysis was performed to rule out oligoclonal expansion as a cause for the observed selection *in vivo*. Still, considering the known carcinogenicity of alkylating agent chemotherapy, this strategy could provide a less toxic alternative to achieve levels of gene marking required for therapeutic benefit in some patient populations.

Immune responses to transgenes

In many situations, the transgene delivered to HSCs will be foreign, and there is the potential for an immune response to gene-modified hematopoietic repopulating cells. In gene-marking studies, a foreign protein such as neomycin phosphotransferase or green fluorescent protein has been expressed in HSCs and their progeny. In cells that express these proteins, they can be processed and presented through MHC class I and II pathways and can trigger cytotoxic, helper, and memory T-cell responses. Processed peptides from green fluorescent protein have been shown to elicit T-cell immune responses in transplanted mice, baboons, and rhesus macaques. Immune responses can also be a problem for clinical applications where a patient may express a truncated form of the therapeutic transgene or may not express the transgene at all. In these cases, the immune system may recognize the therapeutic transgene as foreign. This has been observed in a canine preclinical model for Hurler syndrome, where a strong humoral response was associated with a loss of *in vivo* repopulating cells [107]. In settings where a myeloablative pre-transplant conditioning regimen will be used, pre-existing immune cells are eliminated and a state of tolerance to the foreign transgene can be established. However, even when high-intensity conditioning has been used, cytotoxic T cells specific to a foreign antigen have been observed [108]. Cyclosporine has been used successfully to inhibit the development of immune responses in canines after transplantation of gene-modified cells. Immune responses are not expected to occur in clinical gene therapy situations where there are severe pre-existing immunodeficiencies such as for SCID-X1 and no immune responses have been reported to date in any clinical trial utilizing autologous gene-modified HSCs as discussed later.

Clinical trials of HSC gene transfer

Early studies

The first clinical HSC gene-marking studies demonstrated that persistent gene marking could be obtained in human repopulating cells [10,11]. Early therapeutic clinical trials attempting to transduce HSCs were performed using the multidrug resistance gene *MDR-1* [109–111]. The product of the *MDR-1* gene, P-glycoprotein, is an energy-dependent drug efflux pump that reduces the intracellular concentrations of a wide range of drugs and xenobiotics including chemotherapy agents such as etoposide. The approach was to protect the hematopoietic system from the dose-limiting myelosuppressive effects of chemotherapeutic agents used to eradicate tumors. In these studies, the gene transfer rates were very low, less than 1 in 10^4 cells, similar to earlier studies in the canine and primate models. Additional early clinical HSC gene transfer studies demonstrated low-level but persistent gene marking for ADA-SCID [112,113] and Gaucher disease [114], but therapeutic benefit was not achieved.

In 1995, a report commissioned by the National Institutes of Health concluded that the low efficiency of gene transfer suggested that more emphasis be put on basic aspects of gene transfer, novel vector development, and the study of stem cell biology, but that there was a clear and legitimate need for clinical studies to evaluate various aspects of gene therapy approaches [115]. Improvements to HSC gene transfer were made and several clinical studies were undertaken, including trials for Fanconi anemia [116], leukocyte adhesion deficiency [117], and acquired immune deficiency syndrome [118], but with no clear therapeutic benefit. To date, clinical trials for gene transfer to HSCs have used gammaretroviral and lentiviral vectors. Foamy virus vectors are being pursued preclinically with increasing interest, but currently no foamy virus vectors have been approved for use in an interventional gene therapy clinical trial. Table 8.1 summarizes recent clinical trials for HSC gene transfer.

Genetic diseases

Severe combined immunodeficiency syndrome (SCID)

More than two decades of clinical research in HSC gene therapy for SCID have been conducted, including X-linked and adenosine deaminase (ADA) deficient SCID syndromes, and these have provided a wealth of information which has exponentially stimulated the development and improvement of gene therapy strategies for other diseases. A large international effort beginning in France and involving a total of five clinical trials, treated more than 50 patients, 20 with X-SCID and at least 30 with ADA-SCID [12,119–123] with over 13 years of follow-up in the first patients treated.

Table 8.1 Recent hematopoietic stem cell (HSC) clinical gene therapy studies

Reference	Disease	Patients	Ages	Vector	Env	HSC Source	Mobilization	*Ex vivo* culture			Conditioning	Marking before infusion	Cells infused (/kg*10^6)	Disease response
								Time	FBS	Cytokines				
Cavazzana-Calvo et al., 2000 [12], Hacein-Bey-Abina 2003 [13], Ginn et al., 2005 [197]	SCID-X1	10	1–11 months[*]	Gamma	Ampho	BM	None	3 days	4%	3, F, M, S/3, F, S, T	None	10–40%[*]	14–38[*]	7/10 complete 2/10 partial 4 leukemias
Gaspar et al., 2004 [121]	SCID-X1	4	4–33 months	Gamma	GALV	BM	None	3 days/96 hours	None	3, F, S, T	None	27–58%	8–26	4/4 complete 1 leukemia
Thrasher et al., 2005 [128]	SCID-X1	2	15, 20 years	Gamma	Ampho/ GALV	BM	None	3 days/96 hours	4%/ none	3, F, M, S/3, F, S, T	None	13–30%	2.8, 35	0/2
Chinen et al., 2007 [129]	SCID-X1	3	10–14 years	Gamma	GALV	PB	G-CSF	4 days	None	3, 6, F, S, T	None	39–45%	29–31	1/3 partial
Aiuti et al., 2002; Aiuti et al., 2009 [119,122]	ADA	10	0.6–5.6 years	Gamma	Ampho	BM	None	4 days	4%	3, F, S, T	Busulfan	12–56%	0.9–13.6	5/10 complete 4/10 partial
Gaspar et al., 2006; Gaspar et al., 2011 [136,137]	ADA	6	0.5–3.2 years	Gamma	GALV	BM	None	96 hours	None	3, F, S, T	Melphalan or Busulfan	5–50%	<0.5–5.8	4/6 complete
Candotti et al., 2012; Engel et al., 2007 [123,198]	ADA	10	1.2–20 years	Gamma (MND or GCsapM)	GALV	BM	None	5 days	NR[†]	F, M, S	None or Busulfan	0.1–1.8[‡]	0.7–9.8	3/10 only conditioned patients for whom ERT was withdrawn
Ott et al., 2006 [140]	CGD	2	25, 26 years	Gamma	GALV	PB	G-CSF	5 days	None	3, F, S, T	Liposomal busulfan	39.5–45%	3.6–5.1[¶]	2/2 complete clonal expansion 2/2 loss of transgene funtion, death
Kang et al., 2010; Kang and Malech, 2012 [143,144]	CGD	3	19, 28, 28 years	Gamma	Ampho	PB	G-CSF	96 hours	1%	3, F, S, T	Busulfan	25–73%	18.9–71	2/3 cleared incurable infection with very low-level sustained gene marking
Kim et al., 2008 [145]	CGD	2	9, 18 years	Gamma	NR[†]	PB	G-CSF	NR[†]	NR[†]	NR[†]	Busulfan and Fludarabine	10.5–28.5%	5.4, 5.8	0/2
Grez et al., 2011 [142]	CGD	4	5–27 years	Gamma	Ampho or GALV	PB	G-CSF	NR[†]	NR[†]	NR[†]	Melphalan	5–20%	0.2–10.6	0/4 total loss of gene marking by 42 days after gene therapy
Cartier and Aubourg, 2010 [199]	ALD	2	7,7.5 years	Lenti	VSVG	PB	G-CSF	2 days	None	F, S, M	Busulfan and Cyclophosphamide	33–50%	4.6–7.2	2/2 comparable to HCT
Reviewed in Naldini 2011 [52]	MLD	1	NR[†]	Lenti	NR[†]	NR[†]	NR[†]	NR[†]	NR[†]	NR[†]	Busulfan	NR[†]	NR[†]	1/1 sustained gene marking at 6 months with polyclonal engraftment
Adair et al., 2012 [104]	GBM	3	51–56 years	Gamma	GALV	PB	G-CSF	<96 hours	None	G-CSF, F, S, T	BCNU	74–79%[§]	7.5–21.9	1/3 complete remission 3/3 extended survival
DiGiusto et al., 2010 [175]	HIV	4	25–55 years	Lenti	VSVG	PB	G-CSF	2 days	None	F, S, T	BCNU, etoposide, cyclophosphamide	0.11–0.15%[**]	4.1–8.1	

3, interleukin-3; 6, interleukin-6; ADA, adenosine deaminase; Ampho, amphotropic; BM, bone marrow; FBS, fetal bovine serum; F, Flt3 ligand; GALV, gibbon ape leukemia virus; G-CSF, granulocyte colony-stimulating factor; M, megakaryocyte growth and development factor; NR, not reported; PB, peripheral blood, S, stem cell factor; SCID-X1, X-linked severe combined immunodeficiency; T, thrombopoietin.

[*] For patients that were reported.

[†] Not reported.

[‡] Vector copy number per cell.

[§] Percent gene-modified colony-forming cells in methylcellulose.

[¶] CD34, gp91$^+$ cells infused.

Immunodeficiency has been corrected in 85% of the X-SCID patients and >70% of the ADA-SCID patients and only two patients, both X-SCID, have died. As noted earlier, the success of these studies was hindered by the development of leukemia in five of the X-SCID patients treated, all of whom developed uncontrolled clonal T-cell expansion as a result of the gene transfer and deregulated expression of proximal oncogenes nearby to the site of gene integration. One of these patients died as a result of the leukemia, but the other four patients were cured with standard therapy. However, over the next 5 years, follow-up demonstrated 36 SCID patients who benefitted clinically from these studies, outweighing the hesitation to continue development of this approach. Furthermore, results obtained from the analyses of clonal repertoires in all patients treated have provided a valuable tool for investigating *in vivo* hematopoiesis. Each disease and the associated clinical studies are described in the following sections.

SCID-X1

The first clearly therapeutic HSC gene therapy trial was reported for SCID-X1 [12,124], which is caused by mutations in the common cytokine receptor γ chain (γc, *IL2RG* locus), resulting in the absence of mature T lymphocytes and NK cells. Corrected SCID-X1 lymphoid progenitors have a strong selective advantage due to the restoration of survival and proliferation signals mediated by the γc receptor. Patients with no HLA-identical siblings were eligible for the study and, due to the immunodeficiency, no pretransplant conditioning was given. Autologous CD34$^+$ cells transduced with a gammaretroviral vector pseudotyped with the amphotropic envelope were used. Of the initial 10 patients treated, T-cell development occurred in nine, and T cell reconstitution correlated with the dose of CD34$^+$ cells infused [12]. In these studies, small numbers (1%) of transduced myeloid cells were detected, and analysis of vector integration sites by linear amplification-mediated PCR demonstrated polyclonal T-cell repopulation and transduction of primitive multipotential progenitors with self-renewal capacity [125]. An additional four patients were successfully treated by Gaspar and colleagues in the UK using a gammaretroviral vector pseudotyped with the GALV envelope [121]. These authors recently reported that, of a total of 10 patients now treated, six had restoration of immune function with no requirement for prophylactic immunoglobulin replacement [126]. The success of these studies was attributed in part to the improved transduction methods for gene delivery into CD34$^+$ cells, which included CH-296 and cytokine cocktails that included SCF, MGDF, IL-3, and Flt3 ligand. For both studies, the T-cell repertoire was diverse, and T-cell receptor excision circle (TREC) analysis identified naïve T cells long after transplantation, even in the absence of marked myeloid cells. Thus, a gene therapy approach may allow for better long-term thymopoiesis than is observed with HLA-identical and haploidentical allogeneic transplantation, in which hematopoiesis remains of host origin in the absence of pretransplant conditioning [127].

In two older patients treated at 15 and 20 years of age, SCID-X1 gene therapy failed [128]. In both patients, there was efficient gene transfer to CD34$^+$ cells in vitro, and in one patient there were similar marking levels in B, NK, and myeloid cells to patients less than 1 year old who were successfully treated. Chinen et al. investigated gene therapy as a salvage treatment for three patients aged 10, 11, and 14 years who had previously received T-cell-depleted BM transplants from a parent, but had incomplete immunologic reconstitution with frequent infections and chronic diarrhea [129]. In this study, G-CSF mobilized peripheral blood CD34$^+$ stem cells were transduced with a GALV-pseudotyped gammaretroviral vector expressing γc and infused without prior conditioning. All three patients had detectable retroviral marking in myeloid and lymphoid lineages to at least 12 months post-transplant. The 10-year-old patient had relatively high gene marking in T cells consistent with a strong selective advantage in this lineage. In this 10-year-old patient, CD4$^+$ and CD8$^+$ counts increased, and CD4$^+$CD45RA$^+$ naïve cells and TRECs persisted, indicating restoration of thymic function. Vβ spectratype analysis showed increased T-cell receptor diversity. Thus, a clear therapeutic benefit could be established in this patient, and the authors noted that in addition to the younger age, this patient also had previous full T-cell reconstitution from a prior haploidentical BM transplant. For SCID-X1, the capacity to reinitiate thymopoiesis may be compromised in older patients with loss of thymic potential. Thus, gene therapy should be initiated as early as possible, and previous thymic activity may help predict success in older patients.

A combined 17 of the 20 enrolled SCID-X1 patients have been successfully treated in the French and UK studies. Unfortunately, the finding of leukemia in five patients, four from the French trial, and one from the United Kingdom trial, all of which were vector mediated, initially dampened enthusiasm for retroviral gene therapy. One patient died as a result of the leukemia, but the other four patients responded to treatment and retained gene-modified naïve T cells after leukemic therapy. However, the morbidity and mortality of this disease combined with the limitations of current transplantation protocols supported the continued use and refinement of this approach. The major focus of these continued efforts was on improved vector systems designed to reduce the potential for malignancy from vector-mediated insertional mutagenesis (discussed later). Preclinical data from these studies indicates additional studies of new vector types are forthcoming [67,130,131].

ADA deficiency

In ADA-SCID, an accumulation of deoxyadenosine triphosphate induces cell death by apoptosis in lymphocyte precursors, impairing lymphocyte development and function. ADA-SCID was one of the first diseases proposed for clinical trials because the gene was available and could be expressed from a retroviral vector, the level of protein required for therapeutic effect was estimated at 10–15% of normal levels, and corrected T cells would have a selective advantage. T cells were used initially as targets for the first clinical study of gene transfer to ADA-deficient patients in 1990 [132], and Aiuti et al. observed that, following transplantation of lymphocytes in one patient, discontinuation of polyethylene glycol (PEG)-ADA enzyme replacement therapy led to immune reconstitution [133]. Thus for ADA, the enzyme replacement therapy (ERT) likely reduces the survival advantage provided to gene-modified cells, limiting the potential therapeutic effect from ADA gene transfer.

Aiuti et al. treated two patients using CD34$^+$ BM cells transduced with a gammaretroviral vector and observed sustained engraftment and clinical improvement with restoration of ADA activity in peripheral blood lymphocytes [119]. Unlike previous studies, these ADA patients received non-myeloablative pretransplant conditioning with busulfan (2 mg/kg/day on days 3 and 2 before infusion), and did not receive PEG-ADA enzyme replacement therapy. Within the lymphocyte subsets, increased cell numbers were first detected in B cells and NK cells, followed by T cells. Both patients had corrected cells in multiple lineages including granulocytes, erythrocytes, megakaryocytes, and lymphoid cells. The frequency of gene-modified cells in the lymphoid subsets was higher, suggesting a stronger selective advantage for these cells. Virtually all NK cells in the peripheral blood and BM were transduced. In B lymphocytes, the frequency of transduced B cells was higher in the peripheral blood than in the BM, suggesting a growth or differentiation advantage for corrected peripheral B lymphocytes.

These investigators have now treated at least 30 patients without any evidence of leukemia. Integration site analyses on most of these patients have been published [134,135] and revealed polyclonality with chromatin structure and local histone modifications guiding vector insertion. These results were also compared with insertion sites identified in peripheral blood lymphocytes transduced with the same vector and transplanted into ADA-SCID patients, revealing cell-type specific differences in integration profiles consistent with transcriptional profiles of the individual cell types [135]. Gaspar et al. [136,137] treated six patients with no HLA-matched donor who had a poor response to PEG-ADA replacement therapy using a gammaretroviral vector pseudotyped with the GALV envelope. PEG-ADA was stopped 1 month prior to treatment, and autologous BM CD34$^+$ cells were transduced and infused in five patients after conditioning with melphalan at 140 mg/m^2. The sixth patient received 4 mg/kg busulfan conditioning prior to gene-modified cell infusion. Following gene therapy, T-cell counts rose in four of six patients and were sustained now more than 5 years since gene therapy. The other two patients restarted ERT and remain well. A third study conducted in the United States included 10 ADA-SCID patients and compared two different gammaretrovirus vectors, both based on the myeloproliferative sarcoma virus (MPSV), with one vector using the MPSV LTR and the other using the MPSV-modified MND LTR [123]. The first four patients treated in this study received no conditioning prior to infusion of gene-modified cells and remained on ERT during the transplant procedure. Two of these patients were older (15 and 20 years), while the other two patients were children (4 and 6 years). Only transient low-level gene marking was observed in the older patients, while some gene marking was sustained in the younger patients up to 9 years after gene therapy. However, this sustained marking was also lowlevel, though not as low as observed in the adult patients. This study went on to treat the remaining six patients with ERT withdrawal and busulfan conditioning (65–90 mg/m^2) prior to gene therapy. Three of these patients displayed complete immune reconstitution and remain off ERT up to 5 years after gene therapy. In those patients with sustained gene marking >1%, integration site analysis revealed polyclonal hematopoietic reconstitution with low-level diversity. Although clones displaying common insertion sites including *MDS1/EVI1* and *LMO2* were identified, there were no clinical consequences or noticeable expansion of these clones reported. Of all ADA-SCID patients treated to date, at least 19 patients have not required ERT after gene therapy. The difference in complete immunodeficiency correction compared to X-SCID is thought to be related to extrahematopoietic consequences of this disease and further studies in older patients and patients with diverse pretreatment histories have demonstrated that kinetics of naïve T-cell production were more rapid in younger patients and patients whose prior therapy did not include failed haploidentical BMT or ERT [123] (and reviewed in [138]). These studies support gene therapy for ADA-SCID as a viable approach for patients lacking an HLA-identical BMT donor and suggest intervention as early as possible after diagnosis and determination that a suitable donor does not exist. They also emphasize the need for preparative conditioning prior to gene-modified cell infusion in order to achieve therapeutic levels of engraftment.

X-linked chronic granulomatous disease

Chronic granulomatous disease is caused by a defect in the nicotinamide dinucleotide phosphate phagocyte oxidase (phox) complex, and approximately 70% of patients have mutations in the X chromosome gene encoding the gp91^{phox} subunit (X-linked chronic granulomatous disease). As a result, the antimicrobial activity of phagocytes is impaired due to an inability to generate reactive oxygen species, and patients have recurrent infections with granulomatous lesions. Unlike SCID-X1 or ADA-SCID, gene transfer to myeloid cells is required and the transgene does not confer a growth advantage to gene-modified cells, so high levels of gene transfer are likely necessary to observe a therapeutic effect. Supporting this, early attempts at gene therapy for chronic granulomatous disease in the absence of conditioning resulted in low levels of gene transfer without therapeutic benefit [139].

Ott et al. treated two patients after non-myeloablative conditioning with liposomal busulfan at 4mg/kg/day on days 3 and 2 before infusion [140]. In this study, G-CSF-mobilized CD34$^+$ were transduced with a gammaretroviral vector pseudotyped with the GALV envelope. In both patients, high levels of gene marking were observed and gene marking in peripheral blood leukocytes from patients 1 and 2 increased to 46% and 53%, and then decreased to 27% and 30%, respectively. Biochemical evidence of respiratory burst activity was demonstrated, and although the level of superoxide production observed was lower than in wild-type cells, clinical benefit was established for both patients. Analysis of vector integration sites initially showed a highly polyclonal repopulation, but after 3 months, clones with insertions in the *MDS*1-*EVI*1, *PRDM*16, and *SETBP*1 loci began to dominate. Transactivation of these genes by the strong spleen focus-forming virus promoter in the vector was found to have contributed to clonal expansion. The initial clinical benefit observed in these patients was attributed to this expansion; however, activation of the MDS-EVI1 locus led to genomic instability, monosomy 7, and development of myelodysplasia in both patients [15]. Both patients then demonstrated silencing of the therapeutic transgene by methylation of the viral promoter following the initial resolution of bacterial and fungal infections after gene therapy. One patient died of a severe bacterial sepsis after colon perforation. There was still very high gene marking in this patient, but the function of the transduced cells was greatly decreased. The second patient underwent allogeneic bone marrow transplantation from an unrelated, HLA-matched donor. Due to the use of busulfan prior to infusion of gene-modified cells, for allogeneic BMT a conditioning regimen of fludarabine, melphalan, and antithymocyte globulin (ATG) was used. This patient recovered rapidly following transplant and by day 115 after allogeneic BMT demonstrated 99.5% donor chimerism with almost complete elimination of MDS1/EVI1 clones harboring monosomy 7. A third patient suffering from therapy refractory *Aspergillus nidulans* infection of the lungs was treated with the same vector and protocol by another group after 8.8 mg/kg liposomal busulfan conditioning, resulting in stable gene marking at 20% up to 86 days after gene therapy was administered [141]. However, this patient also developed clonal myeloproliferation (reviewed in [142]).

In a third study conducted at the National Institutes of Health, three patients with incurable infection were treated with 10 mg/kg busulfan prior to infusion of CD34$^+$ cells gene-modified with an MFGS-based gammaretrovirus encoding gp91^{phox} as a salvage therapy [143]. One of these patients had been treated with gene therapy in an earlier study by the same group without conditioning prior to gene therapy approximately 11 years earlier. Despite initially high transduction efficiencies (25–73%) and large cell doses received at the time of cell infusion (18.9–71.0 × 10^6 cells/kg body weight), gene-modified cell levels in peripheral blood declined to 1% and 0.03% in two of the three patients and could not be detected after 4 weeks in the third patient. For the first patient, in whom 1% gene marking remained stable in peripheral blood up to 34 months after gene therapy, clone tracking between different lineages indicated that at least a few HSCs were transduced. A more recent report on this study indicated that the two patients who had detectable though declining gene marking at the time of the first report cleared their initial infections and have sustained gene marking at more than 5 years since gene therapy, albeit at very low levels (0.7–0.03%) [144]. Separately, a study conducted in Seoul, Korea infused two patients with $CD34^+$ cells transduced with a murine leukemia virus-based MT-$gp190^{phox}$ encoding gammaretrovirus after conditioning consisting of 6.4 mg/kg busulfan and 120 mg/m^2 fludarabine [145]. In this study, transduction efficiencies were reported to be 10.5% and 28.5%, respectively; superoxide-producing cells were, again, transiently detectable shortly after gene therapy, declining to 0.1 and 0.4%, respectively, in these two patients. Similarly, a clinical trial in London transplanted four patients with gene-modified cells; three patients with the same gammarertovirus used in the CGD trial where patients developed myelodysplasia and one patient whose cells were transduced with the gammaretrovirus used in the NIH trial (reviewed in [142]). In this study, all four patients received single-agent melphalan at a dose of 140 mg/m^2 prior to infusion of gene-modified cells. Similar transduction efficiencies were reported and cell doses ranged between 0.2 and 10 × 10^6 cells/ kg body weight. Again, low levels of gene marking were observed one month after transplant and declined to undetectable levels by day 42 after gene therapy.

The combined data from these studies, wherein only three patients displaying therapeutic benefit obtained such as the result of insertional mutagenesis and clonal expansion, suggest engraftment as the most significant barrier to successful CGD gene therapy. Additionally, similar gammaretroviruses have been used successfully in other gene therapy applications with similar levels of myelosuppression, substantiating the lack of *in vivo* selective advantage for transplanted CGD cells. In these trials, gammaretroviruses were used for gene transfer and it is possible that extended culture periods required for efficient gammaretroviral transduction may have compromised the multipotency of gene-modified $CD34^+$ cells. It has also been suggested that both HSCs and the niche in which they reside are damaged in CGD patients as a result of constant inflammation [142]. Furthermore, constitutive $gp190^{phox}$ expression in HSCs has been postulated to result in high levels of reactive oxygen species, resulting in cytotoxicity in this cell type. Additional trials utilizing lentivirus vectors to reduce *ex vivo* manipulation in combination with additional conditioning regimens and mature myeloid cell-directed $gp190^{phox}$ expression are planned.

Wiskott–Aldrich syndrome

Wiskott–Aldrich syndrome (WAS) is a recessive primary immunodeficiency with X-chromosome linkage which manifests in recurrent infections, thrombocytopenia, eczema, and autoimmunity. The WAS protein (WASP), which lacks functionality in this patient population, regulates actin polymerization in hematopoietic cells, serving to facilitate cell signaling and locomotion and to form immunologic synapses (reviewed in [146]). Thus, mutations in the *WAS* gene produce a complex biologic manifestation including defective T- and B-cell function, impaired function of the NK-cell immunologic synapse and compromised migratory capability in all white blood cells. Since WASP expression is restricted to hematopoietic cells, gene-modified leukocytes supplying a functional *WAS* gene would have an *in vivo* selective advantage over non-modified cells. Boztug *et al.* initially reported treatment of two WAS patients with mobilized autologous HSCs gene-modified with a GALV-pseudotyped gammaretrovirus expressing a functional *WAS* gene [147]. Three and two days prior to cell infusion, patients were conditioned with 4 mg/kg busulfan. In both patients, high levels of gene-marked, *WAS*-expressing leukocytes were observed, with skewing toward the lymphoid lineage. Functional correction restored NK immune synapse formation, increased T-cell receptor (TCR) usage in T cells and B-cell numbers in these patients. Clinically, both patients demonstrated platelet increases with cessation of clinical bleeding within 6–9 months after gene therapy. Incidence and severity of infections declined in both patients, although one patient experienced pneumococcal meningitis 2 years after gene therapy, hypothesized to be due to a prior splenectomy or insufficient B-cell reconstitution. Both patients also displayed decreased symptoms of autoimmunity. Initial gene-modified cell engraftment was observed to be polyclonal with some skewing towards common integration sites reported in other clinical trials of gene therapy employing gammaretrovirus vectors for gene transfer.

The trial continued to enroll and treat an additional eight patients; however, in 2009 it was reported that one patient had developed a clonal T-cell leukemia with retrovirus insertion proximal to the LMO2 locus, the same genetic locus identified in four out of five leukemic clones observed in the aforementioned X-SCID trial, which also utilized a gammaretrovirus vector for gene delivery [148]. Since that time, at least one additional patient has developed a T-cell leukemia, also with an oligoclonal expansion correlated with an *LMO2*-proximal retrovirus integration event [149]. This suggests that the combination of *in vivo* selective advantage mediated by therapeutic gene expression in combination with proto-oncogene proximal insertion of gammaretrovirus constructs resulting in local gene expression augmentation, specifically the *LMO2* gene, greatly increases the propensity for leukemic transformation in patients treated with gene therapy.

A second, internationally collaborative trial of WAS gene therapy wherein a SIN lentivirus is used to deliver the WAS gene under the control of the WAS promoter is currently underway and to date has treated a total of three patients [52]. Currently, there is no evidence of clonal expansion or leukemia reported in this trial and the therapeutic benefit of lentivirus-transferred WAS gene expression appears to be phenotypically corrective [150].

β-Thalassemia

Hemoglobinopathies of the β subtype are the most prevalent inherited single gene genetic disorders, resulting from mutations in the β-globin gene cluster and manifesting in little to no adult hemoglobin A production. Phenotypic symptoms include poor erythroid precursor maturation and, in Cooley anemia (significantly reduced or absent β-globin production), severe anemia requiring transfusion dependence which can result in iron overload, the leading cause of death in β-thalassemia patients. If an unaffected, matched sibling donor is available, and the patient is well transfused and chelated with good organ function, bone marrow transplantation can be curative, but this clinical scenario does not apply to the majority of β-thalassemia patients, making autologous gene therapy a viable clinical treatment option. However, for high-level, erythroid-specific expression required to be therapeutic, a complex transgene cassette that includes the β-globin gene and its introns, as well as the β-locus control region, must be transferred efficiently. The initial clinical trial employed a lentivirus construct to deliver the required genetic elements and reported treatment of two patients, one who failed to engraft due to technical compromise of the infused gene-modified cells independent of the gene transfer vector, and a second who engrafted with gene-modified cells after conditioning with busulfan (3.2 mg/kg/day for 4 days) [151]. This patient required a total of 10 additional transfusions over the first year after gene therapy, but with a noticeable decrease in frequency over months 6–12 (three transfusions compared to seven transfusions required in months 1–5). After 1 year, the patient became transfusion independent; however, this welcomed clinical benefit was found to be correlated with oligoclonal expansion of a gene-modified cell with a provirus integration in the third intron of the high mobility group AT-hook 2 (*HMGA2*) gene. *HMGA2* expression was increased dramatically in erythroid cells of this patient, but stabilized over time. The bone marrow of this patient did not indicate clinical symptoms of leukemic transformation, with only erythroid hyperplasia commonly observed in β-thalassemia patients noted. This was the first report of insertional gene activation coupled with oligoclonal expansion in lentivirus-transduced HSCs. At present, occurrences of HMGA2-associated vector insertion, without leukemic transformation, have been reported in multiple patients treated with HSC gene therapy for a variety of clinical conditions [104,152].

Fanconi anemia

Despite being an early candidate for HSC gene therapy, like chronic granulomatous disease, Fanconi anemia (FA) represents one of the more challenging monogenic diseases in which to accomplish successful therapeutic benefit from gene therapy. FA is an autosomal recessive disease resulting from mutations that compromise function in any one of 15 different genes, whose products work in concert to coordinate recognition and repair of damaged DNA. The clinical manifestation of FA, first described by Swiss pediatrician Guido Fanconi, is complex and can include congenital defects, short stature, abnormalities of the skin and arms, head, eyes, kidneys, and ears, as well as developmental disabilities. Almost uniformly, 90% of FA patients develop bone marrow failure by age 40. FA patients also display a significantly increased risk for acute myelogenous leukemia and other cancers including epithelial and squamous cell carcinomas [153]. As a result of the defect in DNA repair, FA patients are highly susceptible to DNA damaging agents, such as ionizing radiation and chemotherapy which induces DNA damage for cytotoxic effect, making even traditional bone marrow transplantation challenging. However, the largest barriers to successful HSC gene therapy identified to date have been the lack of HSCs available for gene modification and the fragility of these HSCs when manipulated *ex vivo* [116,154]. In two clinical trials attempted, one to treat FA patients of the C complementation group (*FANCC* gene mutation) [116] and the other to treat patients of the A complementation group (*FANCA* gene mutation) [154], both of which utilized gammaretroviruses for respective *FANCC* and *FANCA* gene transfer, a total of eight patients were infused with gene-modified cells. No conditioning therapy was utilized prior to infusion of gene-modified cells in either trial given the increased susceptibility of FA patients to DNA damaging agents. Both studies demonstrated transient engraftment only in some of the infused patients and, consequently, only temporary clinical benefit in those patients with transient engraftment.

Further preclinical studies have advocated the use of lentivirus vectors to mitigate gene transfer, as these vectors permit shorter *ex vivo* manipulation periods which are postulated to improve survival and engraftment potential of the limited number of HSCs available from FA patients [155,156]. In addition, multiple groups have shown a clear benefit to HSC preservation during *ex vivo* manipulation by addition of antioxidants to culture medium, as well as culture under hypoxic conditions, as this decreases the amount of oxidative stress placed on HSCs *ex vivo* [157,158]. In 2010, an international working group was established to determine the best strategy for HSC gene therapy in FA patients and reported that next steps should include a safety-modified lentivirus directing FA gene expression from a human phosphoglycerate kinase promoter and that minimal *ex vivo* culture with reduced oxidative stress should be attempted [159]. Currently a phase 1 clinical trial is enrolling FA patients to evaluate the feasibility and safety of the recommended approach [158]. However, the lack of engraftment or a proliferative advantage *in vivo* in the prior trials, as well as results from other HSC gene therapy trials such as those for ADA-SCID, X-CGD, and β-thalassemia, suggest that some preparative regimen may be required prior to gene-modified HSC infusion in order to achieve efficient and therapeutically beneficial engraftment in FA patients. Alternatively, *in vivo* selection after transplantation may also improve engraftment and proliferation by eliminating non-modified cells. It has been demonstrated that the sensitivity of FA cells to agents such as cyclophosphamide could provide a means for efficient engraftment and/or *in vivo* selection at very low doses of this drug [160].

Lysosomal storage disorders

X-linked adrenoleukodystrophy

X-linked adrenoleukodystrophy (X-ALD) is a severe genetic disease caused by mutations in the *ABCD1* gene, whose protein product, the ALD transporter, metabolizes very-long-chain fatty acids in the peroxisomal membrane. Loss of functional ALD transporter causes very-long-chain fatty acid accumulation in plasma and tissues as well as progressive demyelination within the central nervous system of ALD males. Sixty-five percent of these patients have an overall lifetime risk of developing fatal cerebral demyelination [161]. Allogeneic BMT, performed at early stages of demyelination, is the only treatment that has been shown to stabilize cerebral demyelination in ALD males. The mechanism for this stabilization arises from replacement of host brain microglial cells with that of donor myelomonocytic cells, although the exact mechanism by which this replacement arrests demyelination is unknown [162,163]. However, since the ALD transporter is not secreted, no cross-correction occurs in patients treated with allogeneic BMT. Furthermore, suitable donors are not available for all patients and for those whom donors are identified, risk for complications associated with allogeneic transplantation, including graft-versus-host disease (GVHD), remain a concern.

Preclinical studies of lentivirus-mediated *ABCD1* gene transfer into mouse ALD1-deficient hematopoietic stem/progenitor cells demonstrated replacement of 20–25% of brain microglia at 1 year after gene therapy [164]. Gene modification of $CD34^+$ cells from ALD patients also resulted in effective ALD transporter expression and these cells migrated to the brain in NOD/SCID mice after transplant, where they differentiated into microglia [165]. The initial clinical study employed a VSV-G pseudotyped HIV-1-derived SIN lentivirus vector expressing the *ABCD1* gene constitutively from a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer binding site substituted (MND) promoter [166]. Two patients were infused with G-CSF-mobilized CD34$^+$ cells transduced with this virus vector at an MOI of 25 after myeloablative conditioning with cyclophosphamide and busulfan due to the lack of a perceived selective advantage for gene-modified cells *in vivo*. Neither patient had an identified HLA-matched bone marrow or cord blood donor available. Hematopoietic recovery occurred within 2 weeks of transplant and immunologic recovery occurred within 1 year. Both patients' genetic mutation resulted in undetectable ALD transporter expression in peripheral blood cells, thus expression of transgenic *ABCD1* was used as a means of determining levels of gene-modified cells in circulation. Approximately 22–30% of peripheral blood cells expressed ALD transporter in the two patients at 2 months after gene therapy with copy numbers <1 per cell on average. Integration site analysis revealed polyclonal reconstitution with no evidence for dominant clones and identification of identical integration sites amongst purified cell populations of different lineages, suggesting true HSC gene modification had been achieved but at a very low level. Importantly, in both patients, arresting of cerebral demyelination occurred by 12–16 months after transplant and remained stable by MRI up to the latest follow-up (36 months). While this approach has been considered a breakthrough in treatment for X-ALD, additional studies aimed at enhancing the number of transduced HSCs, and thus shortening the time to demyelination arrest after transplant, are currently underway. While these initial results demonstrate the utility of lentivirus-mediated gene transfer to achieve therapeutic gene expression with safety (i.e., lack of insertional mutagenesis), additional follow-up is required to fully evaluate the long-term benefits and risks. However, another important feature of this research is the successful engraftment of gene-modified cells without an *in vivo* selective advantage after myeloablative conditioning, highlighting the importance of preparative regimens to achieve therapeutically beneficial hematopoiesis in gene therapy.

Metachromatic leukodystrophy

Metachromatic leukodystrophy(MLD) is an autosomal recessive genetic disorder resulting from mutations in either the arylsulfatase A (*ARSA*) gene or its activator protein, saposin-B (reviewed in [167]). Mutations in these genes result in variable ARSA expression and the accumulation of galatosyl and lactosyl sulfatides, manifesting in accumulation of these molecules in metachromatic granules of oligodendrocytes as well as many other cell types. Clinical symptoms vary with age of onset and affect both the central and peripheral nervous systems. As symptom onset can occur at any time during life and varies with stage of onset, MLD is divided into four onset categories: late infantile, early juvenile, late juvenile, and adult. Late infantile MLD and early juvenile MLD are the most commonly diagnosed stages, and all forms are considered terminal, with only supportive care currently available for patients. Recently, enzyme replacement therapy has entered early-phase clinical trials; however, blood–brain-barrier penetration has been a significant obstacle and efficacy data are pending (reviewed in [167]). There is conflicting evidence that bone marrow transplantation from unaffected donors results in significant therapeutic benefit, and this is thought to be due to the slow replacement of brain microglia and macrophage/histiocytes in the context of the rapidly progressive disease. Furthermore, the risks associated with allogeneic BMT still apply and not every patient has an available HLA-matched donor.

Preclinical studies of gene therapy using lentivirus vectors to deliver the ARSA gene under the control of a constitutive human phosphoglycerate kinase promoter resulted in significant therapeutic benefit in MLD mice, even more so than unaffected, non-gene-modified bone marrow, with reversal of neurologic deficits [168,169]. A phase 1 clinical trial to treat MLD patients was recently opened and initial reports of efficacy and safety are pending [52]. Preliminary reports have indicated that, like the ALD trial, patients were treated with fully myeloablative chemotherapy (busulfan) prior to infusion of gene-modified cells, and that marking in the first 6 months after gene therapy has remained stable [17].

Solid tumors

Glioblastoma multiforme

Glioblastoma multiforme (GBM) is a nearly uniformly fatal brain tumor for which decades of clinical research has provided little therapeutic options. Patients who are not eligible for surgical intervention who choose palliative care only survive a median of 3 months from diagnosis. The most effective therapy includes surgery to remove as much of the primary mass as possible, followed by cranial-focused radiation therapy with concomitant administration of temozolomide, an alkylating agent chemotherapy, followed by adjuvant cycles of temozolomide. However, this intense treatment strategy adds less than 2 years median survival to GBM patients (21 months). Additionally, for approximately 60% of GBM patients, epigenetic changes in tumor cells result in increased expression of endogenous methylguanine methyltransferase (MGMT), a protein which repairs cytotoxic DNA damage caused by temozolomide, rendering tumor cells resistant to standard therapy [170]. A small molecule, O6-benzylguanine, can irreversibly inactivate MGMT and is postulated to restore temozolomide sensitivity in GBM cells; however, early phase trials to assess the efficacy of this therapeutic strategy resulted in severe hematopoietic toxicity [171,172]. An earlier study had reported that MGMT expression is epigenetically silenced in most blood cells and is undetectable in HSCs, making them particularly sensitive to the combination of O6-benzylguanine and temozolomide [173]. Interestingly, toxicity of this regimen was not observed outside of the hematopoietic compartment. Thus, it was envisioned that gene therapy could provide MGMT expression within the marrow to protect the hematopoietic compartment from chemotherapy-induced toxicity, while brain tumor cells remained sensitive. As described earlier, chemotherapy-resistance genes, such as mutant *MGMT*, which confers resistance to alkylating agent chemotherapy, are a promising approach for mediating *in vivo* selection of gene-modified cells in patient populations where selective advantage is not conferred by gene modification. Specific for mutant *MGMT* is the ability to maintain DNA repair capacity while being insensitive to the inhibitory effects of O6-benzylguanine.

The first trial of HSC gene therapy with mutant MGMT was conducted in adult GBM patients who displayed the poor prognosis phenotype of unmethylated (expressed) MGMT in tumor cells [104]. Following surgical intervention and standard radiation without concomitant temozolomide, three patients were treated with single agent BCNU at a dose of 600 mg/m^2 as both a conditioning agent and as chemotherapy, since this agent is known to cross the blood–brain barrier and is active against GBM cells. Two days after BCNU administration, patients received G-CSF-mobilized CD34$^+$ cells transduced with a GALV-pseudotyped gammaretrovirus expressing the mutant MGMT P140K from the endogenous viral LTR promoter. Within 1 month after transplant, gene marking levels were between 35 and 60% of circulating white blood cells (Figure 8.5),

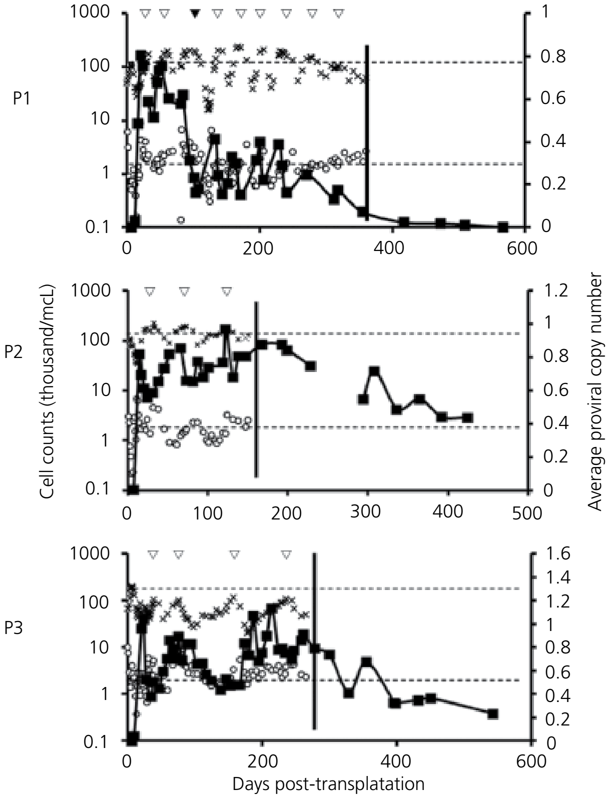

Figure 8.5 Persistance and chemoprotection of P140K gene-modified cells in three glioblastoma patients. Primary *y*-axis represents ANC, lymphocyte, and PLT counts in PB as a function of time and chemotherapy.Cell counts are in thousand per cubic millimeter (mm^3) and are shown for various time points after transplantation of gene-modified cells (*x*-axis). Crosses (x) represent PLTs and open circles (o) represent ANC. Dashed lines (--) represent retreatment threshold of PLTs (upper dashed line) and ANC (lower dashed line). Secondary *y*-axis represents average proviral copy number per genome as determined by real-time PCR at various time points post-transplantation (*x*-axis) in bulk WBCs (closed squares; ■). Inverted open triangles (▽) indicate cycles of O6BG/TMZ chemotherapy. Closed triangle (▼) in P1 graph represents single dose-escalated cycle of TMZ (590 mg/m^2). Source: Adair et al. 2012 [104]. Reproduced with permission from the American Association for the Advancement of Science.

and patients began receiving combination chemotherapy including O6-benzylguanine and temozolomide at a dose of temozolomide that was reported to be the maximum tolerated dose from the previous trials, which did not include gene therapy (472 mg/m^2). All three patients were able to receive more chemotherapy cycles than the average reported for patients in the prior trial without gene therapy, and all survived beyond the expected mean with one patient alive and apparently disease-free after nine chemotherapy cycles. Clonal analysis in these patients did reveal emergence of three dominant clones, two in the same patient, after two or three chemotherapy cycles, including integrations in proximity to the PR-domain-containing 16 (*PRDM16*) and *HMGA2* genes. However, this clonal skewing within the gene-modified cell pool occurred without continued expansion in the white blood cell compartment or evidence for MDS or leukemia in bone marrow or peripheral blood. The report also noted an enrichment for clones harboring provirus insertions in common genomic loci over time and chemotherapy, and these loci overlapped with those reported in other clinical trials using gammaretrovirus vectors for gene transfer. Importantly, none of the patients displayed extrahematopoietic toxicity during the study, underscoring the potential for this strategy in the treatment of cancer. Despite the lack of leukemic transformation in these patients, use of a lentivirus vector to mediate gene transfer is thought to be a safer alternative to avoid insertional mutagenesis and potential for transformation and may be used in future studies.

Infectious disease

HIV

While the development of highly active antiretroviral therapy (HAART) has greatly improved the outcome for HIV$^+$ patients, it is not curative, represents a large expense for patients, and carries associated risks for both therapy-related side effects and virus evolution. Recently, proof-of-principal evidence that transplantation of HSCs could result in a functional HIV cure resulted when an HIV$^+$ leukemia patient was transplanted with allogeneic bone marrow from a donor who carried a mutation in the C-C chemokine receptor type 5 (CCR5) gene following myeloablative chemotherapy and resulting in apparent elimination of detectable HIV in the recipient in the absence of HAART [53]. However, widespread use of this strategy is limited by the percent of mutant CCR5 carriers and the combined difficulty in identifying an HLA-matched bone marrow donor. As noted earlier, targeted gene disruption is one strategy currently being pursued as a means of generating autologous mutant CCR5 HSCs. Another strategy is to transfer genes whose products are capable of interfering with HIV infection to autologous HSCs. Transplantation of autologous HSCs in patients with high-risk or relapsed AIDS-related lymphoma (ARL) receiving myeloablative conditioning was shown to support long-term survival [174]. The same group first attempted gene therapy of HSCs in this population since these patients were already receiving autologous HSCs and myeloablative conditioning prior to cell therapy [175]. In this study, a total of seven patients with high-risk or relapsed ARL were consented to treatment, which included G-CSF (10 mcg/kg) for mobilization followed by leukapheresis to collect a total of 5×10^6 CD34$^+$ cells/ kg body weight. One half of these CD34$^+$ cells were cryopreserved as unmanipulated therapeutic product and the other half was subjected to lentivirus transduction with a VSV-G-pseudotyped vector encoding three HIV-counteracting transgenes. One gene product inhibited viral entry with a CCR5 ribozyme, a second inhibited viral RNA transport by RNA interference of the viral tat/rev element, and the third gene product inhibited initiation of viral transcription by expressing a decoy RNA. One patient displayed progressive disease preventing further treatment and a second patient failed to meet the established collection goal of 5×10^6 CD34$^+$ cells/kg. Of the five remaining patients, one patient was administered only unmanipulated cell product as the transduced cell product failed to meet release criteria (viability) prior to infusion. All five patients received myeloablative conditioning prior to cell infusion including BCNU, etoposide, and cyclophosphamide. The four patients who received gene-modified cell products also received unmanipulated cell products and all patients remained on HAART throughout the study. All patients engrafted by 11 days after transplant, as evidenced by absolute neutrophil count >500/μL for 3 consecutive days. *In vitro* assessment of transduction efficiency in 4-week culture-initiating cells isolated from transduced products was very low (0.11–0.15%). At the time of publication, 18 months of follow-up had been conducted and all patients were in remission from their lymphoma. Gene marking in these patients ranged from 0.02–0.32% of peripheral blood mononuclear cells. Viral load in these patients was undetectable, which is not surprising considering that HAART was maintained throughout the treatment period. One patient displayed an increased viral load of 890 gene copies per milliliter at 15 months after gene therapy, following HAART discontinuation between months 12 and 15. The patient resumed HAART after this test result and returned to undetectable viral load thereafter. It is postulated that both the co-infusion of unmanipulated cell products and continuity of HAART therapy may have contributed to the low-level gene marking observed in these patients.

Vector-mediated insertional mutagenesis

Leukemia in the SCID-X1, chronic granulomatous disease and Wiskott–Aldrich syndrome trials

The ability of retroviruses to efficiently integrate into the genome is also a disadvantage; retroviral vectors are insertional mutagens and can cause leukemias, as observed in the French SCID-X1, CGD, and WAS gene therapy trials. It has long been known that many gammaretroviruses (formerly oncoretroviruses) cause leukemia, but it was previously thought that replication-incompetent vectors had a low risk of insertional mutation leading to malignancy. Leukemias were not observed as a result of vector integration in previous clinical T-cell gene transfer experiments, possibly in part due to the biology of T cells [176]. Leukemias were also not observed in many HSC large-animal gene transfer experiments (reviewed in [61]). This is in contrast to replication-competent viruses, where multiple rounds of infection *in vivo* would greatly increase the risk for malignancy. Leukemia was observed in three of 10 rhesus macaques when replication-competent retrovirus contaminated a vector preparation used for gene transfer to enriched BM cells [177].

In all three trials in which myelodysplasia or leukemia developed, there was no evidence of replication-competent retrovirus, and studies were undertaken to characterize vector integration sites to understand the etiology of the transformed cells. In leukemic cells identified in both the X-SCID and WAS trials, vector integrants were observed near the *LMO2* and *CCND2* genes. The *LMO2* gene encodes a transcription factor involved in early hematopoiesis that had previously been involved in leukemogenesis via chromosomal translocation (reviewed in [178]). It is also unclear if the therapeutic transgene for γc was involved in the leukemias, possibly through aberrant IL receptor signaling. Expression of γc from a lentiviral vector was found to cause lymphoma in a mouse model [179], and a database of viral integrations from mouse tumors identified a leukemia with integrations at both the *IL2RG* and *LMO2* loci [180], suggesting that they could cooperate to contribute to leukemia. Overexpression of γc alone in human CD34$^+$ cells had no effect on T-cell development in vitro, and it has been suggested that integration at LMO2 may represent the primary oncogenic event and that restoring IL-7 signaling with the γc transgene creates a preleukemic condition of immature cells under intense proliferative pressure [181]. However, the development of leukemia with insertions proximal to *LMO2* in the WAS gene therapy trial demonstrated that insertional mutagenesis and genotoxicity of HSC gene therapy with gammaretrovirus vectors extends beyond X-SCID to other diseases. This data suggested that specific diseases, especially those where the therapeutic transgene confers an inherent selective advantage to gene-modified cells, may have inherent risks from a perspective of vector-mediated insertional mutagenesis, and that preclinical models may yield insights into the potential adverse side-effects of proposed clinical trials. However, the report of myelodysplasia as the result of retrovirus integration and insertional activation of the *MDS/EVI1* gene in patients with chronic granulomatous disease treated with gene therapy suggests that selective advantage of the therapeutic transgene may not be the only factor contributing to leukemogenesis. More confounding is the occurrence of expanded clones with or without proximal gene activation, which remain stably elevated for years without evidence for leukemic transformation, such as the *PRDM16* and *HMGA2* clones observed in the gene therapy trials including β-thalassemia and GBM patients. These observations support the growing mass of evidence suggesting that multiple genetic and epigenetic events are required to transform normal cells into tumor cells (reviewed in [17]).

Deregulation of host genes by vector proviruses

An area of active investigation towards safer HSC gene therapy is the development and testing of vectors with an improved safety profile. Gammaretroviral vectors have a preference to integrate close to the promoters of genes [182], lentiviral vectors have a preference to integrate within highly expressed transcripts [183], and foamy virus vectors integrate less frequently near promoters than gammaretroviral vectors and less frequently than both gammaretroviral and lentiviral vectors within transcripts [184]. Although the mechanisms of these integration preferences are not yet well understood, they appear to be defined in part by tethering interactions between the pre-integration complex and host proteins, and also by the state of host chromatin (reviewed in [185]). Integration by retroviral vectors can deregulate host genes by several mechanisms (Figure 8.6), with the potential to lead to clonal dominance, as observed in the CGD trial, and to leukemia as observed in the

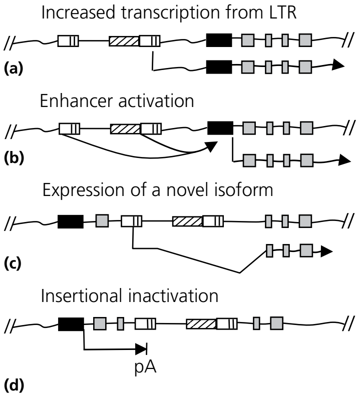
Increased transcription from LTR
(a)
Enhancer activation
(b)
Expression of a novel isoform
(c)
Insertional inactivation
pA
(d)

Figure 8.6 Deregulation of cellular genes by integrated vector proviruses. There are several mechanisms whereby integrated vector proviruses can deregulate the gene expression of proto-oncogenes, tumor suppressors, or genes involved in hematopoiesis. Four examples of deregulation are illustrated. The integrated vector provirus is indicated by two long-terminal repeats (LTRs) with an internal transgene indicated by a hatched box. The cellular gene promoter and exons are indicated by a black box and gray boxes, respectively. In (a), transcription initiated from the vector 3′ LTR transcribes the cellular gene at increased levels. In (b), enhancers in the vector LTRs activate transcription from the cellular promoter increasing transcription. In (c), transcription from the vector 5′ LTR results in a spliced mRNA that creates a novel truncated isoform of the cellular gene that may have distinct properties from the native form, including oncogenicity. In (d), the vector provirus is integrated between the second and third exons, thereby disrupting transcription of the cellular gene via premature polyadenylation using the 3′ LTR polyadenylation signal.

French SCID-X1 trial. Gammaretroviral vector integrants can deregulate the expression of genes over 500 kb from the insertion site [186], and a comparison of the frequency of integrants in canine repopulating cells shows that a significant percentage of gammaretroviral, lentiviral, and foamy virus vectors are observed near genes that have been associated with malignancy [187]. Thus, vector designs that reduce the ability of integrated vector proviruses to deregulate the expression of nearby genes should reduce the potential for malignancy.

Approaches to reduce the potential for vector-mediated malignancy

There are several modifications that can be made to improve vector safety, including using SIN vectors. Unlike the LTR-driven vectors used in the SCID-X1 trials, in SIN vectors the promoter–enhancer sequences are removed, greatly reducing the potential for transcriptional activation by the 3′ LTR and for enhancer activation by both LTRs. Inserting insulator sequences into the LTRs that interfere with enhancer activation of nearby genes has also been proposed as a safety feature, and experiments in a human T-cell line suggest that lentiviral vectors with a chicken HS4 insulator may have a reduced potential for clonal dominance [188]. In the reported gene therapy trial for β-thalassemia discussed earlier, a therapeutic β-globin-expressing lentiviral vector with a chicken HS4 insulator was used. Interestingly, in the dominant clone observed in this study, only one copy of the HS4 250 bp core element was present in this vector, despite the fact it was engineered to have two copies. The single 250 bp core region does not contain the full insulating capability of the 1.2 kb chicken HS4 insulator, which may have contributed to the inability of this vector modification to block clonal dominance. A straightforward approach to improve safety is to use lineage-specific promoters in the context of a SIN vector to restrict gene expression to the desired hematopoietic lineage. This should reduce the possibility of deregulating proto-oncogenes in stem cells and in lineages where gene expression is not required (reviewed in [189]).

Efforts to target the integration of retroviral vectors to defined locations by modifying components of the retroviral pre-integration complex have also been explored. Yeast LTR retrotransposons provide a compelling precedent for targeted integration into a eukaryotic genome. Yeast retrotransposons have many similarities to retroviruses yet have a remarkable specificity for integration. The Ty3 retrotransposon integrates in a small window, two or three nucleotides upstream of Pol III transcripts. To date, targeting the integration of retroviral vectors has been much less efficient. In one study, a zinc finger protein with a unique address was fused to the lentiviral Pol [190]. The integration profile was modified, but titers were greatly reduced, limiting the potential of this approach. Re-targeting of lentiviral vectors has been achieved using engineered target cells that express modified versions of the host protein lens epithelium-derived growth factor (LEDGF). LEDGF contains a domain that binds the HIV integrase and interacts with the HIV pre-integration complex, thereby tethering the complex to sites where LEDGF binds. By modifying the binding specificity of LEDGF, HIV integration can be re-targeted [191–193]. This approach suggests that efficient re-targeting of retroviral vectors can be achieved. However, the challenge is now to devise an approach to efficiently re-target integration in normal CD34$^+$ cells that have not previously been modified with an altered LEDGF.

Another approach is to avoid integration by correcting HSC genes or introducing therapeutic genes at specific chromosomal locations using a gene targeting approach. The frequency of conventional gene targeting by transfection is low in human primary cells, but targeting frequencies can be greatly increased by cleaving the target site with a nuclease to stimulate homologous recombination. Modification of the γc locus was obtained in up to 18% of human hematopoietic cells using a zinc finger nuclease (ZFN) [194], and targeted gene addition using this approach is also remarkably efficient in hematopoietic cell lines [195]. Integrase-deficient lentiviral vectors have been developed for the efficient delivery of DNA donor templates and ZFNs to stem cells. Non-integrating lentiviral vectors that express ZFNs have led to targeting in up to 30% of cells in hematopoietic cell lines, with targeted gene addition in up to 5% of human hematopoietic progenitor cells [196].

Summary and future directions

Advances in the ability to enrich and transduce human repopulating cells have led to recent successes in HSC gene therapy. The unfortunate occurrence of leukemia in three clinical trials of gammaretrovirus-mediated HSC gene therapy has stimulated research into defining the relative safety of different gene transfer approaches and developing safer vectors. However, it also needs to be emphasized that for some diseases like SCID-ADA, even current vector technology appears to be safe and highly efficient. For diseases that have been associated with insertional mutagenesis, novel vector systems and gene targeting/editing approaches are being explored. A number of recently described nucleases including ZFNs, homing endonucleases (HEs), and Tal effector nucleases (TALENs) are currently in preclinical studies. The use of ZFNs to disrupt the CCR5 locus in T cells of HIV$^+$ patients is currently in clinical studies. Thus, over the next 5 years it can be expected that HSC gene therapy for most of the afore-mentioned diseases will shift to newer, safer vector systems like lentivirus vectors, foamy virus vectors, or alpha retrovirus vectors. For some diseases that can be treated with gene disruption, such as CCR5 disruption for generating cells resistant to HIV infection, we would also expect to see nuclease-mediated gene editing studies. These advances will likely translate to the cure of many genetic diseases affecting the hematopoietic system and extend to other diseases like HIV, where gene therapy can potentially restore immune function, as well as certain cancers where HSC gene therapy can improve chemotolerance of blood and marrow cells potentially resulting in more effective chemotherapy dosing and improved survival.

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CHAPTER 9

Overview of Hematopoietic Cell Transplantation Immunology

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Introduction

Immunology plays a central role in allogeneic hematopoietic cell transplantation (HCT). Any appreciation of the immunologic mechanisms involved in engraftment, graft-versus-host disease (GVHD), control of malignancy, the development of tolerance, and immune reconstitution requires some understanding of the cellular and molecular components involved in immune reactions provoked by grafting tissue from one individual to another. Insight into the cellular basis of alloreactivity requires an understanding of immune recognition, the development of the immune system, and the nature of immune responses. This chapter will serve to introduce the reader to the immunology of HCT. Citations are made to selected reviews and reports from the recent literature and to other chapters that provide detailed information.

Fundamental differences between pathogen and transplantation responses

Since the immune system evolved as a defense against pathogens and not under the selective pressure of HCT, the key differences between antipathogen and alloimmune responses deserve consideration (Table 9.1) [1]. A working paradigm for antipathogen immunity holds that pathogens are first encountered by innate cells, including antigen-presenting cells (APCs). These cells detect the presence of pathogen-derived products through an array of sensing mechanisms that decode the nature of the pathogen and initiate the production of specific signals and mediators directed to the particular class of pathogen. These innate cells directly mediate antipathogen immunity and, in addition, they present pathogen-derived antigens to T lymphocytes (T cells), thereby initiating an adaptive immune response. The combined innate and adaptive responses work together to contain and clear the pathogen. In HCT, no known equivalent of a pathogen-derived product alerts either donor or recipient innate cells to the presence of alloantigen. Yet, the infused donor cells both provoke an immune response by recipient immune cells that survive conditioning regimens and generate a potent immune response against the recipient. Pathogens and alloantigens also differ in their persistence and locations. Pathogens typically are tropic only for subsets of cells, and only a fraction of APCs will therefore present pathogen-derived products. In HCT, every recipient cell is a source of alloantigen, every donor-derived APC has ready access to alloantigen, and the supply of recipient antigen is unlimited, akin to chronic infections. Likewise, for rejection responses, all donor cells and APCs are sources of alloantigen. Other relevant differences are worth considering. Mature donor T cells, the primary mediators of GVHD and graft-versus-leukemia effects (GVLEs), are infused and must find their way to sites where alloantigen is presented, whereas they are already present in secondary lymphoid organs at the time of pathogen exposure. Finally, most conditioning regimens and some therapies used to control malignant cells before HCT create a lymphopenic environment, which can have significant effects on T-cell activation and the development of effective responses.

Fundamental differences between hematopoietic cell transplantation and solid organ transplantation

HCT differs fundamentally from grafting of most other organs. In solid organ transplantation, the graft generally contains only limited numbers of cells with immunologic function. The primary clinical concern rests with preventing rejection by the recipient immune system. Lifelong administration of immunosuppressive medication is generally required in order to prevent rejection not only by cellular and humoral mechanisms in the recipient at the time of transplantation, but also by those generated from recipient-derived immunologic precursors after transplantation.

The preparative regimen administered before HCT (see Chapters 19–23) eliminates a variable number of precursors and mature cells of the recipient immune system, depending on the intensity of the regimen. The graft contains large numbers of precursors and mature cells that replace those of the recipient. Thus, the donor graft generates the immune system in the recipient. The primary clinical concerns rest not only with preventing graft rejection by recipient cells that survive the conditioning regimen, but also with preventing donor cells from causing immune-mediated injury (GVHD) in the recipient, while allowing immune reconstitution for recognition and control of pathogens. Immunosuppressive medications

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Table 9.1 Immune responses to transplantation versus pathogens

Feature	Infection	GVHD
Source of responding T cells	Resident	Infused
Specific pathogen	Yes	No
APC maturation linked to antigen presentation	Yes	No
Antigen eliminated	Variable	No
Antigen expression	Generally localized	Ubiquitous
Specific induction of signals for T-cell entry into tissues	Yes	No
Donor–recipient APC chimerism	No	Yes
Immunodominance	Yes	Yes

Source: adapted from Shlomchik 2007 [1].

(see Chapter 81) are administered after transplantation primarily to prevent GVHD. Eventually, it becomes possible to discontinue such treatment in most patients. The subsequent persistence of engraftment and the absence of GVHD, together with recovery of host immune defenses, indicate that a state of immunologic "tolerance" has been achieved between the donor and recipient, although the mechanisms that maintain this tolerance are not fully understood.

Transplantation antigens

Major histocompatibility antigens

Immune reactions provoked by grafting tissue from one individual to another are caused by transplantation or histocompatibility antigens (see Chapter 10). Genes encoding transplantation antigens are located both within and outside the major histocompatibility complex (MHC) [2]. The human MHC is located on the short arm of chromosome 6 and contains a series of genes encoding two distinct types of highly polymorphic cell surface glycoproteins termed human leukocyte antigens (HLAs). HLA class I antigens contain a single polymorphic α chain non-covalently associated with β_2-microglobulin. HLA class I antigens encoded by three loci termed HLA-A, HLA-B, and HLA-C are known to provoke immune reactions in HCT. The role of antigens encoded by other class I loci has not yet been defined. As of August 2012, 1448 HLA-A alleles, 1988 HLA-B alleles, and 1119 HLA-C alleles with protein-changing exon polymorphisms had been described (http://www.ebi.ac.uk/imgt/hla/stats.html).

HLA class II antigens are encoded by three clusters of loci (HLA-DR, -DQ, and -DP) and contain a single α chain non-covalently associated with a β chain. The α chains of the HLA-DR, -DQ, and -DP antigens are encoded by the respective *DRA*, *DQA1*, and *DPA1* genes, while the β chains are encoded by the respective *DRB*, *DQB1*, and *DPB1* genes. Nine distinct *DRB* genes encode polymorphic HLA-DR β chains. Antigens encoded by the *DRB1*, *-DQ*, and *-DP* genes are known to provoke reactions in HCT. The role of antigens encoded by other *DRB* genes has not yet been defined. As of August 2012, 2 HLA-DRA alleles, 860 HLA-DRB1 alleles, 29 DQA1 alleles, 126 DQB1 alleles, 17 DPA1 alleles and 134 DPB1 alleles with protein-changing exon polymorphisms had been described. Extensive polymorphism of HLA class I and class II molecules has evolved as a mechanism to protect both individuals and the population by increasing the range of pathogen-associated antigens that can be recognized by the immune system.

HLA matching

The genes encoding HLA class I and class II antigens are tightly linked, such that they are inherited in families as "haplotypes" with low recombination frequencies [2]. An HLA-matched sibling can be readily identified by an informative family study, especially if family members are HLA-heterozygous and if the parental haplotypes can be identified by reliable markers and clearly assigned by segregation analysis. A given patient has a 0.25 probability that any one sibling inherited the same paternal haplotype and the same maternal haplotype, thereby being "HLA-genotypically identical." Siblings identified in this way are identical not only for the polymorphisms detected by HLA typing, but also for all other polymorphisms in the MHC. The term "HLA-matched" has a different significance when applied to unrelated individuals. In this context, the assessment of matching is based on the relatively limited number of polymorphisms encompassed by the testing. Unrelated individuals who are HLA-phenotypically identical have considerable mismatching at MHC loci that are not assessed by the methods used for HLA typing. Because of the highly polymorphic nature of HLA antigens, the probability that two unrelated individuals have the same HLA-A, -B, -C, -DR, -DQ, and -DP alleles is extremely low.

HLA-haploidentical related pairs have a shared haplotype that is identical by descent for all polymorphisms in the MHC and two distinct non-shared haplotypes, one in the donor and the other in the recipient. Matching of the non-shared haplotypes is based on the relatively limited number of polymorphisms encompassed by the testing, except in cases where the non-shared haplotypes differ because of parental meiotic recombination. In these cases, a portion of the non-shared MHC haplotypes is identical by descent for all polymorphisms.

Structure of HLA and cell biology of antigen presentation

MHC class I and class II molecules bind short peptides for display at the cell surface, where they can be recognized by T cells (Figure 9.1) [3]. Each specific MHC molecule displays a characteristic preference for binding peptides with distinctive characteristics, and this preference in part is responsible for the MHC-restriction of T-cell responses. MHC class I and class II antigens have similar structures [2]. The general structure consists of a floor formed by eight antiparallel β strands overlaid by two α helices in antiparallel orientation to each other, leaving a groove or cleft between them for binding of small peptides. The polymorphic residues of HLA molecules are located predominantly in the β-strand floor and in positions on the α helices that point into the groove between the two helices, where they help to determine the repertoire of peptides that bind to a given HLA molecule.

Since MHC molecules present alloantigen to alloreactive T cells, it is important to understand how protein antigens are processed into peptides and how MHC molecules present these peptides. In nearly all nucleated cells, peptides that bind MHC class I molecules can be generated from endogenous proteins [3]. In a much smaller subset of cells, including some APCs, peptides that bind MHC class I molecules can be generated from endocytosed proteins, from proteins contained in endocytosed cells or cell fragments, or from proteins taken up by surface receptors [4]. This process is called "cross-presentation." Ubiquitin-modified proteins of endogenous or exogenous origin are degraded in the cytosol by proteasomes [5]. These protease enzymes exist as a constitutive form in all cells and as an induced "immunoproteasome" form in cells stimulated with interferons. The peptides produced by immunoproteasomes differ from those produced by constitutive proteasomes and could be particularly relevant in the context of transplantation. The resulting peptides are transferred into the lumen of the endoplasmic reticulum

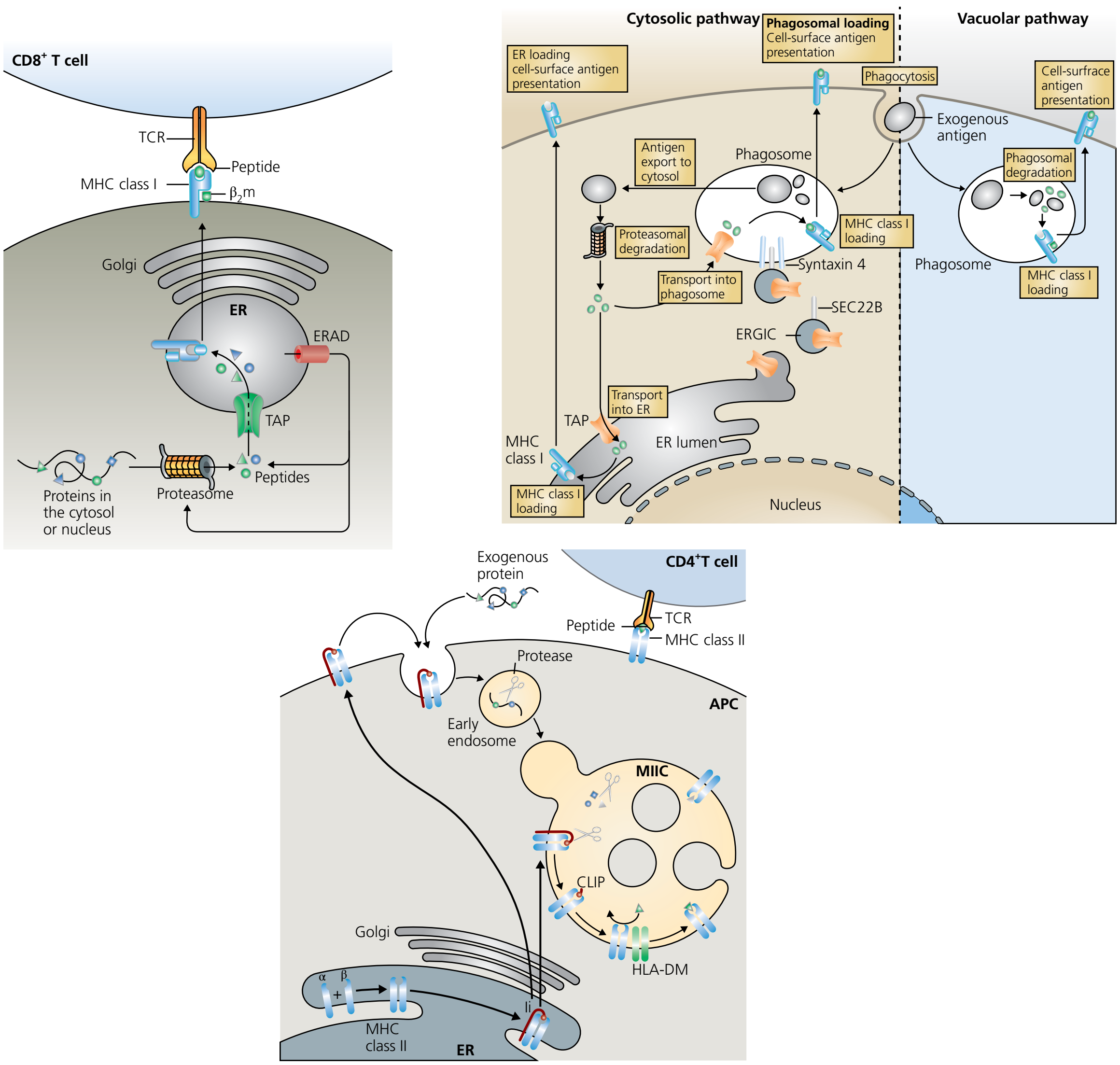

CD8⁺ T cell
TCR
Peptide
MHC class I
β2m
Golgi
ER
ERAD
TAP
Proteins in the cytosol or nucleus
Proteasome
Peptides
Cytosolic pathway
Phagosomal loading
Cell-surface antigen presentation
Vacuolar pathway
ER loading cell-surface antigen presentation
Phagocytosis
Cell-surface antigen presentation
Exogenous antigen
Antigen export to cytosol
Phagosome
Phagosomal degradation
Proteasomal degradation
MHC class I loading
Transport into phagosome
Syntaxin 4
Phagosome
MHC class I loading
SEC22B
ERGIC
Transport into ER
TAP
MHC class I
ER lumen
MHC class I loading
Nucleus
Exogenous protein
CD4⁺T cell
Peptide
TCR
MHC class II
Protease
APC
Early endosome
MIIC
CLIP
HLA-DM
Golgi
α
β
MHC class II
Ii
ER

Figure 9.1 Antigen presentation. Left side: MHC class I presentation. Peptides are generated in the cytosol by proteasome-mediated cleavage of endogenous intracellular proteins. Peptides are also delivered to the cytosol through endoplasmic reticulum-associated degradation (ERAD). Cytosolic peptides are channeled into the endoplasmic reticulum (ER) through the transporter associated with antigen presentation (TAP), loaded into MHC class I molecules and delivered to the cell surface through the Golgi apparatus for antigen presentation to CD8⁺ T cells. Right side: MHC class I cross-presentation. In the vacuolar pathway, material ingested by phagocytosis is degraded into peptides in the phagosome, and loaded into MHC class I molecules and delivered to the cell surface. In the cytosolic pathway, material ingested by phagocytosis is exported to the cytosol and digested into peptides by proteasomes. Peptides are then transported into either endoplasmic reticulum or phagosomes for loading into MHC class I molecules and delivery to the cell surface. Peptide transport into both compartments is mediated by TAP. Incorporation of TAP into phagosomes is mediated via transfer from the ER into the ER-Golgi intermediate compartment (ERGIC). The interaction of SEC22 with syntaxin 4 mediates the transfer of TAP from the ERGIC into phagosomes. Bottom: MHC class II presentation. Exogenous proteins ingested via phagocytosis, endocytosis, pinocytosis and uptake receptors are digested by proteases in early endosomes and in the MHC class II compartment (MIIC). MHC class II molecules are synthesized in the endoplasmic reticulum and complexed with the invariant chain (Ii). The complexes are transported directly or via surface endocytosis to the MIIC. In this compartment, Ii chains are cleaved by proteases, leaving the class II-associated Ii peptide (CLIP) in the groove between the α helices of MHC molecules. CLIP is replaced by internalized peptides through the influence of a molecular chaperone (HLA-DM in humans, H2-M in mice). Peptide-loaded MHC class II molecules are delivered to the cell surface for antigen presentation to CD4⁺ T cells. Source: Adapted from Neefjes et al. 2011 [3] and Joffre et al. 2012 [158] with permission of Nature Publishing Group.

by an adenosine triphosphate-dependent transport mechanism. The transport mechanism functions most efficiently with peptides containing 8–12 amino acids with a hydrophobic residue at the carboxy terminus. Peptides bind MHC class I molecules in the endoplasmic reticulum.

Peptides that bind MHC class II molecules generally originate from extracellular proteins that are taken into cells by endocytosis and other mechanisms [3], although direct presentation of intracellular proteins also occurs. Acidification of endosomes activates proteases that degrade internalized proteins into peptides. Peptides bind MHC class II molecules when endosomes fuse with vesicles containing newly synthesized membrane proteins being transported to the cell surface. Binding of peptides to MHC class I and class II molecules is dictated by the way that the side chains of specific residues of the peptide fit into pockets formed by polymorphic residues lining the groove between the α helices of MHC molecules.

Minor histocompatibility antigens

Minor histocompatibility antigens (mHAs) are the MHC-binding peptide products of coding polymorphisms that distinguish recipients and donors [2,6], whereas major histocompatibility antigens originate from the polymorphisms of intact MHC molecules. mHAs can be generated by a variety of mechanisms, including gene insertions and deletions, differences between Y and X chromosome homologs, amino acid coding changes resulting from single nucleotide polymorphisms, allelic mRNA splice variants, and post-translational modifications of amino acids. Proteins that give rise to mHAs molecules must be processed into peptides for MHC presentation as described above. Recognition of an mHA by T cells is specified both by the polymorphic peptide and the MHC molecule to which it is bound and by the availability of T cells with receptors capable of binding the peptide–MHC combination. Polymorphisms producing a single amino acid substitution in a peptide presented by MHC molecules can be sufficient to generate a T-cell alloimmune response in the absence of any MHC disparity. Only a few mHAs have been defined biochemically. Genetic studies in mice have identified more than 40 loci that encode mHAs, each typically having two known alleles. Human genomes contain more than 15,000 protein-changing single-nucleotide polymorphisms. Because these polymorphisms are distributed throughout the entire genome, siblings other than identical twins always have considerable disparity for mHAs, even when they are HLA-matched, but the immune system responds to only a small number of "immunodominant" antigen mismatches between the donor and recipient.

Differences in responses against major and minor antigens

Immune responses against MHC antigens differ greatly from responses against mHAs [7]. The frequency of T cells that respond to any given MHC antigen has been estimated at 1–10%. For this reason, generation of an in vitro response against MHC antigens does not require in vivo priming. MHC antigens also induce alloimmune antibody responses detectable by serologic testing. The frequency of T cells that respond to any given mHA has been estimated at 0.01–0.10%. Generation of an optimal in vitro response against mHAs typically requires prior in vivo priming, but with repeated stimulation, expanded T-cell populations reactive against mHAs can be generated from naïve T cells [8]. Polymorphic peptides loaded into MHC molecules do not generate antibody responses, but donor B cell-derived antibodies can be generated against recipient-specific proteins [9].

At least two mechanisms might account for the higher precursor frequencies of T cells against major histocompatibility antigens compared with mHAs. First, a given major histocompatibility antigen binds a diverse set of peptides that can all be presented to T cells, and the collective set of peptide–MHC complexes elicits a response from multiple sets of T-cell clones, with each set specific for a different peptide–MHC complex. In contrast, mHAs are composed of a single peptide presented to T cells by a specific MHC molecule, and the complex elicits a response from a single set of T-cell clones, all specific for the same peptide–MHC complex. This framework is consistent with observations that the variety of epitopes and numbers of responding T cells are far greater for major histocompatibility antigens than for mHAs. A second hypothesis to account for the high precursor frequency of T cells reactive against non-self MHC proteins is that certain T-cell clones can recognize a specific MHC molecule in conjunction with a variety of peptides, whereas T-cell clones specific for mHAs recognize a single peptide in conjunction with a specific MHC molecule. According to this scenario, the T-cell receptor (TCR) binding energy is presumed to derive primarily from interactions with the MHC molecule as opposed to the peptide. The ability to recognize a major histocompatibility molecule in conjunction with a variety of peptides greatly increases the cell surface density of epitopes available for stimulation of a T-cell clone and perhaps permits TCRs with lower affinity to contribute to T-cell activation. Regardless of mechanism, the higher frequency of T cells capable of recognizing a major histocompatibility antigen versus an mHA has an important practical consequence: for any given number of donor T cells in the graft, GVHD caused by major histocompatibility antigens is more severe than GVHD evoked by mHAs.

Movement of cells in the immune system

The development of an immune response requires highly orchestrated patterns of cellular migration throughout the body tissues. The thymus and marrow are the primary lymphoid organs where T and B cells, respectively, are produced, and the spleen, lymph nodes, intestinal Peyer's patches, and other specialized sites such as bronchial and mucosal-associated lymphoid tissues are secondary lymphoid organs where lymphoid cells mature and initiate immune responses. During the afferent phase of the immune response, secondary lymphoid organs and tissues function as central meeting points where highly dynamic processes of cell movement allow responding T and B cells to encounter antigen-bearing APCs. Some APCs migrate to secondary lymphoid organs and tissues after acquiring antigen in the periphery, whereas others reside within secondary lymphoid organs and tissues and acquire antigen through direct infection, capture of free antigen, or engulfment of other antigen-bearing cells [10,11]. In the context of HCT, all recipient APCs can directly present alloantigens, and all donor-derived APCs have widespread access to recipient proteins containing alloantigen peptides that can be indirectly presented. During the efferent phase of the immune response, activated T cells leave the secondary lymphoid organs and tissues and migrate to the peripheral tissues, and activated B cells form germinal centers for production of antibody, memory B cells, and plasma cells. Movement of T cells across vascular endothelium into secondary lymphoid tissues during the afferent phase of the immune response and out of these tissues and into peripheral tissues during the efferent phase is regulated by selectins [12], integrins [13], chemokines [14], sphingosine-1-phosphate receptors [15], and certain vitamins.

Innate and adaptive immune responses

As discussed below, triggering of adaptive immunity to many pathogens is linked to prior activation of the innate immune system, which comprises both rapidly engaged antipathogen effectors and APCs that initiate adaptive immunity. The hypothesis that the immune system is endowed with receptors that can identify pathogen-associated molecular products (PAMPs) [16,17] was confirmed first in the identification of transmembrane Toll-like receptors (TLRs) [18]. This family of receptors can recognize a variety of PAMPs, including lipopolysaccharides (LPSs), DNA, RNA, and peptidoglycans [19a]. TLRs encounter ligands either at the cell surface or in endosomes. C-type lectin receptors (CLRs) are a second class of transmembrane cell surface glycoproteins that recognize PAMPS such as mannans and β-2-glucan [19b]. The cytoplasm also contains PAMP recognition systems. Nucleotide-binding oligomerization domain (NOD) leucine-rich-repeat (LRR)-containing receptors (NLRs) recognize a variety of PAMPs and the sequelae of cell stress and mediators of sterile inflammation such as urate and asbestos [20]. Rig-1-like receptors (RLRs) recognize viral RNA [21]. Finally, the cytoplasm can sense nucleic acids through mechanisms that activate signaling pathways converging on STING (stimulator of interferon genes). These detection systems lead to activation of NF-κB, generation of inflammatory mediators such as cytokines and interferons, and upregulation of costimulatory molecules. In sum, these downstream effects both endow the innate cells themselves with antipathogen properties and allow APCs to mold an adaptive T response appropriate to the invading organism or source of stress.

Barrier organs such as the intestinal tract, skin, and lung have highly sophisticated mechanisms that regulate interactions between the innate and adaptive immune systems, since they must be able to distinguish invading pathogens from commensal organisms that may also express PAMPs [22,23]. In the context of an alloimmune T-cell response, every APC is likely to be presenting some alloantigens, and engagement of any of these detection systems could affect the alloimmune response. Consistent with this hypothesis, polymorphisms of some of these receptors in humans have been associated with transplant outcomes, including GVHD. In murine models, alloimmune T-cell responses can occur when APCs lack the ability to respond to any one of the major classes of these receptors, indicating that no single innate receptor system is required [24].

Regulation of natural killer-cell responses

Natural killer (NK) cells are a component of the innate immune system [25] (see Chapter 11). These cells emerge from the marrow, develop in lymph nodes and tonsils, and express CD56 and CD16 but not CD3. NK cells mediate their effects through cytotoxic activity and production of cytokines such as interferon-gamma (IFN-γ) [26,27], tumor necrosis factor-alpha (TNF-α) [28], and granulocyte–macrophage colony-stimulating factor (GM-CSF). NK cells express both activating and inhibitory cell surface receptors, and NK cell function is regulated through the overall balance of activation and inhibitory signals mediated through these receptors (Figure 9.2) [29]. The importance of NK cells for transplantation comes from their ability to recognize MHC class I alloantigens [30].

Human NK cells collectively express a variety of killer lectin-like receptors associated with CD94, killer-cell immunoglobulin-like receptors (KIRs), and natural cytotoxicity receptors. Murine NK cells have killer lectin-like receptors but not KIRs. Individual NK cells express a limited number of receptors through stochastic mechanisms. Receptors with short cytoplasmic tails associate with signaling protein molecules containing immunoreceptor tyrosine-binding activation motifs (ITAMs) that activate NK cells. Receptors with long cytoplasmic tails containing immunoreceptor tyrosine-binding inhibitory motifs (ITIMs) inhibit activation of NK cells. Most lectin-like receptors and KIRs bind to MHC class I ligands. NKG2D is an unusual lectin-like activating receptor that recognizes the MHC class I-like molecules MIC-A and MIC-B and other molecules that are expressed in response to cellular stress [31]. Natural cytotoxicity receptors activate NK cells in response to infection, but the ligands for these receptors have not been well defined.

During their development, NK cells are “educated” to acquire functional competence and self-tolerance such that they do not respond to cells that have normal expression of the self-MHC class I molecules that function as ligands for inhibitory receptors, except when signals from activating receptors such as NKG2D overwhelm the effect of inhibitory signals [29,32–34]. Under certain circumstances, donor NK cells can recognize malignant cells in the recipient and contribute to a GVLE, either because malignant cells in an MHC-mismatched recipient lack expression of donor MHC class I molecules that function as ligands for inhibitory receptors on donor NK cells, or because the expression of activating ligands by malignant cells overwhelms the effect of inhibitory ligands.

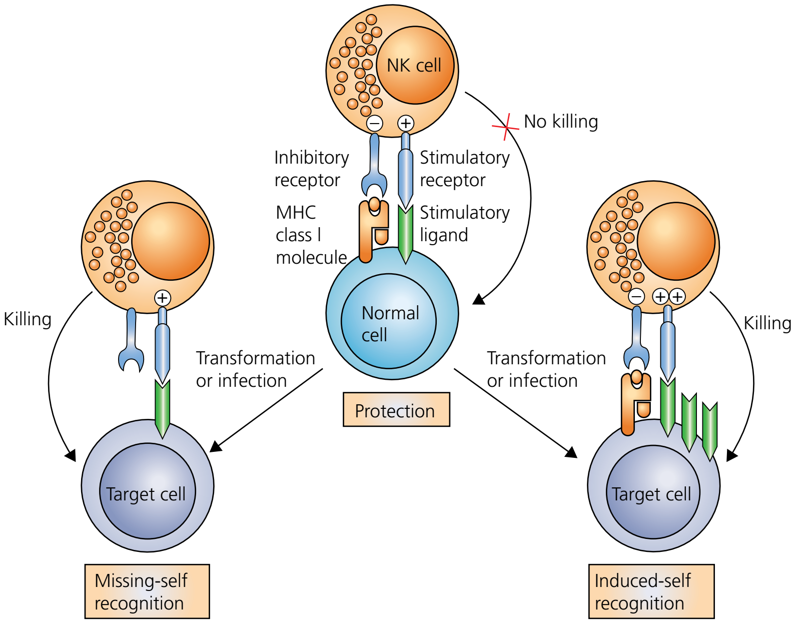


Figure 9.2 NK cell activation. NK cells express both inhibitory and stimulatory or activating receptors. NK cells do not kill normal cells, because binding of self-MHC class I molecules to inhibitory receptors on the NK cell override any activating signals originating from stimulatory ligands on normal cells. NK cells can kill target cells that are altered by infection or malignant transformation either because expression of self-MHC class I molecules is lost or downregulated (left side) or because expression of stimulatory ligands is increased (right side). Source: adapted from Raulet and Vance 2006 [29] with permission of Nature Publishing Group.

Self-tolerance and antigen recognition by T cells

Molecules used for recognition of antigens

Adaptive immune responses are orchestrated and mediated by T and B lymphocytes. In T cells, immune recognition is mediated by the T-cell receptor (TCR); in B cells, immune recognition is mediated by immunoglobulin molecules. In general, each T cell and B cell expresses a single type of receptor for recognizing antigen epitopes. Both types of cell utilize similar mechanisms for generating an enormous diversity of clonally distributed receptors. This diversity is generated initially by somatic rearrangements that link *V* (variable), *D* (diversity), and *J* (joining) segments in various combinations to encode a large repertoire of distinct clonally distributed receptors among T cells and B cells. TCRs recognize peptide antigens presented by MHC molecules, whereas B cell receptors and secreted immunoglobulin (Ig) recognize proteins free of any presenting molecule. High-throughput sequencing of TCR and Ig gene rearrangements can characterize the clonal repertoire of T and B cells and may help elucidate the biology of these responses in immunologic reactions such as GVHD and GVLEs.

TCR signaling

Signal transduction in T cells leads to transcriptional activation of numerous genes by activator protein-1 (AP-1), nuclear factor-kappa B (NFκB), and nuclear factor of activated T cells (NF-AT) [35]. In broad outline, the process begins by the formation of "immunologic synapses" that concentrate TCR–CD3 complexes, CD2, and CD4 or CD8 within small regions of the membrane [36–38]. Molecules associated with the cytoplasmic domains of TCR–CD3 complexes and CD4 or CD8 assemble as a scaffold that initiates a cascade of protein tyrosine phosphorylation events leading to activation of phospholipase Cγ. Phospholipase Cγ cleaves phosphatidylinositol 4,5-diphosphate (PIP_2) in the plasma membrane to form diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). DAG activates protein kinase Cθ, leading to translocation of NFκB from the cytosol to the nucleus. In the nucleus, NFκB family members function as transcriptional regulators for numerous cytokine genes, including *IL-2*. DAG also activates the p21 Ras pathway, leading to activation of Fos, which functions as a component in the AP-1–promoter complex for transcription of *IL-2* and other genes involved in activation. IP_3 releases Ca^{2+} from intracellular stores and allows influx of extracellular Ca^{2+}, thereby activating calcineurin, an enzyme with serine/threonine phosphatase activity. Activated calcineurin dephosphorylates NF-AT, which then translocates from the cytosol to the nucleus, where it activates transcription in conjunction with AP-1. The immunosuppressive drugs cyclosporine and tacrolimus bind to intracellular proteins, forming drug–protein complexes that inhibit the enzymatic activity of calcineurin, thereby preventing activation of NF-AT and interfering with signal transduction in T cells after stimulation with antigen.

Selection of T cells in the thymus

Precursors destined to become T cells originate from hematopoietic stem cells that migrate to the thymus (Figure 9.3). T-cell maturation in the thymus "positively" selects T cells that have receptors with modest affinity for self-MHC–peptide complexes while deleting T cells that have receptors with high affinity for self MHC–peptide complexes ("negative selection") [39–41]. T cells with receptors that fall below the affinity threshold for positive selection die by "neglect." The process of "positive selection" is mediated primarily by thymic cortical epithelial cells. Negative selection is mediated by marrow-derived dendritic cells (DCs) and by thymic medullary epithelial cells. In particular, thymic medullary epithelial cells have "promiscuous" expression of proteins that are "tissue-specific" for organs outside the thymus, thereby eliminating T cells that would be self-reactive in the periphery. Interactions with thymic epithelial cells can also cause developing T cells to become nonresponsive or "anergic" to self-MHC molecules. As an alternative fate, T cells that have TCRs with high affinity for self can be induced to express the transcription factor FoxP3. These cells then differentiate into regulatory T (Treg) cells that suppress T-cell immune responses in the periphery [42]. Negative selection, induction of non-responsiveness, and generation of Treg cells represent the principal "central" mechanisms for establishing self-tolerance (see Chapter 12).

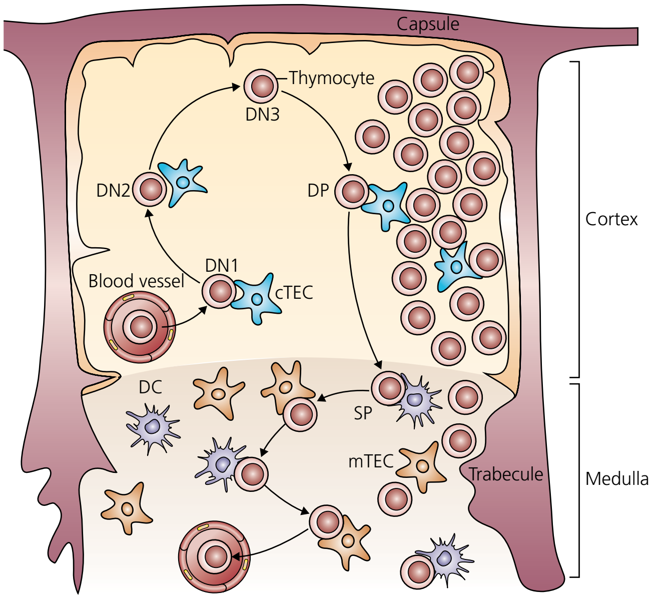


Figure 9.3 Thymic selection. Marrow-derived progenitors enter the thymus through blood vessels and go through three stages of development in the cortex, beginning as "double-negative" (DN) cells lacking expression of either CD4 or CD8 and ending as "double-positive" (DP) cells expressing CD4, CD8, and T-cell receptor β (TCRβ). DP cells with TCRβ receptors that bind to MHC molecules on cortical epithelial cells are positively selected, whereas those lacking TCRβ receptors that bind to MHC molecules on cortical epithelial cells die through neglect. Positively selected DP cells lose expression of either CD4 or CD8, and the "single-positive" (SP) cells expressing either CD4 or CD8 move to the medulla, where they interact with medullary thymic epithelial cells (mTEC) and dendritic cells (DC). SP cells with TCR that have high avidity for MHC molecules on mTEC or dendritic cells die through negative selection. T cells that emerge from the thymus express TCR that have intermediate avidity for self-MHC molecules. Source: adapted from Klein et al. 2009 [40] with permission of Nature Publishing Group.

T-cell activation, effector differentiation, and effector functions

Activation of T cells by antigen-presenting cells

DCs, B cells, macrophages and basophils are the major classes of hematopoietic cells that can prime naïve T cells and activate memory T cells. DCs comprise a heterogeneous collection of different cell types that have in common the expression of CD11c and MHC class II molecules. DC subsets arise from different bone marrow-derived precursors through distinct developmental pathways, frequently further influenced by inflammation [43,44]. DC subsets reside in different locations (e.g., peripheral tissues versus primary or secondary lymphoid organs), and each organ is endowed with its own mix of DCs. Langerhans cells are a subset of epidermal DCs that express langerin. Unlike conventional DCs, Langerhans cells arise locally from a pool of precursors that reside in the skin.

At sites of infection in peripheral tissues, DCs acquire antigen via endocytosis, pinocytosis, and direct infection and then migrate to lymph nodes. Upon arrival in the lymph nodes, DCs directly present antigen to T cells or transfer antigen to other APCs for presentation. Interaction between TCR and MHC–peptide complexes requires close apposition between T cells and APCs [45]. Adhesive interactions exemplified by the respective binding of CD11a/CD18 [leukocyte function-associated antigen-1 (LFA-1)] and CD2 on T cells with CD54 (intracellular adhesion molecule-1) and CD58 (LFA-3) on APCs help to overcome the repulsive forces created by the net negative surface charge of these cells [13]. These molecules also exhibit outside-in signaling, which contributes to T cell activation. Full activation of T cells, including those generating an alloimmune response, requires interactions of a sufficient avidity between the TCRs on T cells and MHC–peptide complexes on APCs [46]. TCR signaling induces a higher affinity conformation of LFA-1, further cementing the interface between T cells and APCs. Engagement between CD28 and its ligands CD80 and CD86 promotes the production of IL-2. CD28 signaling is particularly important for activation of naïve CD4 cells and activation of naïve CD8 cells against lower avidity antigens. IL-2 stimulates proliferation of activated T cells and the differentiation of CD4 cells into various effector T-cell subsets (see below) [47]. IL-2 also stimulates the differentiation of CD8 cells into effectors and their transition into memory cells, and it plays a crucial role in the maintenance of Treg cells.

As demonstrated in murine GVHD models [48–50], initial activation of naïve donor T cells occurs predominantly in secondary lymphoid organs of the recipient. Since the frequency of T cells for a given peptide–MHC combination is very low, specialized mechanisms have evolved to increase the probability that T cells of appropriate specificity have sufficient opportunity to establish contacts with relevant antigen-bearing APCs [51,52]. Successful interactions are promoted by recirculation of T cells both within lymphoid organs on a reticular cell network and between lymphoid organs in blood and lymph. Further, activated APCs release soluble attractants for T cells. Optimal T-cell activation may require recruitment of accessory cells. For example, CD8$^+$ T-cell responses are substantially modified by concomitant local CD4$^+$ T-cell activation, and NK cells can influence the function of APCs, thereby influencing the T-cell response.

T-cell differentiation

T-cell activation and differentiation are not based entirely on TCR signaling. Rather, T-cell activation and differentiation reflect the summation of graded signals from many receptors in response to ligands from APCs and other accessory cells [53,54]. The outcome depends on the strength, duration, timing, and frequencies of these signals. This complex combinatorial signaling provides sufficient information to generate a wide array of T-cell responses. A simplified framework for naïve T-cell activation involves signaling through three types of receptors: TCR, receptors engaged by costimulatory molecules on APCs (Table 9.2) [55–58], and cytokine receptors [59,60]. For CD4 cells, integration of these signals results in the generation of multiple different types of T cells, including T helper (T_H) 1, T_H2, T_H17, T follicular helper (T_{FH}) cells, Tr1, T_H9, and induced FoxP3$^+$ Treg cells. Each subset produces a characteristic set of dominant cytokines. For many subsets, CD4$^+$ T-cell differentiation relies upon activation in the presence of a distinct set of cytokines and induction of a specific transcription factor (Figure 9.4) [61,62]. For example, IFN-γ, IL-12 and IL-18 induce T-bet expression and T_H1 differentiation. IL-4 and IL-33 induce GATA3 expression and T_H2 differentiation. IL-1 and IL-6 induce RORγT expression and T_H17 differentiation. TGF-β induces FoxP3 expression and induces regulatory T-cell differentiation. Frequently, the activity of one type suppresses the development of another [63]. Plasticity adds to the complexity, in that cells can have a mixed phenotype or revert from one type to another [64]. Naïve CD8 cells can also differentiate into some of these analogous subsets. Regulation of CD8 responses, however, seems to be more directed towards influencing differentiation between effector and memory T cells [65]. After initial antigen activation, CD8 cells proliferate vigorously and then die off, with a smaller number of CD8 cells persisting long term as memory cells [66]. Continued inflammation promotes short-term effector differentiation. With less inflammation, activated CD8 cells are more likely to differentiate as memory cells.

Costimulation through interactions of CD28 with CD80 and CD86 is critical for initial activation of T cells, but later costimulation further shapes the character and magnitude of T-cell responses. Costimulatory molecules generally belong to the CD28 or TNF

Table 9.2 Receptors and ligands involved in costimulation

Receptor on T cells	Molecular superfamily	Stage of initial expression by T cells	Ligands on antigen-presenting cells	Functional effect on T cells	Functional effect on antigen-presenting cells
CD28	Ig	Naïve	B7-1/B7-2 (CD80/CD86)	Stimulation	Unknown
CD27	TNF	Naïve	CD70	Stimulation	Unknown
CD40L (CD154)	TNF	Early activation	CD40	Unknown	Stimulation
CTLA4 (CD152)	Ig	Early activation	B7-1/B7-2 (CD80/CD86)	Inhibition	Unknown
ICOS	Ig	Early activation	B7h	Stimulation	Unknown
PD-1	Ig	Early activation	PD-L1/PD-L2	Inhibition	Unknown
CD160	Other	Effector or memory	HVEM	Inhibition	Unknown
HVEM	TNF	Effector or memory	LIGHT	Stimulation	Unknown
OX40 (CD134)	TNF	Effector or memory	OX40-L	Stimulation	Unknown
GITR	TNF	Effector or memory	GITR-L	Stimulation	Unknown
4-1BB (CD137)	TNF	Effector or memory	4-1BBL	Stimulation	Unknown
CD30	TNF	Effector or memory	CD30L	Stimulation	Unknown
TIM-1	TIM	Effector or memory	TIM-4	Stimulation	Unknown
TIM-2	TIM	Effector or memory	Sema4a	Inhibition	Stimulation
TIM-3	TIM	Effector or memory	Galectin-9	Inhibition	Stimulation

CTLA4, cytotoxic T lymphocyte antigen-4; ICOS, inducible costimulatory molecule; PD, programmed cell death; HVEM, herpes virus entry mediator A; GITR, glucocorticoid-induced TNF-related protein; TIM, T-cell Ig domain and mucin domain family; Ig, immunoglobulin; TNF, tumor necrosis factor; LIGHT, ligand for herpes virus entry mediator; Sema, semaphorin; suffix R, receptor; suffix L, ligand.
Source: adapted from Li et al. [58].

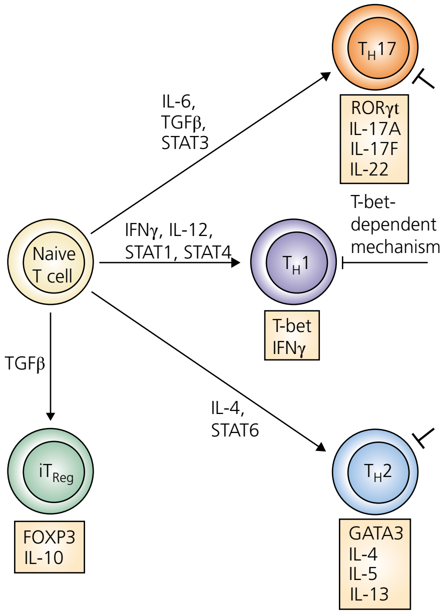


Figure 9.4 T helper cell differentiation. After activation, naïve CD4 cells can differentiate into a variety functionally distinct types of effector T cells, depending on the dominant environmental cytokines that drive characteristic intracellular signaling through signal transducer and activator of transcription (STAT) molecules. Activation dominated by interferon-γ (IFN-γ) and interleukin (IL)-12 drives STAT 1 and STAT4 signaling and produces type-1 T helper cells (T_H1) cells by inducing expression of the transcriptional regulator T-bet. T_H1 cells produce IFN-γ and play a prominent role in cell-mediated immunity. Activation dominated by IL-4 drives STAT6 signaling and produces type-2 T helper cells (T_H2) by inducing expression of the transcriptional regulator GATA binding protein 3 (GATA3). T_H2 cells produce IL-4, IL-5 and IL-13 and play a prominent role in humoral immunity. Activation dominated by IL-6 and transforming growth factor β (TGFβ) drives STAT3 signaling and produces T helper 17 (T_H17) cells by inducing expression of the transcriptional regulator retinoic acid receptor-related orphan receptor-γt (RORγt). T_H17 cells produce IL-17 and IL-22 and play a prominent role in cell-mediated inflammation. Activation dominated by TGFβ produces induced T regulatory (iTreg) cells by inducing expression of the transcriptional regulator forkhead box P3 (FoxP3). iTreg cells produce IL-10 and function as regulators that dampen immune responses. Adapted from Campbell and Koch 2011 with permission of Nature Publishing Group.

receptor families and can be classified according to their stimulatory or inhibitory effects on T-cell survival, proliferation and effector function [58]. Because resting T cells express CD28, they are susceptible to early regulation by interactions with CD80 and CD86. In contrast, other receptors for costimulatory molecules such as CD27, ICOS, OX40, and 4-1BB are expressed only after initial activation. T cells can also condition APCs through engagement of costimulatory receptors [67]. In particular, CD40L on CD4 cells activates APCs and signals immunoglobulin class switching. Some costimulatory receptors deliver negative signals to T cells, as discussed further below [68].

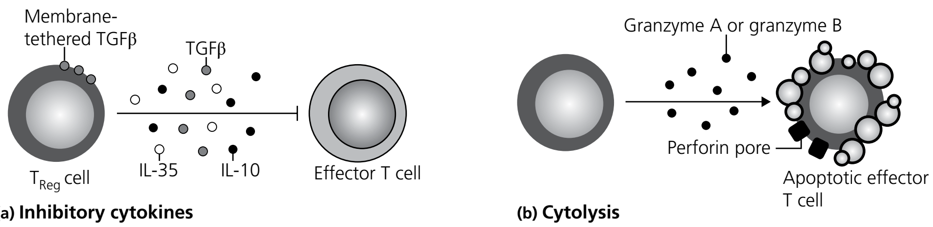
Costimulatory molecule and cytokine receptor signaling

Costimulation signals mediated through CD28 contribute to full activation of T cells. CD28 signaling activates phosphatidylinositol 3-kinase (PI3K), an enzyme that converts PIP_2 to phosphatidylinositol 3,4,5-triphosphate (PIP_3). In the plasma membrane, PIP_3 recruits both phospholipase Cγ and the cytoplasmic tyrosine kinase that activates phospholipase Cγ (see above). Signaling through PIP_3 or Ras activates the mammalian target of rapamycin (MTOR) pathway. MTOR is a serine–threonine kinase that promotes translocation of NFκB to the nucleus, thereby augmenting expression of IL-2 and IL-2R. MTOR exists in at least two distinct complexes. The immunosuppressive drug sirolimus binds to an intracellular protein, forming a drug–protein complex that inhibits one of these complexes. By dampening signal transduction through PI3K and MTOR, sirolimus inhibits certain costimulation pathways and cytokine responses [69], with greater functional inhibition of effector T cells compared with Treg cells [69,70].

Many cytokine receptors have a heterodimeric structure. In some cases, a common chain is used as a partner for several different types of cytokine receptors. For example, receptors for IL-2, IL-4, and IL-15 have the same γc chain as one member of the heterodimer. Binding of a cytokine to the two chains of the receptor brings together the respective dimer components in the plasma membrane. This dimerization activates Janus kinase (JAK) domains in the cytoplasmic tails of the dimeric partners. Phosphorylated JAK domains recruit and activate signal transducers and activators of transcription (STATs). Phosphorylated STAT dimers translocate to the nucleus where they initiate gene transcription.

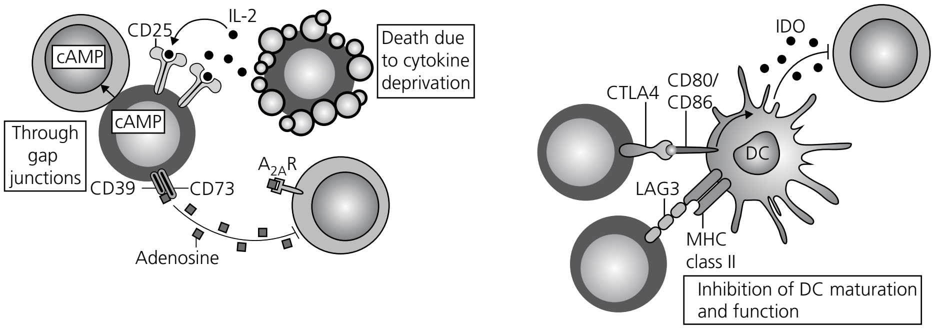
Proinflammatory T-cell effector functions

A detailed description of the functions of each type of T cell that can be generated after antigen exposure is beyond the scope of this chapter and has been the subject of many reviews [64,71–73]. TCRs on CD4 cells are activated only by MHC class II$^+$ cells, and MHC class II expression is far more restricted than MHC class I expression. MHC class II molecules are expressed on APCs, and expression is upregulated by APC activation. Therefore, T_H CD4 cells are well equipped to orchestrate actions of other immune cells [53,74,75]. T_H1 cells, which produce IFN-γ, mediate classical macrophage activation and promote specific types of antibody responses. T_H1-derived IFN-γ elaborated after activation in inflamed tissues can also have bystander effects on cells that do not express MHC class II molecules [27]. Synchronous activation of many CD4$^+$ T cells, which may occur after allogeneic HCT, can induce systemic toxicity through the elaboration of inflammatory cytokines such as IFN-γ and TNF-α. T_H2 cells produce IL-4 and IL-5, promoting IgG1 and IgE responses and helping to recruit eosinophils that are required for antiparasite immunity. T_H17 cells produce IL-17, a cytokine that stimulates neutrophil responses to bacteria and fungi. T_H17 cells have also been implicated in models of inflammatory bowel disease and autoimmune encephalitis. T_{FH} cells promote germinal center reactions and affinity maturation of B-cell receptors. Non-hematopoietic cells can upregulate expression of MHC class II molecules, for example in response to IFN-γ. Through this mechanism, CD4$^+$ T cells can cause direct damage to non-hematopoietic cells. CD4$^+$ T cells can have cytolytic activity mediated through expression of the TNF-family ligands for Fas, TNF-R1, and TRAIL-R and through release of granules containing perforin and granzymes. CD8$^+$ T cells can interact with a wider array of cells, since MHC class I molecules are expressed on nearly all nucleated cells. CD8$^+$ T cells have a critical role in mediating antiviral responses by killing virus-infected cells through engagement of death receptors and the release of granules containing perforin and granzymes [66,76]. CD8$^+$ T cells also produce proinflammatory cytokines, including IFN-γ and TNF-α.



(a) Inhibitory cytokines

(b) Cytolysis



(c) Metabolic disruption

(d) Targeting dendritic cells

Figure 9.5 Mechanisms of T-regulatory cell function. T-regulatory cells (Treg cells) can dampen immune responses (a) by producing cytokines such as transforming growth factor β (TGFβ), interleukin (IL)-35, or IL-10 that inhibit effector T cell responses, (b) by killing effector cells through granzyme or perforin-mediated mechanisms, (c) by metabolic disruption of effector cells through competition for IL-2, delivery of cyclic adenosine monophosphate (cAMP), or signaling through adenosine 2A receptors ($A_{2A}R$) after release of adenosine via CD39- or CD73-mediated mechanisms, and (d) by interfering with maturation and function of dendritic cells when lymphocyte activation gene 3 (LAG3) on Treg binds to MHC class II molecules on dendritic cells, and by inducing dendritic cells to inhibit effector function through production of indoleamine 2,3-dioxygenase (IDO) when cytotoxic T lymphocyte antigen-4 (CTLA4) on Treg binds to CD80 and CD86 on dendritic cells. IDO causes depletion of tryptophan, which inhibits effector T-cell function. Source: adapted from Vignali et al. 2008 [87] with permission of Nature Publishing Group.

Peripheral tolerance and regulation of T-cell responses

Despite the redundancy of mechanisms for inducing central tolerance, some self-reactive T cells emerge from the thymus and migrate to the periphery. Therefore, mechanisms must exist to diminish the risk that these cells will be activated and expanded sufficiently to cause autoimmunity [77]. In addition, excessive tissue damage by cells with low affinity for self must be avoided during the response to infection. The immune system has evolved strategies at nearly every step of the T-cell response to diminish these risks. For example, TCR activation itself is highly regulated, and phosphatases that diminish TCR signaling are activated almost immediately after TCR engagement [78]. As a mechanism for maintaining peripheral tolerance, presentation of self-antigens by DCs outside the thymus and by lymph node stromal cells in the absence of innate alarm signals can result in proliferation followed by deletion or anergy [44,79,80].

Activated T cells also coordinately upregulate inhibitory receptors [58,81]. The CD28 family member CTLA4 is a prototypical inhibitory receptor that is induced early after T-cell activation. Compared with CD28, CTLA4 has higher affinity for CD80 and CD86. The importance of CTLA4 in regulating T-cell responses is evident from the autoimmunity that occurs when it is genetically absent or when binding is blocked by exogenous agents. Fas on T cells also downregulates T-cell responses, and Fas- or FasL-deficient mice and humans have autoimmune syndromes [82]. PD-1, another CD28 family member, inhibits T-cell responses through engagement of its ligands PD-ligand 1 and PD-ligand 2 (also known as B7H1 and B7DC, respectively). The absence of PD-1 causes an autoimmune syndrome that is less severe than that caused by the absence of CTLA4. Reagent-based blockade of PD-ligand 1 can induce antitumor T-cell responses, and experimental systems have demonstrated that both PD-1 and CTLA-4 help to regulate GVHD. TGF-β and B and T lymphocyte attenuator (BTLA) also downregulate T-cell responses. Chronic antigen stimulation leads to a progressive state of T-cell hyporesponsiveness called exhaustion. Several receptors, including PD-1, TIM-3, and LAG-3, contribute to T-cell exhaustion, and their blockade can partially restore T-cell function. PD-1, TIM-3, BTLA, and CTLA4 all regulate alloreactive T-cell responses.

T cells with primary regulatory functions also downregulate T-cell responses and contribute to tolerance [83–86]. FoxP3-dependent thymus-derived Tregs help to maintain tolerance, and their genetic absence or deletion results in severe autoimmunity. FoxP3-expressing Treg cells can also be induced from conventional T cells. This differentiation is provoked only by specific subsets of DCs. The exact mechanisms whereby Tregs act in vivo are not fully elucidated, but evidence supports roles for Treg-derived IL-10, TGF-β, and cytotoxic mechanisms involving perforin and granzymes (Figure 9.5) [87]. Finally, activated T cells can differentiate into FoxP3-negative cells with regulatory function, characterized by expression of IL-10 [85,88].

T-cell memory

T cells exhibit primary and secondary immune response in vivo [63,66,89]. "Primary" responses occur when immunologically "naïve" cells first encounter a specific antigen. The immune response after an initial encounter with antigen develops during a period of approximately 1 week and results in the expansion of specific clones of effectors having TCRs that recognize the antigen.

This initial expansion is followed by clonal contraction. After stimulation of naïve T cells, asymmetric cell division differentiates effector and memory T-cell fates. Memory T cells can be divided into at least two distinct subsets [90,91]. The "central" memory population resembles the antigen-inexperienced naïve T-cell population, migrates preferentially through lymphoid tissue, and is long lived, whereas the "effector" memory population resembles the antigen-experienced effector T-cell population, migrates preferentially in non-lymphoid tissues, and is short lived [92]. Resident memory cells are generally characterized by selection for high TCR affinity, increased expression of adhesion molecules, and altered migratory patterns with distinctive cytokine production and response profiles [93]. Memory cells remain poised to respond quickly after a repeated encounter with antigen. "Secondary" responses by memory cells develop during a period of several days and show greater intensity than the original primary response. CD4 cells are required for maintenance of CD8 memory cell survival and function [94]. IL-7 has an important role in survival of memory CD8 cells, while IL-15 is necessary for proliferation of memory CD8 cells [95].

Selection of donors for allogeneic HCT

The immunogenetic relationship between the donor and recipient profoundly influences the outcome of HCT, much more so than with solid organ transplants [2]. Within the constraints of current practice, however, these differences are most apparent when analyzing immunologic outcomes, such as graft rejection and the incidence and severity of GVHD, and are less apparent when analyzing disease-free survival (DFS). Since MHC matching avoids activation of T cells by major histocompatibility antigens, the best results are seen after transplantation from HLA-genotypically identical sibling donors, but only 30% of patients have such a donor. Some patients without an HLA-identical sibling have an HLA-haploidentical family member with limited mismatching between the non-shared haplotypes (see Chapter 44). Previous studies have shown that patients receiving an HLA-haploidentical transplant incompatible for a single HLA-A, -B, or -DR antigen can have DFS similar to that of patients with marrow from HLA-genotypically identical sibling donors, although HLA mismatching increases the risk of GVHD. Patients receiving transplants incompatible at two or three of these loci have a significantly lower probability of survival when conventional immunosuppressive regimens are used after HCT. In this situation, problems related to GVHD can be overcome by removing T cells from the graft or by giving cyclophosphamide after transplantation to eliminate T cells that are activated after they recognize recipient alloantigens.

HCT from HLA-phenotypically matched unrelated donors or cord blood donors is an alternative for patients who lack a donor in the family (see Chapters 45 and 39). Unrelated HCT has been made feasible by the development of large registries of HLA-typed individuals and cryopreserved cord blood units available to serve as a source of hematopoietic cells. With registries currently available in the United States and in other countries, it is now possible to identify an HLA-A, B-phenotypically matched DRB1 allele-matched unrelated donor for at least 70% of white persons. HLA alleles are more diverse in Hispanic and black populations, and success rates for matching unrelated donors in these populations are lower than in white persons. If a single HLA-A, -B, or -DRB1 mismatch were allowed, then it would be possible to identify donors for many more patients. In certain circumstances, DFS for patients with HLA-A, -B, -C, -DRB1, and DQB1 allele-matched unrelated donors may be comparable to that for similar patients with HLA-genotypically identical sibling donors (see Chapter 45).

Outcomes influenced by genetic disparity between the donor and recipient

Engraftment

Graft rejection after HCT may be manifested as either the lack of initial engraftment or the development of pancytopenia and marrow aplasia after initial engraftment. In humans, rejection, drug toxicity, sepsis, and certain viral infections can all cause graft failure. The key findings that support a diagnosis of rejection in a patient with graft failure are the absence of donor cells and the presence of recipient T cells. The durable persistence of donor cells makes rejection unlikely, even when recipient T cells can be detected.

Experiments in animal models have demonstrated that antibody, NK cells, and T cells of the recipient can all mediate allogeneic marrow graft rejection [96]. Mice with antibody against major histocompatibility antigens of the donor reject donor cells within 3 hours after HCT. NK-mediated rejection does not involve immunologic priming and occurs within 1–2 days after transplantation [30]. The effectors have a life span of less than 1 week but are highly resistant to irradiation. In rodents, NK-mediated rejection can be overcome by treatment of the recipient with cyclophosphamide (CY) or with antibodies that deplete NK cells in the recipient before the transplant. Because NK precursors are sensitive to irradiation, NK-mediated rejection can also be overcome by split-dose irradiation of the recipient, with an interval of 1 week between two irradiation exposures. Rejection mediated by unprimed T cells does not occur until 7–8 days after transplantation.

Rejection by T cells can be enhanced by priming or transfusion-induced sensitization before the transplant. T-cell effectors have a longer life span than NK cells and are generally more sensitive to radiation than NK cells. The increased risk of rejection associated with transfusion-induced sensitization and the decreased risk of rejection associated with the use of higher levels of total body irradiation (TBI) before the transplant clearly implicate recipient T cells as effectors that can mediate HCT rejection in humans. In support of this hypothesis, recipient-derived T cells with antidonor HLA-specific cytotoxic activity have been isolated from the blood of patients with graft failure after HLA-mismatched HCT. A delayed onset of rejection and an occurrence of rejection after grafting from an HLA-identical donor would argue against a role for NK cells in causing rejection. NK-mediated rejection also seems unlikely among patients who have received high-dose CY before the transplant, if it is assumed that this agent has similar effects on human and murine NK cells.

Mechanisms by which recipient NK cells or T cells might cause marrow graft rejection have not been fully elucidated. Although it seems intuitively obvious that cell-mediated cytotoxicity might be involved, recipients deficient in perforin, granzyme B, or FasL function can reject an allogeneic marrow graft. These results indicate redundancy in the mechanisms by which recipient effectors can kill donor hematopoietic cells, as also shown to be the case in GVLEs (see below). This hypothesis was supported by observations that NK cells from certain strains of mice could not mediate marrow graft rejection when perforin- and FasL-mediated mechanisms of cytotoxicity were both absent. Because hematopoietic stem cells do not express Fas under steady-state conditions, any potential involvement of a FasL-mediated mechanism in marrow graft rejection would require the induction of Fas expression, possibly through exposure to IFN-γ or TNF-α. Alternatively, TNF-α released by cytotoxic T lymphocytes (CTLs) or NK cells could have a direct suppressive or cytotoxic effect on hematopoietic stem cells. Graft rejection by CD8 cells after immunization of the recipient against donor alloantigens appears to be mediated by mechanisms other than perforin or FasL.

The risk of graft failure depends on the degree of genetic disparity between the donor and recipient. In particular, graft failure after unrelated marrow transplantation has been associated with HLA class I mismatching. The association between HLA class I disparity and graft failure has been observed primarily among patients with chronic myelogenous leukemia (CML) and other diseases that are not treated with intensive chemotherapy before referral for transplantation. The risk of graft rejection is also greatly increased by transfusion- or pregnancy-induced alloimmunization of the recipient against the donor before the transplant. Alloimmunization presumably occurs when a fetus or transfusion donor happens to have transplantation antigens in common with the transplant donor. Sensitization of the recipient against some alloantigens can be detected by the presence of antibodies that recognize donor T cells or B cells. After sensitization, rejection may be caused either by memory T cells that survive the conditioning regimen or possibly by antibody-mediated destruction of donor cells [97].

The use of more intensive conditioning regimens can reduce the risk of rejection by eliminating a greater proportion of recipient T cells before the transplant, but this approach cannot decrease the risk of antibody-mediated rejection. Post-transplant administration of immunosuppressive agents such as methotrexate and possibly cyclosporine decreases the risk of rejection by interfering with the function of recipient cells that survive the conditioning regimen. Donor T cells can also play a critical role in preventing rejection by recognizing any surviving recipient T cells and eliminating them primarily through a perforin-mediated cytotoxic mechanism. Under certain experimental conditions, donor NK cells and T cells that do not cause GVHD can prevent rejection. Other experiments have shown that precursor-plasmacytoid DCs of the donor can facilitate marrow engraftment.

Acute GVHD

The development of GVHD is a major determinant of outcome after allogeneic HCT (see Chapters 83 and 84 and [1,98–100]). Acute GVHD is caused by mature donor T cells that recognize recipient tissues as "non-self" and cause tissue damage through direct cytolytic mechanisms and indirectly through the release of cytokines and perhaps through the activation of other cells such as macrophages. GVHD can be elicited by T cells that recognize MHC alloantigens and mHAs of the recipient. In the presence of an MHC mismatch, the high precursor frequency of alloreactive donor T cells causes an increased risk of GVHD, but MHC alloantigens can be presented only by recipient cells. Soon after HCT, most of the recipient hematopoietic APCs that trigger GVHD are replaced by donor cells that lack the MHC mismatch. Results in experimental systems have suggested that the responding donor T cells may need continued stimulation by hematopoietic APCs in order to sustain full GVHD activity, and the initial activation of T cells that recognize the MHC mismatch can be followed by some recovery as recipient APCs disappear. Conversely, mHAs can be presented by either recipient or donor cells, and these antigens can be presented throughout the post-transplant period, even after recipient APCs have disappeared. Despite the large number of potential mHAs in any given donor–recipient pair, some are more effectively targeted than others. This "immunodominance" is also characteristic of T-cell responses against pathogens. The determination of whether any given antigen is immunodominant likely depends on the precursor frequency of reactive T cells and on the availability and tissue distribution of antigens.

Donor T cells are initially activated primarily in secondary lymphoid organs of the recipient. GVHD can occur in the absence of either the spleen or other secondary lymphoid organs [101]. Less severe GVHD is elicited in the absence of both the spleen and secondary lymphoid organs [49,50]. In this case, priming may occur in bone marrow. Naïve donor T cells express surface molecules that promote their recruitment to secondary lymphoid organs. CD62L and CCR7 promote entry to lymph nodes and localization to T-cell zones in the spleen and other secondary lymphoid organs, although their expression is not absolutely required to induce GVHD [50,102].

Activation of donor CD8 and $CD4^+$ T cells is driven initially by recipient APCs. Cross-presentation of recipient mHAs by MHC class I molecules on donor APCs does not generate a $CD8^+$ T-cell response that is sufficient to initiate histopathologic GVHD, but indirect presentation of recipient antigens by donor APCs can contribute to the later evolution of GVHD [103]. Early activation of donor $CD4^+$ T cells depends on recipient APCs, since donor-derived APCs are absent when donor T cells are transferred. Either recipient or donor APCs are sufficient to induce CD4-mediated GVHD against mHAs. Despite this redundancy, it is likely that recipient APCs are the initial activators, since it takes time for functional donor-derived APCs to emerge from the graft. This kinetic difference is demonstrated by the insufficiency of donor APCs in CD4-mediated mHA-disparate GVLE models in which the activation of donor CD4 cells by donor APCs occurs too late to control a large burden of rapidly proliferating malignant cells [104,105].

Substantial effort has been devoted to defining the recipient APCs that contribute to GVHD. Classical DCs, plasmacytoid DCs, and Langerhans cells are sufficient to initiate GVHD in MHC-mismatched models. For clinical purposes, the key question is the identification of recipient APC subsets that are required for induction of GVHD. In this regard, Langerhans cells, B cells, and plasmacytoid DCs are not required [106–108]. Surprisingly, GVHD was not prevented when classical DCs were profoundly ablated using multiple model systems and approaches, even with concomitant depletion of plasmacytoid DCs, B cells, and Langerhans cells [106,109]. Some of these experiments were done in systems where hematopoietic APCs were required, suggesting that other recipient hematopoietic APCs could be sufficient. In particular, $CD11c^-$MHC class II^+ myeloid cells, including macrophages, were not well targeted, and it remains to be tested whether these cell populations are sufficient. The possibility that miniscule numbers of targeted recipient APCs were sufficient to induce GVHD is difficult to exclude. Some results have resurrected the idea that non-hematopoietic cells in the recipient can initiate GVHD when functional hematopoietic APCs are absent [109–111]. Additional work has been carried out to identify the donor APCs that can activate alloreactive T cells. The results showed that donor conventional DCs were essential for presentation of recipient mHAs to donor T cells [103,112]. In humans, B cells may have an important role in promoting chronic GVHD (see below). The presence of antibodies recognizing male antigens in male recipients of female allografts correlated with chronic GVHD, and B-cell depletion can be an effective chronic GVHD treatment.

A current paradigm holds that APC activation by innate signals is required for optimal T-cell priming and effector differentiation [113].

It has long been advocated that translocation of LPS from the bowel lumen after conditioning is an essential first step in the development of GVHD. LPSs, and perhaps other TLR ligands, were proposed to function at least in part by activating recipient APCs. More recent work, however, demonstrated that the development of GVHD is not impaired when recipient APCs cannot respond to TLR ligands, IL-1, or type I IFNs [24]. TBI enriches for recipient APCs with higher levels of CD80 and CD86, but it has not been established that this maturation is essential for the development of GVHD, and activation of alloreactive T cells can occur in the absence of any recipient conditioning. In contrast to the lack of major phenotypic changes in GVHD when recipient APCs cannot be activated by TLR, IL-1, or type I IFN, recent work has shown that both complement and ATP may activate APCs that initiate GVHD.

Compared with naïve T cells, memory phenotype T cells have a reduced ability to cause GVHD in mice. In multiple donor–recipient strain pairings, effector memory cells cause little or no GVHD. Data are mixed for central memory T cells, with some studies showing they cause no GVHD and others showing that they cause less severe GVHD compared with naïve T cells [114]. The failure of effector memory CD4 cells to cause GVHD cannot be explained by the absence of CD62L expression and their relative inability to traffic to lymph nodes, since the forced expression of CD62L on effector memory cells did not restore their ability to cause GVHD. Likewise, naïve T cells can cause GVHD in the absence of lymph nodes. Recent data suggest that the reduced capacity of effector memory T cells to cause GVHD relates both to TCR repertoire-dependent and -independent properties [115,116].

During the efferent phase of the disease, activated donor T cells migrate to target organs where they cause tissue injury in the skin, liver, and gastrointestinal tract. Chemokines expressed in target tissues bind to chemokine receptors on T cells and help attract T cells into these tissues. Activated T cells from both the CD4 and CD8 subsets produce proinflammatory cytokines, and both subsets generate CTLs. No single effector mechanism can account for the entire clinical spectrum of acute GVHD (see Chapters 13 and 83). In some patients, the disease presents as a syndrome of high-grade fever, confluent erythematous rash, and vascular leak with pulmonary infiltrates and edema during the first 10 days after the transplant. In this situation, the disease can be viewed appropriately as a "cytokine storm" that generally shows striking improvement after treatment with high-dose glucocorticoids. In most patients, however, the disease presents in much less dramatic fashion, with a slowly progressive morbilliform rash or with anorexia, nausea, and vomiting. The role of cytokine storm in these more typical cases of acute GVHD without overt systemic toxicity remains to be determined.

Cytokines play complex roles in the pathogenesis of GVHD, and much effort has been devoted to determining which cytokines and which T_H subsets contribute to GVHD [117,118]. In mice with GVHD, IFN-γ-producing T cells dominate, with much smaller numbers of T_H2 cells and even fewer IL-17-producing cells. Data from experiments with T cells deficient in specific cytokines or with reagent-based cytokine neutralization are difficult to interpret, because these cytokines can have autocrine and paracrine regulatory functions and can suppress the expansion of other T_H subsets. For example, while IFN-γ has pleotropic proinflammatory effects, neutralization of IFN-γ and the use of donor T cells that cannot produce IFN-γ exacerbated GVHD. These results suggest that IFN-γ actually has a complex dual role to promote and control GVHD in mice [27]. The presence of eosinophils in duodenal biopsies from patients with GVHD supports the involvement of type 2 cells in the pathogenesis of the disease. In one study, GVHD was more severe in the absence of IL-17a, coincident with a larger IFN-γ response, suggesting that the former suppresses the latter. Another study, however, showed little difference in GVHD recipients of IL-17a-deficient T cells compared with wild-type T cells [119]. The overall results in different systems indicate that T_H1, T_H2, and T_H17 cells can all cause GVHD, although clinical manifestations and the histopathologic patterns may differ and in the absence of one or another cytokine.

Cell-mediated cytotoxic mechanisms clearly contribute to the pathogenesis of GVHD. In an MHC-matched, mHA-mismatched model, expression of MHC class I molecules by non-hematopoietic target tissues was required for GVHD mediated by $CD8^+$ T cells. In contrast, expression of MHC class II molecules by non-hematopoietic target tissues was not required for GVHD mediated by $CD4^+$ T cells. Substantial effort has been devoted to understanding cytolytic mechanisms, mostly in studies with gene-deficient T cells. GVHD is less severe when induced by perforin-deficient T cells compared with wild-type T cells. In a model of GVHD caused by mHAs, FasL-deficient donor T cells caused cachexia but did not cause B-lymphoid hypoplasia or inflammatory lesions in the skin or liver. These results are difficult to interpret, because both perforin and Fas have a role in regulating T-cell responses. The severity of hepatic GVHD was reduced in Fas-deficient recipients, but the severity of GVHD in other organs was increased. Neutralization of FasL prevented hepatic lesions in wild-type recipients but had no effect on intestinal GVHD, whereas neutralization of TNF-α improved intestinal GVHD but had no effect on hepatic GVHD. Neutralization of both FasL and TNF-α prevented GVHD in all organs. Taken together, these results indicate that multiple cytopathic and inflammatory mechanisms are involved in both the afferent and efferent pathways leading to GVHD.

Under certain conditions, activated donor NK cells can reduce the severity of GVHD [30,120]. Mechanisms proposed to explain this effect include production of TGF-β or IL-4 by donor NK cells or rapid elimination of recipient DCs or alloreactive T cells by donor NK cells after the transplant. Donor $CD25^+CD4^+$ Treg cells can also reduce the severity of GVHD in mice. GVHD can be ameliorated by adding large numbers of Treg cells to the graft and is exacerbated by removal of these cells from the graft. The ability of Treg cells to prevent GVHD is partly mediated by their ability to produce IL-10. Results of preliminary clinical trials suggest that Treg cells can diminish GVHD in humans [121,122]. More extensive testing of Treg cells will require the development of better methods for isolating large numbers of these cells or expanding them in culture.

Clinical studies have shown that the risk of acute GVHD depends on the degree of genetic disparity between the recipient and donor. The risk of GVHD is greater with unrelated donors than with related donors, mostly because of unavoidable MHC disparity. The risk of GVHD is higher with multiple disparities than with a single disparity. The type of post-transplant immunosuppression and compliance with the prophylactic regimen influence the risk of GVHD. The most effective regimens currently in widespread use include an antimetabolite such as methotrexate or mycophenolate mofetil in combination with a calcineurin inhibitor (cyclosporine or tacrolimus) or sirolimus (see Chapter 81). Multi-drug regimens are more effective than single-drug regimens. Acute GVHD can be prevented by removing mature T cells from the donor marrow (see Chapter 82). The benefit brought about by a reduced risk of GVHD, however, can be offset by increased risks of graft failure and recurrent leukemia, and by delayed immune reconstitution (see below).

Research studies are attempting to determine whether the donor cells that cause GVHD can be distinguished from those needed to prevent rejection, eliminate malignant progenitors, or initiate immune reconstitution. In the past, many clinical trials have employed methods aimed at global T-cell depletion, even though GVHD is initiated by the relatively small subset of donor cells that recognize recipient histocompatibility antigens. In the future, it may be possible to prevent GVHD by specific depletion of donor T cells that recognize recipient alloantigens, by interference with positive costimulatory signals needed for optimal activation of T cells, by interference with mechanisms involved in the migration of T cells in vivo [123], by administration of agents that increase activation-induced apoptosis in donor T cells that recognize recipient alloantigens, by infusion of Treg cells [121,122,124,125], or by treatment with agents that facilitate expansion of Treg cells in vivo [126]. Possible interference with GVLEs is a concern with all of these approaches. Alternatively, the severity of GVHD might be decreased with the use of agents that protect gastrointestinal integrity [127,128] or decrease intestinal inflammation [129].

Chronic GVHD

The biologic mechanisms leading to chronic GVHD are not as well understood as those leading to acute GVHD [100,130,131]. Although acute GVHD has been recognized as a risk factor for chronic GVHD, evidence suggests that chronic GVHD is not simply the end stage of acute GVHD. Not all cases of acute GVHD evolve into chronic GVHD, and chronic GVHD can develop in the absence of any prior overt acute GVHD. Chronic GVHD is characterized by distinctive clinical and pathologic abnormalities that are not associated with acute GVHD. In the skin, the initial phase of chronic GVHD is characterized by an intense mononuclear inflammatory infiltrate with destructive changes at the dermal–epidermal junction, accompanied by irregular acanthosis, hyperkeratosis, or atrophy. Evolution of the disease is characterized by increasing dermal fibrosis, ultimately resulting in dermal sclerosis (see Chapter 26). Other pathognomonic hallmarks of chronic GVHD include destruction of tubuloalveolar glands and ducts in the skin, salivary glands, and lacrimal glands.

Several hypotheses have been invoked to explain the pathogenesis of chronic GVHD in humans, based on clinical and experimental observations, although no single animal model encompasses all features of chronic GVHD observed in humans. T cells in the graft clearly contribute to the disease, although the target antigens that provoke chronic GVHD might differ from those that provoke acute GVHD. Fibrotic characteristics suggest that chronic antigen stimulation could play a role. Sclerotic manifestations of chronic GVHD may be caused by the activity of profibrotic receptor tyrosine kinases such as fibroblast growth factor receptor and platelet-derived growth factor receptor (PDGFR). For example, cutaneous sclerosis has been associated with the presence of agonistic autoantibodies against PDGFR and, in some cases, sclerotic manifestations have improved after treatment with tyrosine kinase inhibitors. The causal relationship between the presence of these antibodies and the development of sclerotic manifestations remains controversial, and it is possible that the improvement observed in some patients after treatment with tyrosine kinase inhibitors occurred through mechanisms unrelated to agonistic autoantibodies. In humans, the use of growth factor-mobilized blood cells is associated with an increased risk of chronic GVHD compared with marrow, but experimental studies with mice have indicated that T cells do not account for this effect. Phase II studies demonstrating clinical improvement in manifestations of chronic GVHD in some patients after treatment with B-cell-specific monoclonal antibodies have suggested a role for B cells in the pathogenesis of the disease. Excessive production of TGF-β or other profibrotic cytokines could contribute to the development of fibrosis. Thymic damage caused by TBI, acute GVHD, or immunosuppressive medications could lead to failure of negative selection, allowing the production of donor-derived T cells that recognize recipient-specific alloantigens or antigens shared with the donor [132]. Chronic GVHD could result from inadequate production or function of natural or induced Treg after HCT [133,134]. Taken together, these results suggest that multiple pathways involving T cells, B cells, non-lymphoid cells, and Treg cells can lead to manifestations of the clinical syndrome recognized as chronic GVHD.

Graft-versus-leukemia effects

The therapeutic advantage of allogeneic HCT results not only from the ability to deliver more intensive chemotherapy and radiotherapy, but also from antineoplastic effects mediated by the graft (see Chapter 16). Experimental evidence for this GVLE was reported years before the first clinical transplants. Clinical evidence for GVLEs came initially from retrospective observations that leukemic relapse occurred less frequently in patients who developed GVHD than in those who did not. Donor T cells clearly are major mediators of GVLEs. Removal of T cells in the donor marrow has been associated with an increased risk of relapse in addition to a reduction in GVHD. The risk of relapse after T-cell-depleted marrow transplantation for acute leukemia is comparable to the risk in patients who do not develop GVHD after transplantation with unmodified marrow. Experimental animal systems also show unequivocally that alloreactive donor T cells mediate GVLEs.

In patients with acute leukemia, GVLEs tracks closely with GVHD. In contrast, patients with chronic phase (CP) CML who received T-cell-depleted grafts had a higher risk of relapse than those who received T-cell-replete grafts but did not develop GVHD. These results suggest that in patients with CP CML, alloreactive T-cell responses of small magnitude may be sufficient for GVLEs while remaining insufficient for clinically apparent GVHD. Malignant CP CML stem cells (and their normal counterparts) are thus extremely sensitive to attack by allogeneic T cells.

The mechanisms whereby alloreactive T cells can mediate GVLEs have been well studied in murine models [135]. Both CD4- and CD8-mediated GVLEs require recognition of recipient peptide–MHC complexes by donor TCRs [104]. T cells must also make adhesive interactions with ICAM-1 on leukemia cells. The key T-cell effector molecules have varied with the leukemia model used and with the strain pairing. Essential roles for perforin, FasL and TRAIL have been reported in experiments with gene-deficient T cells, but the results of these experiments can be difficult to interpret, since deficiency of these genes in donor T cells can have effects that extend beyond cytotoxic activity [82]. In contrast, experiments with gene-deficient leukemias induced by the retrovirus-mediated expression of oncogenic translocations showed no essential role for Fas, TRAIL-R, TNF receptors, or perforin, indicating that the T-cell effector mechanisms responsible for GVLEs were highly redundant.

NK cells can also mediate GVLEs [83]. A protective effect against AML but not ALL relapse was first observed in HLA-haploidentical HCT with profound T-cell depletion, high CD34 doses, and no calcineurin-based immunosuppression in donor–recipient pairs where the HLA class I types of the donor and recipient predicted for the presence of donor NK cells that lacked an appropriate inhibitory

HLA class I molecule in the recipient [136]. Importantly, alloreactive NK cells do not induce GVHD. Recent work indicated that NK cells can protect against AML relapse in recipients of T-cell-replete grafts from HLA-mismatched unrelated donors [137]. In this study, GVLEs were associated with expression of the activating receptor KIR2DS1 by donor NK cells, but the effect was not observed with donors with strong expression of MHC class I ligands that bind the activating receptor, since this condition induces hyporesponsiveness in developing NK cells that express this specific receptor. Finally, the effect was observed only when recipient cells expressed at least one MHC class I ligand that binds to the activating receptor.

Efforts to gain a therapeutic advantage from interventions designed to increase GVLEs have had mixed success. Among patients with high-risk malignancies, the administration of non-irradiated peripheral blood buffy coat cells from the marrow donor during the first 4 days after transplantation caused an unacceptably high risk of GVHD and transplant-related mortality. Among patients who have recurrent malignancy after transplantation, remissions can occasionally be induced simply by withdrawing immunosuppressive medications. In patients who do not have GVHD, remissions can be induced by infusion of donor lymphocytes (see Chapters 69 and 70). Such remissions occur in approximately 70–80% of patients with CP CML. Remissions occur less often in patients with CML in blast crisis, myeloma, myelodysplasia, and acute myeloid leukemia and much less frequently in patients with acute lymphoblastic leukemia. Donor lymphocyte infusions can cause acute or chronic GVHD and transient marrow aplasia, depending on the number of T cells administered and the degree of HLA mismatching between the recipient and donor.

From a clinical standpoint, a central question is whether GVLEs can be separated from GVHD [138,139]. The separation of T-cell-mediated GVLEs from GVHD could be achieved by using donor T cells that recognize MHC class I-restricted mHAs expressed only on hematopoietic cells and malignant cells in the recipient [140]. Alternatively, T cells could be altered in a way that disables functions that contribute to GVHD but are not required for GVLEs. Finally, T cells could be altered in a way that restricts their ability to migrate to GVHD target tissues, such as the bowel, where malignant cells are less likely to reside. Such strategies have been tested successfully in animal models. Donor NK cells might be especially well suited for mediating GVLEs without GVHD, since they do not cause GVHD.

Development of tolerance

Tolerance after HCT is defined functionally as the presence of immune reactivity without rejection or overt GVHD in the absence of immunosuppressive medications [141] (see Chapter 12). At any given time, immune reactivity potentially involves three main populations of T cells in the recipient: residual recipient T cells, progeny of mature T cells contained in the allograft, and donor-derived T cells that mature from donor progenitor cells in the thymus. Alloreactive residual recipient T cells and progeny of mature T cells in the graft can be controlled by several mechanisms. Under normal physiologic conditions, active suppression is required for peripheral tolerance, since autoimmunity develops in the absence of Treg cells, and the same applies after HCT. Consistent with this hypothesis, donor-derived T helper and T cytotoxic cells that recognize recipient alloantigens can be detected by in vitro assays in all patients after allogeneic HCT regardless of whether clinically evident GVHD has occurred. As discussed above, reconstitution of Treg cells may have an important role in preventing chronic GVHD. Other mechanisms that potentially control alloreactive residual recipient T cells and progeny of mature T cells in the graft include clonal deletion or induction of anergy or exhaustion through chronic antigen stimulation [142–146].

In the thymus, developing T cells originating from donor-derived progenitors are negatively selected by non-hematopoietic thymic components that present recipient antigens through both direct and indirect mechanisms, and by donor-derived DCs that present donor antigens through direct mechanisms and present recipient antigens through indirect mechanisms [147]. Some evidence has suggested that central tolerance can be impaired by GVHD, either because of damage to non-hematopoietic thymic components or because of functional abnormalities in donor-derived DCs. These results raise the possibility that autoimmune responses against epitopes other than mHAs could contribute to late GVHD in humans.

Immune reconstitution

Pathways of T-cell reconstitution

After HCT, all patients develop profound immunodeficiency caused by the preparative regimen, the effects of donor T cells, and the administration of immunosuppressive medications. Reconstitution with donor cells corrects this immunodeficiency (see Chapter 15). Reconstitution of innate immunity with myeloid-derived cells, NK cells, and natural IFN-α/β-producing cells occurs within the first month after transplantation, and reconstitution of adaptive immunity follows later. Risk factors for delayed reconstitution of adaptive immunity include the use of cord blood cells, unrelated donors or grafts containing low numbers of CD34 cells or T cells, older age of the recipient or donor, the occurrence of acute or chronic GVHD, and high-dose glucocorticoid treatment.

Reconstitution of T cells can occur both through thymus-independent expansion of the mature T-cell population in the graft and through maturation of marrow-derived cells in the recipient thymus [148]. Clinical and experimental studies have indicated that the initial reconstitution of T cells after transplantation occurs primarily through proliferation of mature donor T cells in the graft, and the contribution of new T cells from the thymus becomes apparent later. Memory T cells transferred with the graft contribute immediate protective effects, whereas naïve T cells transferred with the graft and new T cells produced through the thymus after HCT confer protection only after priming by infection or immunization. Production of new T cells in the thymus persists throughout life but declines gradually with age, and this decline is at least partly intrinsic to the thymus and not the hematopoietic system. Irradiation, medications used for immunosuppression before and after HCT, and GVHD impair recovery of thymic function after the transplant, with more profound effects in adults than in children. The thymic pathway of T-cell reconstitution is more important for recovery of CD4 cells than for CD8 cells. For these reasons, the balance between contributions from the thymus-independent and thymus-dependent pathways of T-cell reconstitution differs between $CD4^+$ and $CD8^+$ T cells and depends on the age of the patient, the number of T cells in the graft, and the time interval from transplant.

Thymic production of T cells in humans can be assessed by an increase in the volume of the thymus by imaging studies, an increase in the percentage or number of cells with T-cell receptor excision circles (TRECs), and an increase in the percentage or number of cells that express the $CD45RA^+$ $CD30^+$ $CD45RO^-$ phenotype characteristic of recent thymic emigrants. TRECs are formed during the process of *TCR-A* and *-B* gene rearrangement in T cells during development in the thymus. Since TREC DNA is not copied during cell division, the percentage of TREC-containing cells decreases as recent thymic emigrants proliferate. For this reason, the most informative measurements of thymic output require assessment of both TREC content and T-cell proliferation. In lymphopenic mice, naïve T cells emerging from the thymus proliferate because of homeostatic stimulation and convert from a naïve phenotype to a "pseudomemory" phenotype in the absence of specific antigen stimulation. This process is driven by insufficiency of the peripheral T-cell repertoire. T cells that have recently emigrated from the thymus proliferate and convert to a pseudomemory phenotype unless the peripheral repertoire already contains T cells with the same TCR specificity. The presence of naïve T cells in the periphery therefore indicates both thymic output and sufficiency for at least some specificities in the T-cell repertoire.

The time for restoration varies for different components and functions of the immune system. In recipients transplanted with unmodified marrow from an HLA-identical sibling, proliferative responses after in vitro stimulation of T cells with mitogen begin to reach the normal range after approximately 4–6 months, the number of peripheral blood CD4 cells begins to reach the normal range after 7–9 months, and production of immunoglobulin G by in vitro stimulated B cells recovers after 7–9 months. Recovery of immune responses is delayed by several months when T-cell-depleted marrow is used for transplantation, and recovery of CD4 cells is accelerated when hematopoietic growth factor-mobilized stem cells are used for transplantation. Long-term reconstitution of immunity against certain pathogens probably represents the acquisition of new memory cells generated by exposure to antigens after the transplant.

During the first 3 months after HCT, selective proliferation among different T cells in the thymus-independent pathway of reconstitution causes severe skewing in the distribution of TCRs among repopulating T cells. During this time, the spectrum of *TCR* gene rearrangements remains abnormally limited and unstable. Changes in the T-cell repertoire during this period most likely result from alloantigen-driven proliferation, especially in patients with GVHD.

Throughout the first 3 years after the transplant, the progeny of mature T cells in the graft are gradually diluted by T cells that have originated through maturation of marrow cells in the thymus. With this replacement, the spectrum of *TCR* gene rearrangements gradually becomes normal. The rate at which the spectrum of *TCR* gene rearrangements returns to normal depends on the number of mature T cells in the graft and the level of thymic function. Thymus-dependent replacement of cells that originated from the thymus-independent pathway would be expected to occur more slowly with grafts that contain a large number of mature T cells and more rapidly with grafts that contain small numbers of mature T cells. Abnormalities in the spectrum of *TCR* gene rearrangements can persist for more than 3 years in patients with severely impaired thymic function. For reasons that are not yet clear, the spectrum of *TCR* gene rearrangements also remains abnormal in patients with persistent mixed chimerism, even in the absence of GVHD. The diversity of immunoglobulin gene rearrangements in B cells is also reduced after HCT.

Effects of GVHD on immune reconstitution

GVHD impairs T-cell immune reconstitution through at least several distinct mechanisms. GVHD causes injury to thymic epithelium, thereby reducing T-cell reconstitution and diversification of the TCR repertoire through the thymus-dependent pathway [149]. GVHD decreases the total number of T cells that can be accommodated within the periphery, perhaps by causing microenvironmental abnormalities that reduce the number of functional peripheral T-cell niches. Stimulation by recipient alloantigen can cause activation-induced apoptosis in a large fraction of donor T cells that recognize recipient alloantigens, and during GVHD, donor T cells that do not recognize recipient alloantigens are induced to express Fas and become susceptible to fratricidal or bystander apoptosis caused by donor T cells that express FasL. GVHD also impairs the function of APCs, particularly for presentation of antigens to $CD4^+$ T cells [150]. Both acute and chronic GVHD block B lymphopoiesis. Patients with chronic GVHD do not develop immunocompetence until chronic GVHD resolves and until withdrawal of immunosuppressive medications has started.

Opportunistic infections as an indicator of impaired immune reconstitution

Opportunistic infections are a key clinical indicator of immunodeficiency after HCT. Pathogens involved in opportunistic infections prominently include Epstein–Barr virus (EBV) (see Chapter 90), cytomegalovirus (CMV) (see Chapter 87), adenovirus and BK virus (see Chapter 93), HHV6 (see Chapter 92), and fungal organisms (see Chapter 86). The development of lymphoproliferative disorders caused by EBV infection is a particularly striking example of opportunistic infection associated with immunodeficiency after HCT [151]. The risk of this often-fatal complication is increased in patients who receive T-cell-depleted grafts and in patients treated with agents that deplete T cells after HCT, indicating that transfer and persistence of pathogen-specific immunity by memory T cells from the donor have an important role in controlling EBV infection. Risk factors for CMV, fungal, and adenovirus infection are very similar. Prolonged lymphopenia represents a common thread among these risk factors. In particular, CD4 lymphopenia has been strongly correlated with the risk of opportunistic infections after HCT. In addition, more subtle abnormalities of T-cell function are likely to increase the risk of opportunistic infections. Examples include qualitative defects in T-cell signal transduction and reduced production of TNF-α by activated CMV-specific CD8 cells.

Approaches for enhancement of immune reconstitution

In certain conditions, EBV-specific immunity can be reconstituted and regression of EBV-associated lymphoproliferative disorders can be induced by infusion of a relatively small number of donor lymphocytes. Alternatively, immune responses against specific pathogens can be reconstituted by infusion of antigen-specific T-cell lines or T-cell clones (see Chapters 15 and 68). Immune reconstitution might also be accelerated through a variety of other strategies. In mice, administration of keratinocyte growth factor has been shown to protect thymic epithelium from the effects of irradiation and GVHD, thereby improving immune reconstitution through the thymus-dependent pathway after the transplant [152]. Thymic epithelium is a source of IL-7, a growth factor involved in production of T cells and maintenance of T-cell homeostasis in the periphery. Exogenous administration of this cytokine does not reverse the effects of GVHD on thymic stromal function but can enhance T-cell production through direct effects on T cells as they develop in the thymus [153]. Exogenous administration of IL-7 also stimulates proliferation of mature T cells, protects T cells from apoptosis, and enhances T-cell function. Although exogenous administration of IL-7 exacerbated GVHD after HCT in non-irradiated mice, this effect was not observed in irradiated mice in which endogenous levels of IL-7 are already increased because of lymphopenia. Expression of the IL-7 receptor α chain is reduced in donor T cells that have proliferated in response to recipient alloantigens but not in other T cells. For this reason, IL-7 has little effect on T cells that recognize recipient alloantigens but stimulates proliferation of other T cells that may be important for immune reconstitution. Effects similar to those observed with exogenous administration of IL-7 also occur with exogenous administration of IL-15 after HCT. Under some conditions, however, exogenous administration of IL-15 exacerbates GVHD in irradiated recipients. Other potential approaches for enhancing immune reconstitution after HCT include androgen blockade, administration of flt3 ligand or IL-22 [154], and administration of synthetic oligodeoxynucleotides containing unmethylated cytosine–guanine motifs that can expand naïve and memory populations by preventing T-cell death.

In most recipients without chronic GVHD, immunologic function has recovered sufficiently to allow responses after administration of diphtheria, pertussis, and tetanus vaccine and pneumococcal vaccine at 1 year after HCT (see Chapter 106). To some extent, antigen-experienced memory T cells transferred from the donor contribute to this immune reconstitution, and responses to polysaccharide–protein conjugate vaccines in the recipient after the transplant can be enhanced by vaccination of the donor before the transplant. Administration of live viral vaccines, such as measles, mumps, rubella, and oral polio, is deferred until at least 2 years after transplantation, even in healthy recipients, as a precaution to avoid inadvertent infection with these attenuated strains. These vaccines should not be given to patients with chronic GVHD or to anyone taking immunosuppressive medications.

Summary and conclusions

Although this overview of the immunology of HCT has focused on the individual outcomes of engraftment, GVHD, control of malignancy, tolerance, and immune reconstitution, these remain highly interconnected as different aspects of a single overall process. For this reason, the evaluation of interventions designed to influence one particular immunologic outcome of HCT will require equivalent scrutiny of other immunologic outcomes. Historically, clinical HCT has evolved from the biomedical knowledge and understanding gained from animal studies. Murine models have the advantages of well-defined immunogenetics and a wealth of reagents and methods for dissecting the cellular and humoral mechanisms involved in HCT immunobiology, whereas canine and non-human-primate models more closely mimic the immunologic and medical challenges of HCT in a large outbred species. The wide range of experimental manipulations afforded by animal models has allowed rapid progress in elucidating principles that improve the biomedical understanding of HCT immunology in all of its interconnected complexity. The constraints of any given experimental model and the differences between species [155–157] must be carefully considered when results are interpreted and when conclusions are translated for clinical application.

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CHAPTER 10
Histocompatibility

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Introduction

The human major histocompatibility complex (MHC) is a 7.6-megabase (Mb) region on the short arm of chromosome 6 that contains more than 300 genes, approximately 20–30% of which have immune-related function [1,2] (Figure 10.1). These functions include antigen processing and presentation (the classical *HLA-A*, *B*, *C*, *DM*, *DO*, *DP*, *DQ*, and *DR* genes; *PRSS16*; *PSMB*; *AP*; and *UBD*), inflammation (*ABCF1*, *AIF1*, *DAXX*, *IER3*, *LST1*, *LTA*, *LTB*, *NCR3*, and *TNF*), leukocyte maturation (*DDAH2* and *LY6G*), the complement cascade (*BF*, *C2*, *C4A*, and *C4B*), maternal–fetal immunology (the non-classical class I *HLA-E, F*, and *G* genes), stress response (*MICA*/*MICB*), immune regulation (*NFKB*, *RXRB*, and *FKBPL*), and the immunoglobulin superfamily (*AGER*, *BTN*, *C6orf25*, and *MOG*).

HLA antigens are expressed on virtually all nucleated cells, and this ubiquitous expression contributes to their fundamental role in the acceptance and rejection of transplanted tissues. Most individuals are heterozygous for two different parentally derived alleles at each HLA locus. For a given HLA locus, the antigens encoded by the two HLA alleles are codominantly expressed, and a heterozygous individual will therefore possess two different antigens at that locus. The extensive polymorphism of HLA genes, coupled with their tendency to be strongly linked to one another, has important implications in donor–recipient histocompatibility matching for hematopoietic cell transplantation (HCT).

Evidence for an MHC in humans was first recognized in the early 1950s following observations that sera from patients with febrile transfusion reactions could cause the agglutination of leukocytes from their transfusion donors, in addition to other individuals [3]. Subsequent studies showed that leukocyte antibodies could also be found in the sera of multiparous women. The term "HLA" was derived by combining the human-1 (*HU-1*) and leukocyte antigen (*LA*) designations used by Dausset and Payne, respectively, to describe the newly discovered leukocyte antigen system [3–7] (Table 10.1). Since 1964, a series of 16 collaborative International Histocompatibility Workshops has greatly extended our knowledge of the HLA system and contributed to the standardization of typing methods and nomenclature [8–21] (Table 10.2).

HLA antigens are inherited in classical Mendelian fashion (Figure 10.2). During an immune response, cells of the immune system interact through recognition of cell surface molecules encoded by genes of the human MHC. T cells recognize foreign antigens presented as peptide fragments in association with MHC molecules. Before T cells can recognize an antigen, the antigen must be processed by an antigen-presenting cell, bound to a "self" MHC molecule, and transported to the cell surface. Many different peptide antigens can be processed and presented to T cells, including those derived from bacteria, viruses, toxins, and foreign cells and tissues, as well as from autologous tissue and cellular products. Definition of the peptide-binding motifs that characterize HLA molecules provides important information about the role of the HLA system in peptide presentation, immune responsiveness, and disease susceptibility [22–24]. The role of the MHC in antigen presentation helps explain the extensive polymorphism of the MHC at the population level: the greater the polymorphism of the MHC, the larger is the array of foreign peptides that can be presented to the immune system. In this way, MHC genes influence T-cell immune responsiveness by the selection of antigens that can be bound and presented for T-cell recognition. MHC molecules have a major effect in transplantation due to the fundamental role that they play in T-cell activation and initiation of an alloresponse.

With the advent of DNA typing methods, it is now possible to define each class of HLA molecule by its unique sequence [25] (http://www.ebi.ac.uk/imgt/hla). For a given HLA locus, the unique sequence variant is termed the "allele" (e.g., A*02:01), and the alleles expressed by the two parental haplotypes constitute the "genotype" (e.g., HLA-A*02:01, *03:01). HLA "antigens" refer to molecules defined by their reaction with HLA antibodies. The combination of two antigens at a given locus, encoded by the alleles of the two parental chromosomes, is termed the "phenotype" (e.g., HLA-A2, A3). The remarkable diversity among genes of the HLA system has greatly exceeded expectations and reflects the fundamental role that the MHC plays in immune responsiveness and susceptibility and resistance to disease [26]. An appreciation of HLA polymorphism is also central to understanding how histocompatibility antigens function as transplantation determinants through their interaction with T cells (Chapter 9), natural killer (NK) cells and killer-cell immunoglobulin-like receptors (KIRs) (Chapter 11) and by the presentation of minor histocompatibility antigens (Chapter 16).

HLA genes: structure and function

The MHC was first sequenced in 1999 [27,28] (http://www.hapmap.org/; http://www.sanger.ac.uk/HGP/Chr6/).The "central" portion of the MHC measures 4 Mb in length and houses the classical and non-classical HLA genes. The "extended MHC", or xMHC, comprises a total of 7.6 Mb of sequence, extending from the

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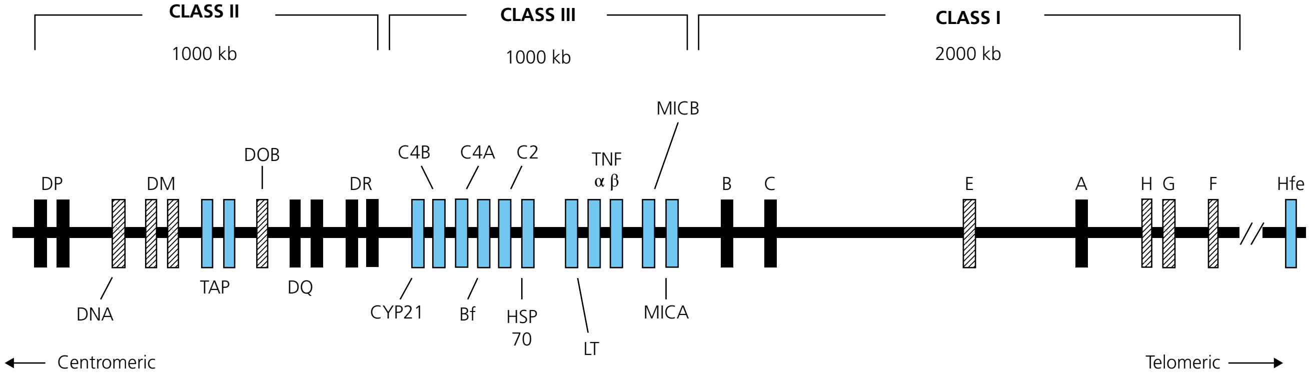

Figure 10.1 The human major histocompatibility complex (MHC) on the short arm of chromosome 6. The class II *HLA-DP*, *-DNA*, *-DM*, *-DOB*, *-DQ*, and *-DR* genes and the class I *HLA-B*, *-C*, *-E*, *-A*, *-H*, *-G*, and *-F* genes are shown in black; those encoding classical transplantation antigens are shown as solid bars, others are cross-hatched. Other HLA region-associated genes are shown in gray: transporter of antigenic peptides (TAP); 21-hydroxylase (CYP21; also termed 21-OH or P450-C21B); complement component C4; properdin factor B of the alternate complement pathway (Bf); complement component C2; heat-shock protein (HSP70); tumor necrosis factor (TNF) complex and lymphotoxin-A (LTA); MICB; MICA; and the hemachromatosis gene (Hfe). The distance from the *HLA-F* gene telomeric to the *Hfe* gene is approximately 4000 kb.

Table 10.1 Definition of commonly used terms in histocompatibility

Term	Definition	Example
Allele code	A system for grouping related alleles together, often defined by the typing technology	HLA-DQB1*02AB signifies HLA-DQB1*02:01 or 02:02
Ancestral haplotypes (AH)	Highly conserved HLA haplotypes derived from a prototype ancestor	HLA-A1B8DR3 (the "8.1" AH)
Direction (vector) of mismatching	Host-versus-graft (HVG): donor alleles not present in the recipient	Recipient: HLA-B7,7 Donor: HLA-B7,8
	Graft-versus-host (GVH): recipient alleles not present in the donor	Recipient: HLA-B7,8 Donor: HLA-B8,8
Epitope	A moiety of a molecule that is immunogenic	Cross-reactive: epitopes shared by two or more HLA antigens Private: epitopes unique to a single antigen
Genotype	An individual's HLA allele sequence(s)	HLA-A*01:01, 02:01
Haploidentical	Relatives who share one chromosome 6 and are variably matched for the non-shared HLA haplotype	Recipient: A1,2; B7,8; DR2,3 Donor: A1,11; B8,44; DR3,4
Haplotype	Markers that travel together on the same chromosome 6 strand	HLA-A1, B8, DR3
Heterozygous	Two different HLA alleles/antigens at the HLA locus	HLA-A*02:01,02:05 (NB: this genotype has the homozygous A2,2 phenotype)
HLA	HU-1 (human-1 antigen) and LA (leukocyte antigen)	Human leukocyte antigens that govern tissue type
HLA allele	An alternative form of a gene; a unique sequence variant	HLA-A*02:01
HLA antigen	The expressed HLA protein for which alloantisera are defined	HLA-A2
HLA class I	The telomeric (further from the centromere) region of the MHC encoding HLA genes	HLA-A, HLA-B, HLA-C ("classical class I genes") HLA-E, F, G; MICA, MICB ("non-classical class I")
HLA class II	The centromeric (closer to the centromere) region of the MHC encoding HLA genes	HLA-DR, HLA-DQ, HLA-DP
HLA class III	The region of the MHC mapping between class I HLA-B and class II HLA-DR	Tumor necrosis factor (TNF); complement pathway genes
Homozygous	Two copies of the same allele/antigen at the HLA locus	HLA-A*01:01,01:01
Identical-by-descent (IBD)	Genotypically identical siblings who have inherited the same maternal and paternal chromosome 6	Patient's and sibling's maternally derived haplotype is A1B8DR3, and paternally derived haplotype is A2B7DR2
Identical-by-state (IBS)	Phenotypically matched unrelated individuals who fortuitously share the same HLA antigens	HLA-A1,2 B7,8 DR2,3 patients and donors
Leukocyte antibody cross-match	An assay that quantifies antibodies in the sera of patients that are directed against the transplant donor's HLA class I and II antigens	
Linkage disequilibrium (LD)	The non-random association of alleles or antigens	HLA-B8 with HLA-DR3 HLA-B8 with HLA-C7 HLA-DR3 with HLA-DQ2
Match grade	The total number of HLA alleles/antigens for which the transplant donor and recipient are matched	Five locus definition (total of 10 possible determinants at HLA-A, -B, -C, -DRB1, -DQB1): 10/10 (fully matched); 9/10 (one HLA mismatched allele or antigen); 8/10 (two mismatched alleles or antigens); etc. Four locus definition (total of 8 possible determinants at HLA-A, -B, -C, -DRB1): 8/8; 7/8; 6/8; etc. Three locus definition (total number of 6 possible determinants at HLA-A, B, DRB1): 6/6; 5/6; 4/6; etc.
Mixed lymphocyte culture (MLC)	A functional assay that measures proliferative responses between two cell populations	
MHC	Major histocompatibility complex	A 7.6-Mb region of chromosome 6p21.3 that encodes the histocompatibility genes important in transplantation

Continued

Table 10.1 *Continued*

Term	Definition	Example
Natural killer immunoglobulin-like receptor (KIR)	A multi-gene system encoded on chromosome 19q13.4 responsible for innate immunity	Inhibitory receptors: KIR2DL3, KIR2DL2, KIR2DL1, KIR3DL1 Activating receptors: KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1
Non-inherited maternal antigen (NIMA) match	HLA antigen(s) that is mismatched between a patient and donor, but is shared between the patient and the donor's mother	Patient: HLA-A1,2 Donor: HLA-A1,3 Donor's mother: HLA-A1,2
Phenotype	An individual's HLA proteins	HLA-A1,2
residue	An amino acid of the HLA protein	Residue 116 of class I
Single-nucleotide polymorphism (SNP)	The simplest form of human genetic variation. An SNP is a biallelic nucleotide change	A vs. T A vs. C A vs. G T vs. G etc.
TagSNP	An SNP that is in 100% LD with another SNP(s) so that they serve as proxies for one another	
Typing resolution	Low: broad family of alleles equivalent to antigen	HLA-A2 if defined by serology; A*02 if defined by molecular methods
	Intermediate: group of related alleles within an antigen family equivalent	HLA-A*02:01/02:05
	High: DNA sequence is defined (minimum 4 digits, often 6 digits)	HLA-A*01:01:01

Table 10.2 The International Histocompatibility Workshops

Workshop	Year	Chairman	Venue	Advances	Ref.
1st	1964	D.B. Amos	Durham, NC, USA	Study of the Hu-1, LA, and FOUR antigens; MLC; microcytotoxicity serology technique	[8]
2nd	1965	J.J. van Rood	Leiden, Netherlands	Computerized data analysis; skin grafting	[9]
3rd	1967	R. Ceppellini	Turin, Italy	Family studies; HLA in renal transplantation	[10]
4th	1970	P. Terasaki	Los Angeles, CA, USA	Definition of 27 HLA-A, -B, and -C specificities	[11]
5th	1972	J. Dausset	Evian, France	Worldwide typing of 49 populations	[12]
6th	1975	F. Kissmeyer-Nielsen	Aarhus, Denmark	Description of Dw specificities	[13]
7th	1977	W. Bodmer	Oxford, UK	Definition of DR1-7 specificities; HTC testing	[14]
8th	1980	P. Terasaki	Los Angeles, CA, USA	Definition of HLA-MB (DQ) and MT (DR52/53); HLA in renal transplantation and disease association	[15]
9th	1984	E. Albert, W. Mayr	Munich, Germany; Vienna, Austria	New class I and II specificities; transplantation HLA class II in renal	[16]
10th	1987	B. Dupont	Scanticon, NJ; New York, NY, USA	Establishment of RFLP/T-cell clones and HTC methods; creation of standardized homozygous cell line panel	[17]
11th	1991	T. Sasazuki, K. Tsuji, M. Aizawa	Yokohama, Japan	HLA class I PCR typing; anthropology	[18]
12th	1996	D. Charron	St. Malo and Paris, France	Sequencing; class I DNA typing; HLA in medicine	[19]
13th	2002	J. Hansen	Victoria, BC, Canada; Seattle, WA, USA	Virtual DNA analysis; identification of SNP markers; HLA in anthropology, disease association, HCT	[20]
14th	2005	J. McCluskey	Melbourne, Australia	MHC and anthropology, disease, infection, HCT, cancer, non-classical HLA genes	[21]
15th	2008	M. Gerbase-DeLima, M. Moraes	Buzios, Brazil; Rio de Janeiro, Brazil	Anthropology, disease, HCT, non-classical genes	
16th	2012	S.G.E. Marsh, D. Middleton	Liverpool, UK	HCT, MICA, antibodies, haplotypes, KIR, data management methods, bioinformatics, NGS, aging	

HCT, hematopoietic cell transplantation; HLA, human leukocyte antigen; HTC, homozygous typing cell; MLC, mixed lymphocyte culture; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single-nucleotide polymorphism; NGS, next-generation sequencing.

HIST1H2AA gene at the telomeric end, to the *RPL12P1* gene at the centromeric end. HLA genes are clustered in three distinct regions, designated class I, class II, and class III (Figure 10.1). Genes within the class I and class II regions share structural and functional properties and are considered part of the immunoglobulin gene superfamily. Although distinct in sequence and structure, both class I and class II genes encode polypeptides that are critical in controlling T-cell recognition and determining histocompatibility in transplantation.

A characteristic feature of HLA genes is their extreme polymorphism (http://www.ebi.ac.uk/imgt/hla). HLA diversity is a reflection of the primary immunologic function of MHC molecules, which is to bind and present antigenic, and potentially pathogenic, peptides for recognition by antigen-specific T-cell receptors (TCRs). The differing structural properties of HLA class I and class II molecules account for their respective roles in activating different populations of T lymphocytes. Cytotoxic T lymphocytes recognize antigenic peptides presented by HLA class I molecules, whereas helper T lymphocytes recognize antigenic peptides presented by HLA class II molecules. HLA class I and class II molecules are characterized by distinctive α and β polypeptide subunits that combine to form αβ heterodimers of the mature molecule. Class I molecules were originally defined by typing with alloantisera and class II molecules defined by testing in functional assays such as the mixed lymphocyte culture (MLC) reaction [29].

The class I region

Classical HLA genes

At least 17 loci, including several pseudogenes, exist in the HLA class I region (Figure 10.1). Three of these loci, termed class Ia, encode HLA-A, -B, and -C alloantigens important for matching in transplantation. Genes of the *HLA-A*, *-B*, and *-C* loci are highly

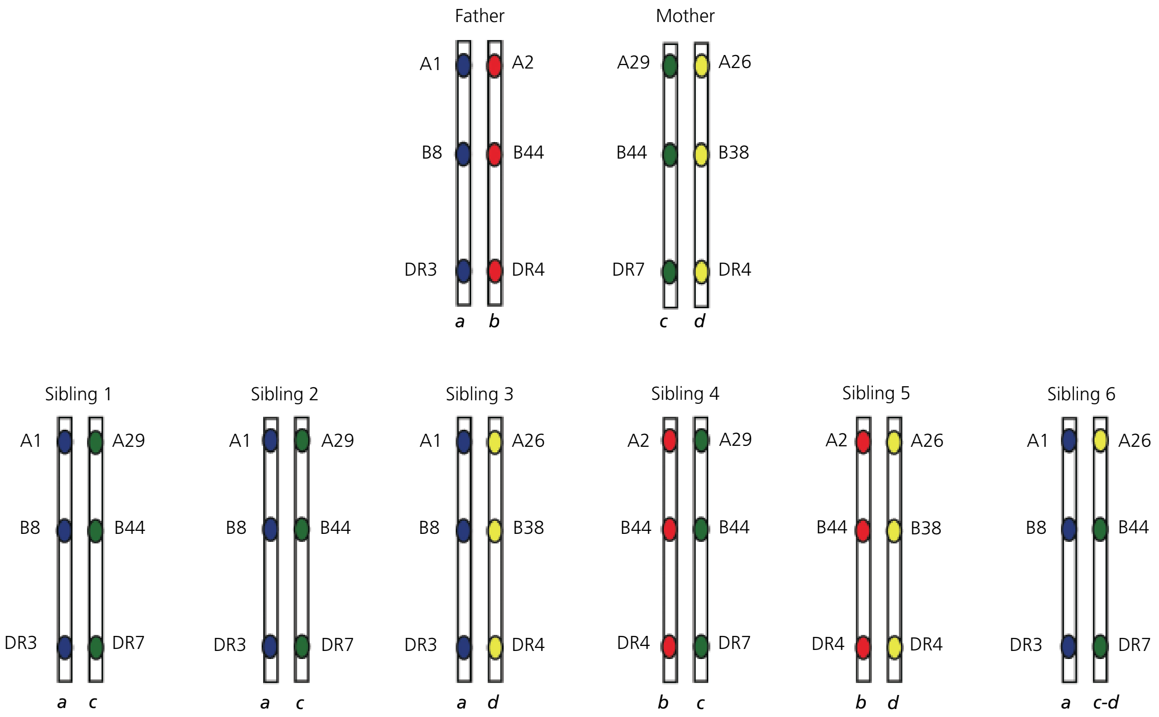

Figure 10.2 A family study. The paternal haplotypes are designated ***a/b*** and the maternal haplotypes ***c/d***. Sibling 1 is the propositus. Compared with sibling 1, sibling 2 is ***a/c*** (HLA genotypically identical), sibling 3 is ***a/d*** (haploidentical), sibling 4 is ***b/c*** (haploidentical), sibling 5 is ***b/d*** (complete mismatch), and sibling 6 is ***a/c–d*** (maternal ***c–d*** recombinant). Siblings 1 and 4 are mismatched at HLA-B in the graft-versus-host (GVH) vector only. Siblings 1 and 6 are mismatched at only HLA-A for both GVH and host-versus-graft vectors; they are matched from HLA-B to DR due to the maternal recombinant haplotype.

polymorphic, with *HLA-B* the most polymorphic locus in the human genome (http//www.ebi.ac.uk/imgt/hla) [25]. *HLA-A*, *-B*, and *-C* genes consist of eight exons and seven intervening introns. Exon 1 is a leader sequence; exons 2, 3, and 4 encode the α1, -2, and -3 domains of the class I molecule, respectively; exon 5 encodes the transmembrane portion, and exons 6, 7, and 8 encode the cytoplasmic tail. The expressed HLA class I molecule consists of a single polymorphic α (heavy) chain approximately 338–341 residues in length that is non-covalently bound at the cell surface to a β_2-microglobulin light chain. The gene for β_2-microglobulin is located outside the HLA complex on chromosome 15. Structurally, class I molecules comprise two α-helical regions overlaying an eight-strand antiparallel β-pleated sheet that together form the functional groove for peptide binding [22]. Nucleotide substitutions within exons 2 and 3 of class I genes are not distributed randomly but are concentrated in discrete hypervariable regions. The corresponding polymorphic sites within the α1 and α2 domains facilitate the binding of peptide fragments for presentation to the TCR [30]. These polymorphic sites determine the allospecificity of the molecule and form the basis for their nomenclature (http://www.ebi.ac.uk/imgt/hla). Polymorphic residues 77–80 of HLA-C molecules and 77–83 of HLA-B molecules (the latter constituting the Bw4 epitope) define the specificity of NK receptor recognition of HLA-C and -B ligands (described below and in Chapter 11).

Non-classical genes

The class I region contains at least three non-classical genes – *HLA-E*, *-F*, and *-G* (class 1b) [31] – and two class I-like genes – *MICA* and *MICB* [32]. *HLA-E*, *-F*, and *-G* loci are very similar to class Ia genes in terms of overall organization and the relationship between exon sequence and function of the encoded molecule. Class Ib genes serve specialized roles in immune responses, and have a prominent role in both adaptive and innate immune pathways. One difference between classical Ia and non-classical Ib molecules is their tissue expression: whereas class Ia molecules are expressed on all nucleated cells and are therefore present in most tissues, class Ib molecules are more restricted in distribution and tend to be tissue specific. Of the three, HLA-E is the most ubiquitously expressed [33]. HLA-G is expressed specifically in placental tissue [34], whereas HLA-F is expressed in the bladder, liver, and placenta and on lymphoblastoid cells [35]. HLA-E and -G molecules serve as ligands for NK cell receptors and thereby regulate NK function [36,37]. HLA-E molecules bind specifically to the conserved leader sequence peptides from class Ia MHC molecules to promote cell surface interactions with the CD94/NKG2 class of NK cell receptors.

MICA and *MICB* genes share amino acid sequence homology with the classical class I proteins but are distinguished from HLA-A, -B, and -C by the lack of association to β_2-microglobulin [38] and the absence of peptide-binding function [32] due to a shallow peptide-binding groove. MIC proteins are expressed in the intestinal epithelium and play a fundamental role as cell stress response genes inducible by heat shock [32]. MICA and MICB products are ligands for the NKG2D-activating receptor involved in NK immunosurveillance and in co-stimulation of T-cell responses [39].

The class II region

The HLA class II region is comprised of nine distinct genes: *DRA*, *DRB1*, *DRB3*, *DRB4*, *DRB5*, *DQA*, *DQB*, *DPA*, and *DPB*. The class II region also encodes *TAP* and *LMP* that play a role in the loading of class I molecules with peptides. Six additional genes or gene fragments are either non-functional pseudogenes or do not encode proteins known to participate in transplant-related immune interactions. Class II genes have been collectively referred to as HLA-D region genes since they were initially described in the mid-1970s after the description of the HLA-A and -B (1965) and -C (1971) loci. Class II genes are divided into five families, designated DR, DQ, DO, DN, and DP, based on their degree of sequence homology and their location within the HLA-D region. Class II HLA-DR, -DQ, and -DP genes, like class I genes, are highly polymorphic (http://www.ebi.ac.uk/imgt/hla) [25].

Class II molecules consist of a single polymorphic (DQ and DP) or non-polymorphic (DR) α chain non-covalently bound to a polymorphic β chain [23]. The β chains of HLA-DR, -DQ, and -DP antigens are encoded by the *DRB*, *DQB*, and *DPB* genes, respectively. Polymorphic HLA-DR β chains are encoded by four distinct DRB genes termed *DRB1*, *DRB3*, *DRB4*, and *DRB5*. HLA-DRB genes are inherited as a genetic unit, or *haplotype*. Within the HLA-DRB region, variable numbers of genes are expressed from one haplotype to another (Figure 10.3). For example, DRB haplotypes that type as DR1 include four genes: a polymorphic *DRB1* gene, a non-polymorphic *DRA* gene, and two pseudogenes. In contrast, DRB haplotypes that type as DR3 include five genes: a polymorphic *DRB1* gene, a non-polymorphic *DRA* gene, a polymorphic *DRB3* gene, and two pseudogenes. Further polymorphism within the HLA-D region can result from *trans* pairing of a polymorphic DQ α chain encoded by one parental chromosome with a polymorphic DQ β chain encoded by the other parental chromosome.

Polymorphic sites within class II molecules are localized in specific regions of the α1 and β1 domains of the α and β chains, respectively, to enable binding of a large array of peptides [30]. The polymorphic α1 and β1 domains also determine the allospecificity of the class II molecule. Antigens encoded by *DRB1* and *DQB* genes are known to be potent stimulators of immune reactions in HCT (Chapter 45).

The class III region

The HLA class III region is located between class II (centromeric boundary) and class I (telomeric boundary) (Figure 10.1). The class III region comprises some 60 genes within approximately 700 kb of DNA, making it the most gene-dense region of the human genome. More than 14% of the sequence within this region is coding, with an average of 8.5 genes per 100 kb of DNA. The class III region genes display positive linkage disequilibrium (LD) with HLA loci, and haplospecific polymorphisms have been described for several common xMHC haplotypes [40]. The class III region has been associated with susceptibility to numerous autoimmune and infectious diseases [26]. Those with proven function include genes encoding the complement components C2, C4, and Bf, tumor necrosis factor, certain transport proteins, and heat shock proteins.

The history of HLA

The description of the first HLA group in 1958 stimulated worldwide interest in the genetic structure and biologic function of the HLA system. It became apparent that the scope of work necessary to elucidate the HLA system would require broad collaboration beyond what would be possible within individual laboratories. This led to the organization of an international group of investigators willing to share reagents and unpublished data, beginning in 1964 (Table 10.2). The first HLA antigens had been defined by individual investigators using their own reagents, antisera, and cell panels, and employing a variety of locally developed laboratory assays. An exchange of reagents was necessary to compare antisera and standardize antigen definition, establish a common nomenclature, and develop standardized testing methods. The International HLA Workshops thus provided a critical mechanism for promoting this collaboration, in addition to creating a forum for new technology and dissemination of reagents worldwide.

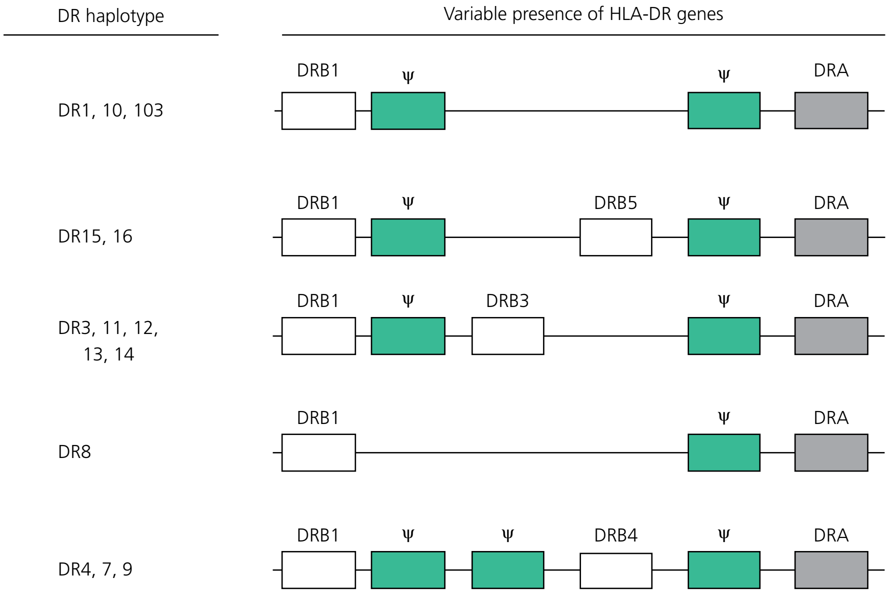

Figure 10.3 Variable expression of *DRB* genes according to HLA haplotype. Clear boxes, *DRB* genes; gray boxes, non-polymorphic *DRA* gene; black boxes, pseudogenes.

The early period: 1964–1972

HLA antigens were discovered in the late 1950s using agglutination assays employing leukoagglutinating [immunoglobulin M (IgM) antibodies. The 1st HLA Workshop (1964) introduced a complement-dependent microdroplet assay that required only microliter amounts of cytotoxic (IgG) antibodies of relatively high specificity and reproducibility. Cytotoxic antisera were carefully selected for HLA specificity and were usually obtained from multiparous women who had been naturally immunized to HLA alloantigens through pregnancy. The in vitro MLC was also studied in the 1st Workshop. The MLC assay involved culturing responder leukocytes, later identified as T lymphocytes, with second-party stimulator cells and measuring the ensuing proliferative response. The magnitude of the response was considered an indication of the disparity between the two cell populations for certain HLA antigens, although the exact nature of these antigens was unknown.

The 3rd HLA Workshop (1967) elucidated that HLA antigens were products of two closely linked loci. A nomenclature committee of geneticists and immunologists was organized to establish standardized nomenclature. The designation of "HL-A" (H standing for the Hu1 system of Dausset, L standing for leukocyte, and A for the first recognized locus within this system) was subsequently adopted [41].

By the 4th HLA Workshop (1970), 11 official HL-A specificities (HL-A1, -A2, -A3, -A5, -A7, -A8, -A9, -A10, -A11, -A12, and -A13) had been defined, with numeric designations being assigned in sequential order of discovery and reporting. At least eight other specificities were given provisional "Workshop" designations (e.g., w27) indicating probable but not conclusive characterization. There was also evidence of a third sublocus within the HL-A system [42]. Following the 5th Workshop (1972), the nomenclature committee confirmed the existence of at least two subloci within the HL-A system and assigned the known antigens to two distinct segregant series, the first, or LA, series, and the second, or FOUR, series. The identity of the locus encoding determinants causing stimulation in the MLC reaction remained to be elucidated.

The middle period: 1975–1984

This period was highlighted by the refinement of serologic methods for class II region determinants, the development of additional cellular assays for the class II region, and a dawning recognition of the enormity of HLA diversity which ultimately ushered in the DNA era of HLA testing. Four International Workshops were held between 1975 and 1984 (Table 10.2). By the 6th Workshop (1975), it was recognized that disparity for HLA-D region antigens led to the lymphocyte activation and proliferation measured in the MLC assay [43]. The 6th Workshop was the first to include the use of homozygous typing cells (HTCs) to study determinants of the newly defined HLA-D locus [44]. Identification of HLA antigens expressed on B cells but not on T cells was termed "Ia-like" or "D region-associated B-cell antigens," and their description paved the way for the future description of the HLA-DR locus. Following the 6th Workshop, the World Health Organization Nomenclature Committee adopted the term "HLA" to reflect the multi-locus nature of the MHC.

Although the direct MLC had been used as a tool for donor selection in HCT since the late 1960s, its utility as a test of histocompatibility was largely restricted to confirming HLA-D region compatibility between HLA-A, -B, -DR serologically matched donors and recipients. Data were ultimately published indicating that the MLC was not consistently predictive of the risk of clinically severe acute graft-versus-host disease (GVHD) [45]. In the late 1980s, DNA-based methods supplanted both the direct MLC and the use of D-region homozygous typing cells for the evaluation of donor–recipient compatibility in HCT. In contrast, the leukocyte antibody cross-match continues to be used as an important indicator of histocompatibility in HCT. Historically, leukocyte antibody cross-matches were performed using a complement-dependent cytotoxicity assay testing recipient serum against donor lymphocytes. More recently, cross-matches have been performed utilizing the highly sensitive and specific flow cytometry. By using fractionated T and B lymphocytes as target cells in the cross-match assay, antibodies directed against both HLA class I and class II antigens can be demonstrated. A positive antidonor result in the complement-dependent cytotoxicity assay, indicative of the presence of donor-directed HLA antibodies in the HCT recipient, is highly predictive of graft failure [46]. Similarly, a positive patient antidonor cross-match by flow cytometry is predictive of graft failure [47].

In the mid-1980s, serologic typing became more refined as large numbers of highly specific HLA antisera were accumulated and tested against diverse panels of target cells. It was soon recognized that operationally monospecific antibodies could detect alloepitopes shared by more than one HLA class I gene product [48]. The term "cross-reactive" was coined to describe antigenic epitopes shared by two or more distinct HLA antigens, "public specificities" to describe epitopes shared by more than one distinct antigen, and "private specificities" to describe epitopes that were unique to a single antigen. The identification of private and public specificities provided a means to cluster serologically cross-reactive antigens into cross-reactive groups (CREGs). In the early unrelated donor transplant experience, CREG-mismatched unrelated donors were considered when matched donors were not available [49]. By the 9th HLA Workshop (1984), more than 124 HLA specificities at the A, B, C, D (MLC-defined), DR, DQ, and DP loci had been defined [50].

The later period: 1987 to the present day

The HLA Workshops provided a unique framework for testing functional assays for alloimmunogenicity. Primary among these was the limiting dilution assay, a modification of the MLC in which patient and donor cells are co-cultured for an initial 3–6-day priming phase, followed by plating at limiting dilution which then generates a readout of patient-versus-donor and/or donor-versus-patient alloimmune reactivity. The frequency of both cytotoxic T-lymphocyte precursor and helper T-lymphocyte precursor cells can be measured [51,52]. The presence of elevated patient antidonor cytotoxic T-lymphocyte precursor and/or helper T-lymphocyte precursor frequencies might signal an increased risk of rejection of the allograft, whereas the presence of elevated donor anti-patient precursor frequencies might signal an increased risk of GVHD. Today, cellular methods have been supplanted by DNA assays, although the former may have some utility in donor screening when several otherwise equally matched donors are available [53].

The discovery of polymerase chain reaction (PCR) technology in the mid-1980s propelled the HLA field into the DNA era. At that time, the development of PCR-based methods was feasible for HLA-DR because of the availability of sequence information at this locus. The 10th Workshop (1987) was the first to introduce DNA-based laboratory methods for tissue typing. Three major technologies emerged and became integrated in both clinical and research applications: sequence-specific primer (SSP) methods [54] (Figures 10.4 and 10.5); sequence-specific oligonucleotide probe (SSOP) hybridization [55,56] (Figures 10.6 and 10.7), and direct

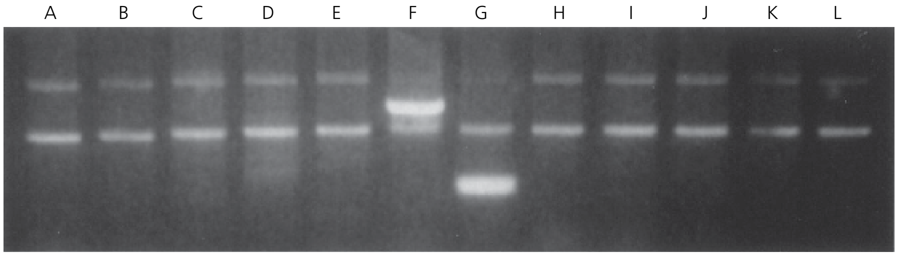

Figure 10.4 Sequence-specific primer (SSP) typing for HLA. In the SSP method, a panel of amplification primers is designed to detect all known polymorphisms encoded by an HLA locus or group of alleles. Polymerase chain reactions (PCRs) are first performed for each of the primer pairs. The PCR products are then electrophoresed on a gel, and the presence or absence of a PCR product of the appropriate size is scored. Assignment of an HLA type is made by examining the pattern of positive and negative PCR reactions. Advantages of the SSP method include its relatively low cost, its technical simplicity, and the speed with which low-resolution typing can be achieved. High-resolution-level typing requires as many primers as there are polymorphisms, and large numbers of PCR reactions are therefore necessary, increasing both assay time and cost. This figure shows the results of a typical SSP assay in which genomic DNA from a single sample was amplified with a panel of HLA-C primer pairs, electrophoresed on an agarose gel, stained with ethidium bromide and visualized with ultraviolet translumination. All primer pair amplicons (lanes A–L) showed amplification of the 796-base pair (bp) fragment from the internal control primers, demonstrating successful PCR conditions in each sample. The sample was typed as C*06 and C*07 by the identification of specific amplification products in lane F (1062 bp fragment specific for C*07:01/07:02/07:03) and lane G (304 bp fragment for C*06).

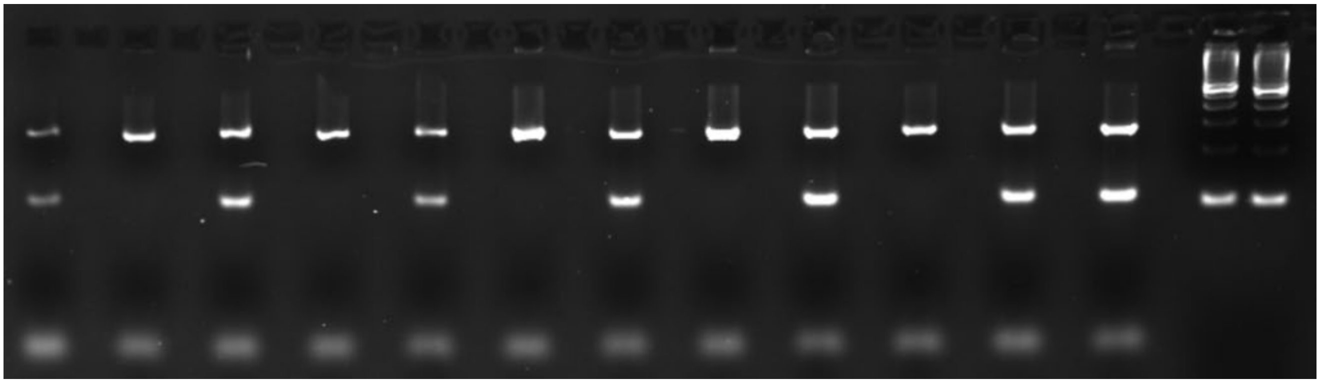

Figure 10.5 Sequence-specific primer (SSP) typing for KIR2DS2 allele typing as described in [87]. Each lane represents a unique DNA sample. An amplified PCR product of 110 bp is represented by the lower band and is indicative of the presence of KIR2DS2 in the sample.

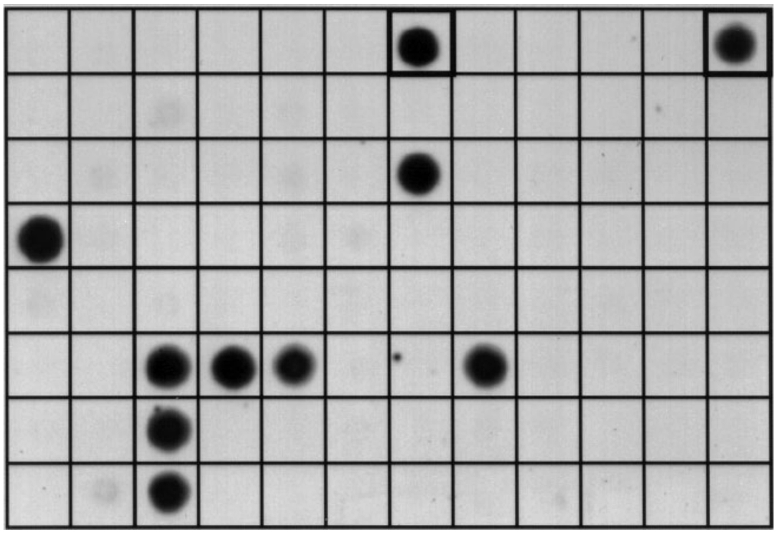

Figure 10.6 Sequence-specific oligonucleotide probe (SSOP) "forward blot" typing. With this method, the HLA gene of interest is amplified by PCR and immobilized on nylon membranes. Oligonucleotide probes labeled with a reporter molecule (either radioactive or non-radioactive) are then allowed to hybridize to the membranes. Probes with sequences complementary to the membrane-bound DNA will hybridize, whereas those with as little as a single nucleotide mismatch will not. The pattern of positive and negative hybridization is used to deduce the HLA type of the target DNA. In this method, only the known polymorphic sites in an HLA gene are probed, and therefore no information is obtained for regions of the gene not defined by the sequence-specific probes. Although novel patterns of probe hybridization may indicate the possible presence of a new allele, alleles with polymorphisms outside the probed regions may escape detection. Since many samples being examined for a given HLA gene can be tested on the same set of membranes, the SSOP method is particularly well suited to high-volume HLA typing. This figure shows the results of SSOP typing of genomic DNAs of 12 known control samples and 82 unknown samples that were amplified with HLA-A locus-specific primers, blotted on a series of nylon membranes and probed with a panel of HLA-A locus probes. Positive hybridization of probes labeled with alkaline phosphatase was detected by chemiluminescence and exposure to X-ray film. In this example, a probe specific to a sequence found only in A*29 and A*43 at codons 62 and 63 of exon 2 showed positive hybridization with two control samples (outlined in bold) and eight of the unknown test samples.

automated sequencing-based typing (SBT) [57,58] (Figure 10.8). As the use of these methods in the research and clinical settings increased, so did the discovery of novel HLA alleles. As a result, the HLA Nomenclature Committee introduced new guidelines for the naming of HLA alleles. The allele was defined by the genetic locus (DRB1) followed by an asterisk separating the locus from the allele name. The first two digits of the allele name correspond to the broadly defined serologic specificity group to which the allele belongs, with the second two digits uniquely identifying the allele. In 1989, official designations were, for the first time, assigned to HLA alleles that had been sequenced. A total of 56 HLA class I and 78 HLA class II alleles were recognized. In 1990, the discovery of "silent" (synonymous) nucleotide substitutions in HLA genes (i.e., those that do not result in changes in amino acid composition of the encoded HLA molecule) required the addition of a fifth (and later sixth) digit to the allele name.

Figure 10.7 Sequence-specific oligonucleotide probe (SSOP) "reverse probe" format typing. In this assay, the sequence-specific probes are immobilized on a solid-phase support. Sample DNA is labeled during PCR amplification and allowed to hybridize to the immobilized panel of probes. As with forward SSOP, HLA alleles are deduced from the pattern of positive and negative probe reactions. In this figure, genomic DNA was amplified with DRB locus-specific biotinylated primers and hybridized to a panel of probes for *DRB1*, *DRB3*, *DRB4*, and *DRB5* sequences immobilized on a nylon membrane strip. The pattern of positive hybridization reactions, detected by color formation, was interpreted with a computerized analysis program. The sample was typed as DRB1*15 or -*16 by the presence of band A, DRB1*04 by bands B and C, DRB4*01 by band D, and DRB5*01:01 or *01:04 by bands E and F.

DNA-based methods were a major theme of the 11th Workshop (1991). This workshop explored the extent of diversity of HLA class II genes and was the first workshop to introduce high-throughput strategies for HLA class II typing. In 1994, the first HLA null allele, DRB4*01012N, was recognized. To accommodate the growing number of polymorphisms within coding and non-coding regions of HLA genes, additional digits were added to the nomenclature system. Beginning in 2002, the expansion of HLA allele designations to eight digits provided greater flexibility for cataloguing variation. Many of the alleles defined by DNA-based typing methods do not have a serologically defined equivalent, placing a further requirement for flexibility and adaptability on the HLA nomenclature system. Application of PCR-based methods in the mid-1990s carried through to the 12th HLA Workshop (1996), in which methods for characterizing classical class I alleles were a breakthrough for population-based analysis. The 12th Workshop included studies of human diversity, HLA in cancer, transplantation, and disease, in addition to a thorough exploration of newly defined HLA genes, alleles, and haplotypes.

With DNA-based typing technology solidly established in HLA research, the 13th and 14th HLA Workshops focused international collaborative efforts on furthering understanding of the functional significance of the MHC in disease models. The 13th Workshop (2002) launched new research initiatives, including studies of cytokine gene polymorphism, HCT, disease, cancer, reproduction, peptide repertoire, the KIR system, and minor histocompatibility antigens. A major contribution of the 13th Workshop was the

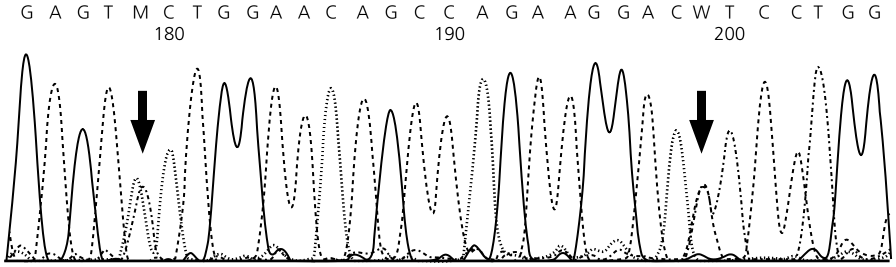

Figure 10.8 Automated fluorescent sequencing-based typing (SBT). The HLA gene is first amplified in a PCR. Cycle sequencing of the PCR product is then performed using primers that are labeled with a fluorescent label (dye primer, usually 5′-primer). Alternatively, the dideoxynucleotides ddATP, ddTTP, ddGTP, and ddCTP can be individually labeled with fluorescent label (dye terminator). In dye primer sequencing, the label is incorporated into the 5′- end of the HLA sequence, whereas in dye terminator sequencing the addition of a labeled dideoxynucleotide at the 3′ end terminates the sequencing reaction. In both approaches, the sequencing reaction is electrophoresed on a polyacrylamide gel using an automated sequencer. The fluorescent signals are captured and interpreted as a sequence of DNA bases by a computer program. Assignment of the HLA allele(s) is performed by comparing the derived sequence with all known sequences for a given gene. This figure shows the partial sequence chromatogram for polymorphic positions 179 and 199 (arrows) of *DRB1* exon 2 (residues 60 and 67) from an HLA-DRB1*08:01,12:01-positive sample. The genomic DNA was sequenced using direct automated dye primer fluorescent chemistry on an Applied Biosystems Model 377 sequencer (ABI, Foster City, CA, USA). The laser-induced fluorescent signal emitted from each sequenced fragment is captured and interpreted by software and identified on the chromatogram above the peak as an A, C, G or T nucleotide base, except at heterozygous positions. Using the International Union of Biochemistry codes, heterozygous position 179 has M, indicating signals from A (DRB1*08:01) and C (DRB1*12:01), whereas the W at position 199 indicates that T (DRB1*08:01) and A (DRB1*12:01) were incorporated.

creation of a public database (*db*MHC) in collaboration with the National Institutes of Health, the National Center for Biotechnology Information, and the National Library of Medicine. By 2008, the number of newly identified alleles mandated a new nomenclature to accommodate the rapid discovery of novel sequences. The WHO Nomenclature Committee for Factors of the HLA System developed a nomenclature that permits unlimited numbers of unique alleles to be individually named, as described below. The most recent 16th International HLA Workshop (2012) focused on the biological implications of MHC diversity in solid organ transplantation and autoimmune disease, and the development of informatics to support data analysis. The International Histocompatibility Working Group in Hematopoietic Cell Transplantation (IHWG in HCT) conducted studies on the role of HLA and KIR genes in unrelated donor transplantation (Chapter 45) [59]. New studies were initiated for cord blood and haploidentical related donor transplantation, paving the way for analysis of MHC region and genome-wide investigation of genes of importance in transplantation.

Nomenclature

As of April 2015, more than 3107 HLA-A, 3887 HLA-B, 2623 HLA-C, 1726 HLA-DRB1, 780 HLA-DQB1, and 520 HLA-DPB1 alleles had been defined (http://www.ebi.ac.uk/imgt/hla) [25]. To accommodate the rapid discovery of sequences, the WHO Nomenclature Committee on Factors of the HLA System established a new nomenclature in 2008 (http://www.ebi.ac.uk/imgt/hla). A hyphen separates the HLA prefix from the gene (HLA-A). Following the gene, an asterisk delimits four kinds of information (in order): the serological equivalent; the unique protein; synonymous substitutions, and non-coding variation affecting expression. Each of these numbers are delimited from one another by a colon (:). For example, HLA-A*02:101:01:02N signifies an HLA-A allele of the A2 family; it has a unique coding sequence named 101, a synonymous change named 01, and is the 02 null (N) allele. The use of colons to separate these four categories permits an unlimited number of novel alleles to be named.

Nomenclature for the KIR genetic loci is analogous to that adopted for HLA and is the responsibility of the HUGO Genome Nomenclature Committee (HGNC) [60]. KIR genes are descriptive of their molecular structure. The name begins with the KIR acronym followed by the number of immunoglobulin-like domains and the letter "D" for "domain". After D, the letter "L" designates molecules with a "long" cytoplasmic tail, whereas "S" is used for "short" cytoplasmic tails, and "P" for "pseudogenes". The name concludes with the number of the gene that encodes the protein with the aforesaid structure. For example, "KIR2DL1" indicates a protein with two domains, a long cytoplasmic tail and is the first protein described. The full description of the KIR genetic system is presented in Chapter 11.

Hallmarks of the MHC: linkage disequilibrium (LD) and haplotypes

If each HLA allele occurred randomly, more than 5.7×10^{31} unique HLA-A, -B, -C, -DRB1, and -DQB1 phenotypes would be possible and the prospects of identifying a matched unrelated donor would be insurmountable. However, the HLA region is characterized by positive LD, in which two or more alleles occur in non-random association and are observed at a higher frequency than would be predicted by chance alone, that is, by the product of their individual allele frequencies. In studies of unrelated individuals where no family study is available, LD is often used as a mathematical prediction of the departure from expectation given the known frequencies of the phenotype or genotype in the population [61].

Strong positive LD between HLA-B and -C and between HLA-DR and -DQ increases the probability that a patient and donor matched for HLA-A, -B, and -DR will also be matched for HLA-C and -DQ. Conversely, mismatching at HLA-B or -DR increases the chance of HLA-C or -DQ mismatching, respectively. HLA-A1, -B8, and -DR3, the most common North American white person haplotype, serves as a good example of how LD may aid in the identification of unrelated donors. Matching for two loci (e.g., A1 and B8) will almost always determine matching for the third (e.g., DR3), fourth (e.g., C7), and fifth (e.g., DQ2) loci. Moreover, an antigen (e.g., HLA-B8) that shows characteristic LD with another antigen is often found to be encoded by a specific and characteristic allele (e.g., HLA-B*08:01). Using the example of the HLA-A1, -C7, -B8, -DR3, and -DQ2 haplotypes, the alleles that encode the phenotypically defined antigens of this group are HLA-A*01:01, -C*07:02, -B*08:01, -DRB1*03:01, and -DQB1*02:01, respectively. The success of identifying suitable unrelated donors is a function of the haplotypic combination of alleles that the recipient has inherited. When patients possess HLA haplotypes with rarer combinations of alleles, it may be feasible to match for some but not all loci. LD phenomena also explain the highly complex patterns of multilocus HLA mismatching among unrelated donors and transplant recipients that have been uncovered in the retyping effort of legacy samples [62,63].

HLA alleles are inherited en bloc as a haplotype on the same strand of DNA. A rigorous identification of haplotypes can only be achieved by ascertainment of segregation of markers in a family study. The family study plays an important early role in planning for an allogeneic transplant. A family study provides the definitive means to validate the patient's genotype and to determine the availability of HLA genotypically identical siblings who might be considered donors. The probability that a patient and sibling will inherit the same two parental haplotypes is 25% ("HLA genotypically identical" or "identical-by-descent") (Figure 10.2). The probability that a patient and sibling have inherited one identical paternal or maternal haplotype and one non-identical (non-shared) haplotype is 50% ("haploidentical"). The probability that a patient and sibling have inherited neither of their parental haplotypes in common is 25%. HLA genotypically identical siblings are identical by descent and therefore share not only the same HLA alleles and antigens, but also all variation linked to the haplotypes. Haploidentical siblings are identical by descent only for the shared haplotype; they are variably mismatched for the non-shared haplotype, depending on fortuitous sharing of maternal and paternal HLA alleles and antigens. In rare situations, a maternal or paternal recombination event gives rise to HLA mismatching between two otherwise matched siblings. Unrelated donors and patients who share the same HLA antigens and alleles are "identical-by-state" because they do not share the same parental haplotypes.

Haplotypes include the known HLA alleles that are physically linked on the same DNA strand in addition to undetected interlocus variation. The terms "ancestral haplotypes" (AHs) and "conserved extended haplotypes" refer to the definition of specific conserved long stretches of DNA sequences thought to be derived from a prototype ancestor [64]. AHs include HLA-B7, -B8, -B13, -B35, -B44, and -B57, observed at high frequency in white populations [40,64]. New ethnically specific haplotypes are generated by the shuffling of interlocus ("frozen") blocks of conserved regions at points of low LD. Recombinational events together with gene conversion, genetic drift, and balancing selection result in haplotypes that share not only the same HLA alleles, but also blocks of highly conserved sequences in strong positive LD with those HLA alleles [40,65–67]. In this way, HLA alleles are markers for untyped genetic variation including novel genes that are potentially important in transplantation (described below). A specific HLA allele may also be found on one or several different haplotype backgrounds [66,68,69]. Although haplotype-specific alleles and blocks have been well characterized for many of the common haplotypes, the extent of block sharing among other less common haplotypes on unrelated chromosomes is unknown.

Intergenic variation within the MHC

The classical HLA genes that currently form the basis of histocompatibility testing in support of allogeneic hematopoietic cell transplantation represent a minority of the total gene content of the human MHC. Mapping the MHC for new genes of importance in transplantation has been facilitated by information on the content and organization of microsatellites and single nucleotide polymorphisms (SNPs) (Chapter 45) [2].

Microsatellites were the earliest form of non-coding variation to be studied in detail within the MHC. Microsatellites are di-, tri-, tetra-, and pentanucleotide repeats and complex repetitions, and have been instrumental in the construction of the second-generation human genome map [70]. Since 1996, over 400 microsatellites have been identified within the central MHC, of which 241 are polymorphic and occur on average one every 16 kb. Although it is not likely that microsatellite markers themselves confer susceptibility to disease, microsatellites can serve as functional coding and regulatory elements. Microsatellites located in the upstream promoter regions of coding sequences may provide enhanced function to expression vectors and binding sites for regulatory proteins, and affect transcription [71].

Microsatellites have been powerful tools for mapping novel transplantation determinants within the MHC [72,73]. In an analysis of 31 markers in 344 HLA-matched unrelated donor–recipient pairs, microsatellites were in strongly positive LD over much of the MHC [73]. Five common White haplotypes (HLA-A1, -B8, -DR3; -A3, -B7, -DR2; -A2, -B44, -DR4; -A29, -B44, -DR7; and -A2, -B7, -DR2) were examined using a new measure called "haplotype-specific heterozygosity," which predicted informative microsatellites. The HLA-A1, -B8, -DR3 and HLA-A3, -B7, -DR2 haplotypes could be predicted with over 90% probability using three microsatellite loci (D6S265/ D6S2787/D6S2894 and D6S510/ D6S2810/D6S2876, respectively). These results demonstrate that microsatellites are informative markers for HLA haplotypes and that sequence variation exists outside the classical HLA loci.

SNPs are the most abundant kind of inherited variation [74]. Over 11 million SNPs have been deposited in the dbSNP public repositories at a density of 1 per 180 base pairs (bp) (http://www.hapmap.org/; http://www.sanger.ac.uk/HGP/Chr6/) [28]. An estimated 4% of these SNPs occur in coding and 96% within non-coding regions of genes [75]. SNPs represent the simplest form of human genetic variation. They represent two different alleles, for example, A versus G, T versus A, C versus T. Since they are very efficient and cost-effective to genotype [76] (Figure 10.9), intense efforts have been made to define the linkage of SNPs in genomic DNA, to specify the organization of SNPs into haplotype blocks, and to identify tagSNPs that could serve as proxies for haplotype blocks. Most often, haplotypes are used as proxies for ungenotyped markers because they may allow the identification of susceptibility markers that might escape detection when tested individually. In this way, the use of haplotypes for disease mapping is especially informative for association studies of complex human diseases wherein the full extent of genetic variability within individuals is unknown [77].

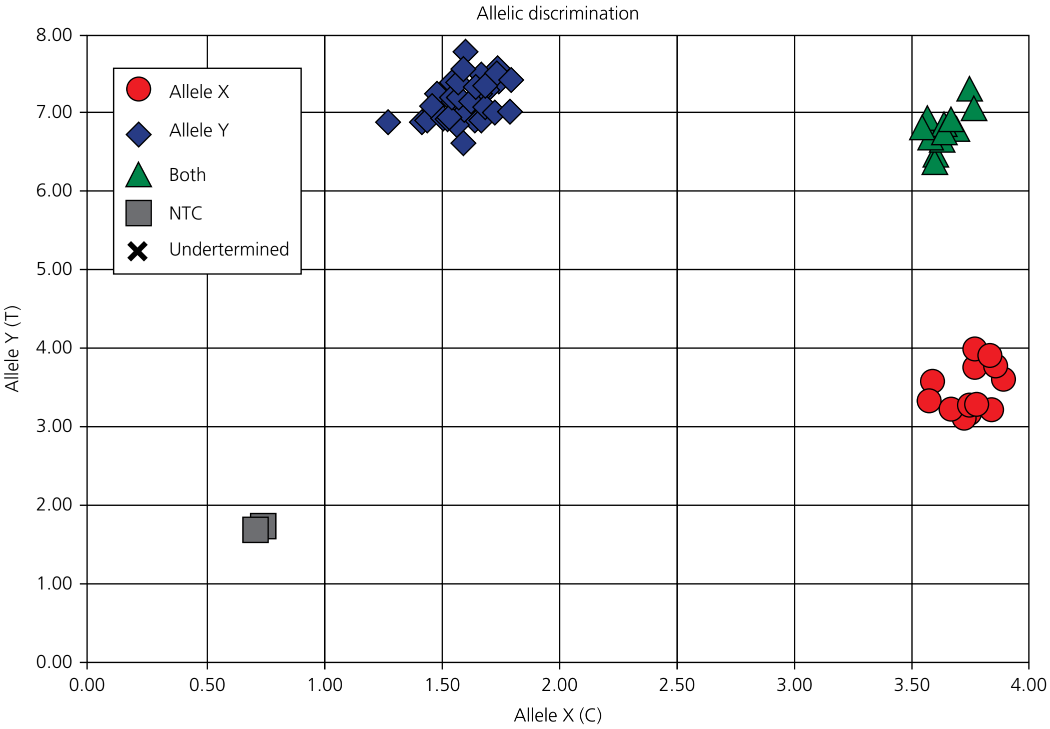

Figure 10.9 SNP genotyping using the TaqMan chemistry [76]. Simple bialleleic SNPs are genotyped as homozygous "allele X" (circles), homozygous "allele Y" (diamonds) or heterozygous (triangles).

The HapMap and 1000 Genomes projects paved the way for the analysis of genome-wide SNPs to study the content and architecture of the human genome, the extent of positive LD, and the organization of variants on haplotypes in ethnically diverse populations. Analysis of SNPs within the MHC reveals striking haplotype organization, with "blocks" of SNPs extending across 40–80 kb. HLA haplotypes have characteristic conservation for SNPs, and HLA haplotypes differ from one another in the size and content of their blocks. Sequencing of eight HLA homozygous cell lines [2] uncovered haplotypic diversity among discrete blocks across the MHC, such that haplotypes defined by their HLA-A, -B, and -DR tissue type exhibit further haplotypic diversity within blocks measuring 20–60 kb in length.

KIR genes and haplotypes

HLA-A, -B and -C serve as ligands for KIR receptors, and hence bridge the innate with the adaptive immune systems. NK cell receptors have inhibitory or activating potential, and it is the balance of these effector functions that results in the NK-cell response to altered or loss of self MHC antigens [78]. Inhibitory NK cell receptors include the KIRs and the immunoglobulin-like transcripts encoded on chromosome 19q13.4. Genetic KIR variation is generated through both allelic and haplotype diversity [60,78]. The inhibitory receptors KIR2DL3, KIR2DL2, and KIR2DL1 recognize epitopes presented by HLA-C antigens, whereas the KIR3DL1 receptor recognizes the HLA-Bw4 expressed by some HLA-B antigens, discussed below. The immunoglobulin-like transcript-1 receptor recognizes HLA-A, -B, -C, -G, -E, and -F as its ligands. A second structurally distinct group of inhibitory receptors consists of CD94 covalently bound to NKG2A, known as the C-type lectin receptors; these genes are encoded on chromosome 12p12–p13 and serve as the receptor for HLA-E.

The inhibitory KIR receptors and their HLA-C and -B ligands are the best studied. The ligand specificity of inhibitory KIR receptors is governed by residues 77 and 80 of HLA-C and by the HLA-Bw4 epitope present on some HLA-B and -A molecules [78]. High-resolution typing of the transplant recipient and donor for HLA-A, -B, and -C genes provides all the information required to determine the presence/absence and heterozygosity/homozygosity of ligands, donor–recipient mismatching for ligands, and specificity for inhibitory KIRs. Based on HLA-C residue 77/80 polymorphism, two groups of HLA-C ligands are distinguished. The Ser77/Asn80 polymorphism is encoded by HLA-C group "C1" molecules (HLA-C1; C3 except C*03:07, *03:10, *03:15; C7 except C*07:07, *07:09; C8; C12 except C*12:05, *12:041/2; C13; C14 except C*14:04; C*15:07; and C16 except C*16:02), and is the ligand for the inhibitory KIR2DL2 and 2DL3 receptors. The Asn77/Lys80 polymorphism is encoded by the HLA-C group "C2" molecules (HLA-C2; C*03:07; C*03:15; C4; C5; C6; C*07:07, *07:09; C*12:05, *12:041/2; C15 except C*15:07; C*16:02; C17; and C18) and is the ligand for the KIR2DL1 receptor. The third ligand group is specified by the presence of the HLA-Bw4 epitope present in B5, B13, B17, B27, B37, B38, B44, B47, B49, B51, B52, B53, B58, B59, B63, B77, B*15:13, B*15:16, B*15:17, B*15:23, and B*15:24 sequences; the Bw4 ligand is recognized by the inhibitory KIR3DL1 receptor.

Two broad groups of KIR haplotype are recognized and are referred to as "group A" and "group B" [60,78]. Group A haplotypes encode primarily inhibitory receptors and the activating KIR2DS4 gene. Group B haplotypes exhibit extensive diversity by encoding more activating genes, including KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, and KIR3DS1. Inhibitory KIR receptors have greater affinity for class I ligands than do activating KIR receptors. HLA serves a dominant role in the selection of the peripheral repertoire of NK-cell inhibitory KIRs through its function as a ligand. During normal development, NK cells that express inhibitory KIRs with specificity for self-class I are selectively enriched. Although both inhibitory and activating KIRs are expressed on NK and some T cells, inhibitory KIRs are dominant.

The known ligands for the activating receptors are MHC class I molecules. KIR2DS1 and KIR2DS4 have specificity for HLA-C, CD94/NKG2C for HLA-E, and NKG2D for MHC class I polypeptide-related sequences A and B, and retinoic acid early transcript-1/UL16 binding protein (RAET1/ULBP).

Models for donor inhibitory KIR-mediated killing

Donor NK-cell alloreactivity against host cells can arise in both the HLA-mismatched the HLA-matched settings. As described in Chapters 11 and 44, the sentinel discovery by the Perugia team that recipient target cells are susceptible to lysis via donor NK-cell recognition of HLA ligands has provided a model for understanding the HLA–NK genetic systems [79,80]. In the original observations, graft-versus-host (GVH) vector KIR ligand mismatching occurs when HLA class I ligands are present in the donor but absent in the recipient. In the setting of HLA-mismatched transplantation (haploidentical mismatched related donors and recipients and HLA-mismatched unrelated donors and recipients), recipients may lack the appropriate ligand that is present in the donor due to the HLA mismatch (Table 10.3). The recipient's target cells are susceptible to alloreactive donor NK-cell killing (GVH) because the donor NK cells are not inhibited. The recipient target cells include residual host leukemia or tumor cells. As an example, in the mismatched setting between a donor who has both a C1 (HLA-C8) and a C2 (HLA-C6) epitope and a recipient who has only C1 (HLA-C8, C8 homozygous), donor inhibitory KIRs are not engaged and recipient targets are lysed. With regard to the Bw4 epitope, donor inhibitory KIRs are not inhibited with a Bw4-positive donor and a Bw4-negative recipient. For example, this situation is present in a B44,18-positive donor and a B18,18-positive recipient [donor Bw4 (B44), Bw6 (B18); patient Bw6 (B18), Bw6 (B18)], and also in a B44,52-positive donor and a B18,35-positive recipient [donor Bw4 (44), Bw4 (B52); patient Bw6 (B18), Bw6 (B35)]. High-resolution typing of class I provides the necessary data to test hypotheses related to the clinical importance of ligand mismatching and ligand absence.

Histocompatibility testing of donors for allogeneic transplantation

Typing methods

Because of the broadly reactive nature of HLA antibodies, two or more unique sequences (e.g., HLA-A*02:01 and *02:05) may have the same serologically defined phenotype (e.g., HLA-A2). An increasing number of class I and class II alleles have been discovered with the use of DNA-based typing methods and have not been phenotyped by serologic methods. To bridge the transition in nomenclature from serologic to DNA-based typing methods, "serologically equivalent" designations have been defined [81].

DNA-based typing methods vary according to the level of discrimination they provide in defining the nucleotide sequence of an HLA gene. When the DNA typing method allows the identification of a serologically defined HLA antigen-equivalent (e.g., HLA-A2),

Table 10.3 Models for natural killer immunoglobulin-like receptor (KIR) ligands in unrelated donor hematopoietic cell transplantation

Model	Mechanism	Ligand			Examples
		Recipient	Donor	Vector	
Mismatched ligand	Due to the donor–recipient HLA class I mismatch, the recipient's target cells lack the class I allotype present in the donor	C1, C1 and Bw4, Bw4	C1, C2 and Bw4, Bw6	No C2 in recipient; GVH	HLA-B15 versus B13 mismatched haploidentical donor and recipient: Donor B*27:05 (Bw4), C*01:01 (C1); B*15:01 (Bw6), C*04:01 (C2) Recipient B*27:05 (Bw4), C*01:01 (C1); B*13:01 (Bw4), C*03:02 (C1)
		C1, C2 and Bw4, Bw6	C1, C2 and Bw6, Bw6	No Bw4 in donor; HVG	HLA-B18 versus B52 mismatched haploidentical donor and recipient: Donor B*15:01 (Bw6), C*04:01 (C2); B*18:01 (Bw6), C*07:01 (C1) Recipient B*15:01 (Bw6), C*04:01 (C2); B*52:01 (Bw4), C*12:02 (C1)
Missing ligand	Despite HLA identity, host cells are missing the class I ligand to provide the inhibitory signal	C1, C1; Bw4, Bw4 or C1, C1, Bw4, Bw6	No C2 ligand for donor KIR2DL1No C2 ligand for donor KIR2DL1 and no Bw4 for 3DL1		Recipient B*13:01 (Bw4), C*03:02 (C1); B*27:05 (Bw4), C*01:01 (C1) or B*13:01 (Bw4), C*03:02 (C1); B*08:01 (Bw6), C*07:01 (C1)
		C1,C1; Bw6,Bw6			Recipient B*39:01 (Bw6), C*12:03 (C1); B*07:02 (Bw6), C*07:02 (C1)
		C2, C2; Bw4, Bw4 or C2, C2; Bw4, Bw6	No C1 for donor KIR2DL2		Recipient B*44:05 (Bw4), C*02:02 (C2); B*13:02 (Bw4), C*06:02 (C2) or B*44:05 (Bw4), C*02:02 (C2); B*15:01 (Bw6), C*04:01 (C2)
		C2,C2; Bw6, Bw6	No C1 for donor KIR2DL2 and no Bw4 for donor 3DL1		Recipient B*18:01 (Bw6), C*05:01 (C2); B*13:02 (Bw6), C*06:02 (C2)

C1, HLA-C group 1 allotypes: the KIR2DL2 and 2DL3 receptors recognize Ser77 and Asn80.
C2, HLA-C group 2 allotypes: the KIR2DL1 receptor recognizes HLA-C allotypes encoding Asn77 and Lys80.
Bw4: The inhibitory receptor KIR3DL1 recognizes the HLA-Bw4 epitope expressed on certain HLA-B and HLA-A molecules.
GVH, graft-versus-host; HVG, host-versus-graft.

the method is termed "low resolution." As described below, SSP methods and SSOP approaches that employ a restricted number of probes may provide only limited sequence information about a particular HLA gene, equivalent to that achievable by serology. HLA typing methods that provide information beyond the serologic level but short of the allele level are termed "intermediate resolution." For example, an SSP method that uses a comprehensive panel of PCR primers, or an SSOP method that employs a wider array of probes, might identify the presence of either HLA-A*02:01 *or* -*02:09 in an amplified DNA sample, but might be unable to discriminate one allele from the other. This intermediate-resolution result would be characterized as "HLA-A*02" or "HLA-A*02:01/09." Typing methods that generate nucleotide sequence information allowing the precise identification of an HLA allele (e.g., HLA-A*02:01) are termed "high resolution." High-resolution typing results may be achieved by direct automated sequencing of an HLA gene (*SBT*) or by the use of large panels of oligonucleotide probes that test all known regions of variability within a gene. In order to interpret HLA-typing results and select donors for transplantation, it is necessary to know whether the typing was carried out at low, intermediate or high resolution. A patient and donor who are "matched" for HLA-A and -B antigens by low-resolution typing methods may be mismatched for HLA-A and/or -B alleles (see Chapter 45). Histocompatibility guidelines for unrelated HCT have been revised to accommodate DNA-based typing methods [82].

SSP

SSP provides a simple and low-cost method for low-to-intermediate resolution of HLA alleles. The SSP methods employ a panel of PCR primers that can amplify specific HLA loci or alleles [54,83–86] (Figure 10.4) and KIR genes [87,88] (Figure 10.5). The SSP method is based on the design of PCR primers whose sequence is complementary to one or more nucleotide bases that distinguish HLA alleles, KIR alleles, or KIR genes. Depending on the need, panels of primers can be designed to distinguish the major serologically equivalent families at a given HLA locus; alternatively, primers can be designed to determine the specific allele among a family of alleles within a given serological family. For example, SSP typing methods can differentiate between HLA-A1, -A2, -A11, and so on. SSP primers can also identify the A*02:01 allele from the A*02:05 allele within the HLA-A*02 family. After PCR amplification with the primers, the products are electrophoresed on a gel. The presence of an amplified product is signaled by a dye and is evidence that the HLA allele is present. The HLA tissue type is determined by evaluating the patterns of positive and negative amplifications to the entire panel of primers (Figure 10.4.) When SSP primers are designed to specific polymorphisms that distinguish between KIR genes, then an amplified product is evidence that the KIR gene is present (Figure 10.5). SSP methods are highly cost-effective for ascertaining the presence of KIR genes and typing the serologically equivalent HLA alleles. SSP methods for comprehensive typing of HLA alleles are less cost-effective than probe- or sequencing-based methods, owing to the need for the large number of PCR reactions.

SSOP

SSOP methods provide high resolution of alleles and the capacity for increased throughput. SSOP methods for typing HLA alleles employ a solid-phase support to immobilize PCR-amplified products [55,56,89,90] (Figures 10.6 and 10.7). Similar SSOP-based methods have been developed to type KIR genes [91]. Oligonucelotide probes range between 15 and 30 nucleotide bases in length, and are complementary to known sequence polymorphisms of HLA alleles. The target DNA is PCR amplified, immobilized on a solid-phase support, and allowed to hybridize to the probes. When the probe has exact complementarity to the target DNA, the probe anneals to the target, and hybridization is detected by way of a marker that is attached to the probe. Alternatively, probes can be immobilized on a solid-phase support, with which the target DNA is allowed to hybridize. Whether the probe or the target is immobilized, either approach requires a comprehensive panel of probes that are descriptive for the family of HLA alleles being tested. Allele assignment is made by interpreting the reactivity of the target DNA to the entire panel of probes. SSOP methods provide information on positions known to be polymorphic; if a novel substitution is located in a region of an HLA sequence to which a probe is not designed, then that substitution escapes detection. A recent variation of probe-based typing technologies is the Luminex® system, which uses beads that are coated with oligonucleotides. After the target DNA is PCR-amplified, it is allowed to hybridize to the beads in a 96-well plate system. Hybridization of the target DNA with the beads is detected by way of fluorescent dyes. The use of plates that can handle up to 96 samples, robotics for sample handling, and automated data analysis permit efficient typing.

SBT

The only methods that permit comprehensive evaluation of an HLA or KIR sequence, nucleotide for nucleotide, are sequencing-based methods [57,58,92,93]. For HLA genes, SBT methods are highly accurate and powerful for uncovering novel variations. Among the most widely used SBT methods is automated fluorescent cycle sequencing. For HLA class I genes, exons 2, 3, or 4 can be separately PCR-amplified, or primers may be designed to amplify longer fragments containing both exons 2 and 3. For HLA class II genes, the polymorphic exon 2 is PCR-amplified. KIR genes contain 8–9 exons; PCR amplification may involve amplifying several overlapping groups of exons. Following PCR amplification, cycle sequencing is performed using fluorescently labeled primers or fluorescently labeled dideoxynucleotides. The sequenced templates are electrophoresed on a gel dedicated to an automated sequencer (Figure 10.8). Newer sequencing methods, known as "next-generation sequencing (NGS)" permit millions of single DNA molecules to be sequenced in parallel, and offer long clonal read lengths that enable the sequence of one exon to be linked to that of the neighboring exon, akin to phasing [94]. Allele assignments are made with the aid of software. A novel sequence-based approach for KIR genes used mass spectrometry to type 17 KIR genes and define novel alleles of KIR genes [95].

Donor selection

Donor selection begins with a full evaluation of available family members of the transplant recipient (Figure 10.2). A family study includes HLA typing of the father, mother, full siblings, and additional relatives, where indicated. The family study confirms the recipient's genotype and haplotypes, and provides information about the existence of genotypically matched siblings and/or haploidentical family members. When more than one equally suitable related donor is available, additional selection criteria including the number of HLA disparities on the mismatched maternal or paternal haplotype, and the presence or absence of disparity and of homozygosity for HLA KIR ligands, may be considered. When several equally matched unrelated donors are available, non-HLA factors, including donor age, parity, gender, and cytomegalovirus serostatus, may be considered in donor selection (Chapter 45).

For any donor who is HLA-mismatched to the patient, the vector of HLA incompatibility has biological meaning (Table 10.4). The concept of the vector or "direction" of HLA mismatching was shown by Anasetti and Hansen [96] in 1994 in risk of graft failure and acute GVHD in related haploidentical transplants. The vector of a mismatch can be defined for host-versus-graft (HVG) and GVH alloreactivity. The presence of donor antigens or alleles not shared by the recipient determines HVG allorecognition. The presence of recipient antigen or alleles not shared by the donor provides the immunologic basis for GVH allorecognition. Mismatching between a donor and recipient can be described as "bidirectional" if both HVG and GVH vectors are present at a given HLA locus. Mismatching is called "unidirectional" if one but not the other vector is present. Unidirectional mismatching in the GVH vector occurs when the donor is homozygous and the recipient is heterozygous and shares one allele or antigen with the donor (e.g., patient A*02:01, *02:05 versus donor A*02:01, *02:01). Unidirectional mismatching in the HVG vector occurs when the patient is homozygous and the donor is heterozygous and shares one allele with the patient (e.g., patient A*02:01, *02:01 versus donor A*02:01, *02:05).

Table 10.4 Vector of HLA mismatch

Vector	Definition	Examples	
		Donor	Recipient
HVG	Presence of donor alleles or antigens not present in the recipient	B*08:01,44:02[a]	B*08:01,44:05
		B*08:01,44:02[b]	B*08:01,08:01
GVH	Presence of recipient alleles or antigens not present in the donor	B*08:01,44:02[a]	B*08:01,44:05
		B*08:01,08:01[b]	B*08:01,44:05

GVH, graft-versus-host; HVG, host-versus-graft.

[a] These combinations contain bidirectional (both HVG and GVH disease) mismatch vectors.

[b] Unidirectional mismatches.

Haploidentical siblings may be variably mismatched for the non-shared haplotype depending on whether the maternal and paternal HLA haplotypes fortuitously encode the same HLA antigen(s) or allele(s). The haplotypes of a child may be described in relation to the mother ("inherited maternal HLA antigens") or father ("inherited paternal HLA antigens"). Non-inherited maternal antigens (NIMAs) or non-inherited paternal antigens (NIPAs) refer to the HLA antigens expressed on the non-shared haplotypes. NIMA and NIPA have clinical significance. Exposure to NIMA in utero is tolerizing, whereas that to NIPA is immunizing. Donor-specific suppression of T-cell responses against NIMA is associated with lower risks of acute and chronic GVHD and transplant-related mortality after transplantation from mother to child compared with father to child [97]. In addition to maternal and paternal sharing of HLA antigens, selection of haploidentical family members can also include consideration for mismatched or missing HLA KIR ligands encoded by HLA-B, -C and certain -A alleles. For these purposes, the availability of high-resolution typing of HLA class I alleles of the recipient provides the necessary information to determine whether a patient is Bw4 positive or negative, and whether the recipient is C1/C2 heterozygous, C1/C1 homozygous, or C2/C2 homozygous.

Histocompatibility testing for unrelated donors includes five loci: *HLA-A*, *-B*, *-C*, *-DRB1*, and *-DQB1*. Donor typing and matching for the two determinants at each locus give rise to the 10 possible alleles considered relevant in HCT. Donors and recipients who have the same 10 alleles at the five loci are commonly described as "10/10 allele-matched." The term "8/8" refers to high-resolution matching at the four loci *HLA-A*, *-B*, *-C*, and *-DRB1*. The term "6/6" refers to three typed loci *HLA-A*, *-B*, and *-DRB1:* however, the resolution may not necessarily be high at all three genes as in the case of cord blood transplantation, where HLA-A and -B typing is usually performed at low resolution and -DRB1 at high resolution. In addition to HLA matching of unrelated donors, pretransplant assessment of the presence of anti-HLA antibodies in recipient serum is vital, as a positive cross-match places the patient at high risk for non-engraftment [46,47].

Conclusions

Genes of the HLA system encode a complex array of histocompatibility molecules that play a central role in immune responsiveness and in determining the outcome of tissue transplantation. The primary goal of histocompatibility testing for patients undergoing HCT is the identification of a suitable HLA-matched donor to reduce the risk of post-transplant complications resulting from HLA incompatibility. The recent advent of DNA-based methods that allow discrimination of HLA genes at the allele level has enhanced our ability to perform typing and matching with speed and precision. The future of histocompatibility testing in support of allogeneic transplantation will incorporate consideration not only of the classical HLA genes, but also of functional variation that is linked to HLA loci on haplotypes, and KIR genes. Ultimately, the key to understanding the histocompatibility barrier in transplantation will require information on the content of the MHC region and the organization of that genetic variation on haplotypes.

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CHAPTER 11

Natural Killer Cells and Allogeneic Hematopoietic Cell Transplantation

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Introduction

Natural killer (NK) cells are large, granular lymphocytes that express CD56 (neural adhesion protein, NCAM) and lack the CD3 receptor (i.e., $CD56^+CD3^-$). NK cells make up ~10–15% of the peripheral blood (PB) lymphocytes and are a critical component of the immune system. NK absence or dysfunction results in recurring, life-threatening viral infections [1]. NK cells have also been implicated in tumor surveillance since they preferentially kill malignant cells while sparing normal, non-transformed cells. This is accomplished by displaying numerous non-rearranged, germline surface receptors with unique specificity. These receptors detect the presence or absence of MHC class I and also "stress" receptors, both of which are perturbed during malignant transformation and/or viral infection. In addition to cytotoxic responses, NK cells are an important source of cytokines, which feed back to the adaptive immune system including T cells, B cells, and dendritic cells (DCs). NK cells directly interact with these cells and thereby shape adaptive immune responses. As one of the earliest lymphocyte populations to recover after allogeneic hematopoietic cell transplantation (HCT), NK cells may be important in the early immune events that occur after HCT. The purpose of this chapter is to provide up-to-date information on NK-cell biology as it relates to allogeneic HCT. Since there are both similarities and differences between murine and human NK cells, we will focus mainly on human studies but, where necessary, relevant murine information will be included.

NK-cell subpopulations: $CD56^{bright}$ and $CD56^{dim}$ NK cells

NK cells have been subdivided into two major subsets on the basis of CD56 staining intensity and the expression of the low-affinity immunoglobulin (Ig) receptor CD16 ($F_c\gamma RIII$). Approximately 85–90% of all NK cells express CD16 and the majority of these cells also show dim CD56 expression. Consequently, the $CD56^{dim}CD16^+$ fraction of NK cells accounts for the bulk of the circulating NK cells. A smaller fraction (~10–15%) of NK cells shows high CD56 expression. $CD56^{bright}$ cells have low to absent CD16 expression (i.e., $CD56^{bright}CD16^{-/+}$). These two NK-cell subsets differ in their responses to exogenous cytokine stimulation, cytotoxicity, and cytokine production, suggesting that they may have distinct roles in immune responses (reviewed in [2]).

Cytokines, such as IL-2 and IL-15, are essential for the growth, development, and activation of NK cells. For these cytokines to transmit intracellular signals, the expression of a trimeric protein complex is required. This complex is made up of the common γ chain (CD132) and the β chain (CD122) in addition to a "private" chain [IL-2α (CD25) or IL-15α]. Whereas $CD56^{bright}$ and $CD56^{dim}$ NK cells do not differ in the expression of the common γ chain (CD132), there are differences in the expression of the other two chains (α and β) of the IL-2 receptor. Only $CD56^{bright}$ NK cells display all three chains of the high-affinity IL-2 receptor (αβγ) and proliferate in response to picogram quantities of IL-2 [3–5]. In contrast, $CD56^{dim}$ NK cells express only the β and γ chains of the IL-2 receptor and show poor proliferation under these same conditions [3–5]. About half of the $CD56^{bright}$ NK cells express the stem-cell factor receptor (CD117, c-KIT) and the IL-7 receptor (CD127) and respond to these cytokines, whereas $CD56^{dim}$ lack these receptors and are therefore insensitive to these early-acting cytokines.

$CD56^{bright}$ and $CD56^{dim}$ NK cells also differ functionally. Compared with $CD56^{dim}$ NK cells, $CD56^{bright}$ cells produce significantly more INF-γ, TNF-α, TNF-β, GM-CSF, IL-10, and IL-13, following activation with monocyte derived cytokines, such as IL-12, -15 and -18 [2,6,7], yet $CD56^{dim}$ NK cells produce cytokines in response to target-cell engagement. In contrast, $CD56^{dim}CD16^+$ NK cells have ~10-fold more perforin and granzyme B than $CD56^{bright}$ cells [7]. Accordingly, freshly isolated $CD56^{dim}$ NK cells have higher cytotoxicity than the $CD56^{bright}CD16^-$ cells [4]. However, after short-term culture in cytokines, both NK-cell subpopulations show similar killing ability [4]. As mentioned above, $CD56^{bright}$ and $CD56^{dim}$ NK cells differ in the expression of the low-affinity Ig receptor CD16. Therefore, $CD56^{dim}CD16^+$ NK cells have the ability to recognize and kill antibody-coated targets through antibody-dependent cellular cytotoxicity (ADCC). Antibody-coated B cells (anti-CD20; rituximab), breast cancer cells (anti-Her2neu; trastuzumab) and osteosarcoma cells (anti-EGFR; cetuximab) are more effectively killed by NK cells [8–10]. Collectively, the above studies suggest that these two NK-cell subpopulations have distinct immune functions, with $CD56^{bright}$ NK cells producing cytokines and the $CD56^{dim}$ NK cells having more of the cytotoxic/effector role. In addition, CD16 delivers a potent activating signal on $CD56^{dim}$ NK cells; therefore, novel strategies to impart specificity to NK cells are being contemplated through the use of CD16 antigen bispecific immune engagers.

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NK cell Cytotoxicity

A hallmark feature that distinguishes NK cells from most other cell types is the ability to kill malignant cell lines (K562) directly without prior antigen priming or cytokine stimulation. One of the first steps in NK-cell cytotoxicity is the establishment of a stable conjugate between the NK cell and the target cell, known as an “immune synapse” (IS). Studies have demonstrated that the early events in the creation of the IS involve the binding of CD2 and β_2-integrins (CD11a and CD11b) on the surface of NK cells with their ligands on target cells. These proteins organize into a ring-like structure at the point of contact between the NK cell and the target cell, forming the periphery of the IS [11]. Following this numerous NK-cell surface receptors are recruited to the center of the IS. Assuming that the balance of activating signals is greater than inhibitory receptor signals, actin polymerization facilitates the redistribution of cytotoxic granules to the center of the IS [11], for eventual release.

Target killing by NK cells occurs through two separate mechanisms: (1) the granule release pathway and (2) the membrane expression of TNF superfamily death receptors. NK cells contain lysosomal granules that store a number of cytotoxic proteins, including serine proteases (granzymes A, B, H, K, and M), perforin, and granulysin. Following activation, these granules are redistributed within the NK cell and released into the IS. Once released, these serine proteases enter the target cell and induce apoptosis. The two best characterized membrane receptors involved in the second method of killing are FasL and TNF-related apoptosis-inducing ligand (TRAIL) [12]. FasL binds to the Fas receptor (CD95), which is broadly expressed, including on human hepatocytes. In the case of TRAIL, two receptors (DR4 and DR5) are selectively expressed by malignant cells [13]. Binding of either FasL or TRAIL to the surface of malignant target cells results in activation of caspase-dependent death pathways, culminating in target cell apoptosis. Numerous malignant target cell lines and clinical acute leukemia and myelodysplastic syndrome (MDS) samples express the Fas or TRAIL receptors [14–16].

MHC-specific NK-cell receptors: KIR and CD94/NKG2A

The “missing self” hypothesis

There has been significant progress in the understanding of the receptors that control NK-cell triggering. Through the pioneering work of Karre and Ljunggren, it was noted that a subclone of a malignant cell line was more sensitive to NK killing than the parental line. Further experiments showed that the more sensitive subclone had lost MHC class I expression. This observation lead to “the missing self” hypothesis, which predicted that NK cells are inhibited by self-MHC [17]. Such a hypothesis reasonably explained how NK cells might be tolerant of normal tissues, but could also kill malignant or virally infected targets with downregulated or lost MHC class I. Since this original observation, MHC class I-specific receptors have been identified on NK cells from a number of species (reviewed in [18,19]). Interestingly, some of these receptors are unique between mouse and humans, suggesting their recent (and parallel) evolution, perhaps in response to infectious challenges [20]. In humans, two groups of MHC-specific receptor systems have been identified and characterized: (1) the killer Ig receptor (KIR) family and (2) a c-lectin receptor made up of a heterodimeric complex between NKG2A, -C, and -E molecules and CD94 (Figure 11.1).

KIR receptors

The KIR gene locus is present on human chromosome 19q13.4 and comprises a multigene family of up to 15 individual cell surface receptors (reviewed in [19–21]). KIR genes are further subdivided based on the structure of both the extracellular and intracellular

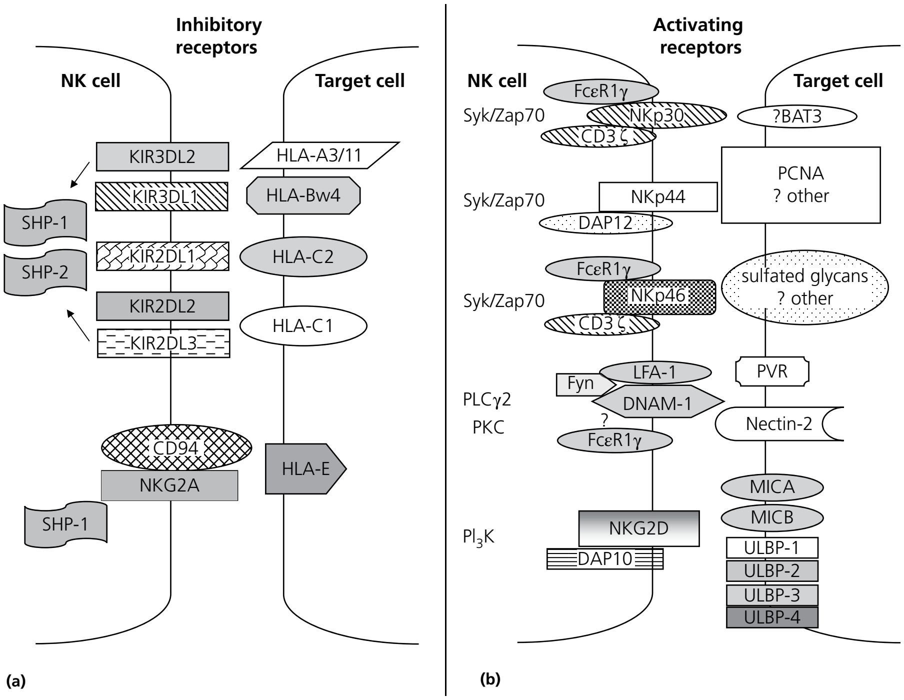
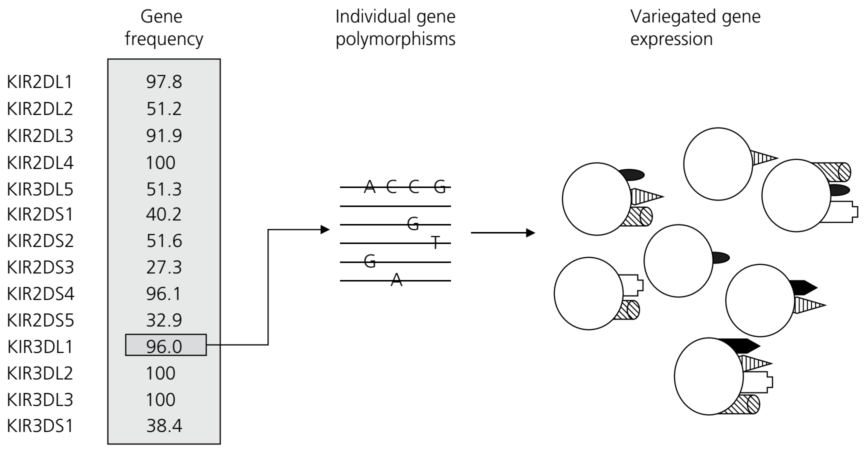


Figure 11.1 NK cell receptors and their ligands. Shown are the various inhibitory receptors (a) and the activating receptors (b) and their ligands. Also shown are the associated adapter proteins and the intracellular signaling pathways that are activated.



	Gene frequency
KIR2DL1	97.8
KIR2DL2	51.2
KIR2DL3	91.9
KIR2DL4	100
KIR3DL5	51.3
KIR2DS1	40.2
KIR2DS2	51.6
KIR2DS3	27.3
KIR2DS4	96.1
KIR2DS5	32.9
KIR3DL1	96.0
KIR3DL2	100
KIR3DL3	100
KIR3DS1	38.4

Figure 11.2 The complexity of KIR. Individuals vary in the number and type of genes that they express and data are shown for >450 patients types (unpublished observations) (left). In addition, there is considerable diversity within an individual KIR gene at the nucleotide level (i.e., polymorphisms) (middle) and at the cellular level (right).

domains. These domains regulate ligand binding and signaling, respectively. In general, KIR genes are referred to as either KIR2D or KIR3D, depending on the number of Ig domains in the extracellular portion. KIR receptors also vary with respect to the length of their intracellular signaling domains and are classified as either "long" or "short." KIR with long intracellular signaling domains contain ITIM (immunoreceptor tyrosine-binding inhibition motif) regions that recruit inhibitory signaling proteins, such as SHP-1 and SHP-2 [22,23], resulting in an significant attenuation of NK cell activation (reviewed in [21]). In contrast, KIR with short tails lack ITIMs and instead associate with adapter proteins, such as DAP12, to mediate signals that activate NK cells [24]. Thus, KIR with *short* intracellular tails are activating whereas those with *long*, ITIM-containing regions are inhibitory. A nomenclature committee has assigned a cluster of designation (CD) number of CD158 for the KIR genes with individual loci designated by a small letter ± a number (e.g. KIR3DL1 = CD158e1] [25].

Given the vast polymorphism of human MHC, it was difficult to conceive how a single KIR could recognize multiple MHC alleles from different individuals. X-ray crystallography shows that like the T-cell receptor (TCR), KIR interacts with the face of the MHC molecule, but more to the right-hand side and outside the peptide-binding groove [26]. This corresponds to a motif conferred by a conserved stretch of amino acids at positions 77–83 of HLA-B and at position 80 of HLA-C [19]. Thus, despite the vast polymorphism of MHC class I alleles, alignment of this amino acid stretch falls into two different groupings, known as Bw4 and Bw6 for HLA-B. Likewise, depending upon whether there is an asparagine (Asn) or a lysine (Lys) at position 80, HLA-C can be divided into C1 and C2 groups, respectively. These dimorphisms in HLA-B and -C convert the recognition of a highly diverse, multi-allelic MHC class I molecules into a biallelic system. However, further complexity is present since polymorphism of KIR genes and HLA determines the strength of inhibitory signaling. To date, HLA-Bw6 alleles do not appear to be recognized by KIR. In contrast, both HLA-C1 and -C2 are ligands for different KIR receptors (KIR2DL2/KIR2DL3 and KIR2DL1, respectively). While the specificity of many of the inhibitory KIR receptors is known to be MHC class I, the KIR family also contains receptors for which the specificity is not known, including most of the activating (or short) KIR, except for KIR2DS2 that binds HLA-C2 at low affinity.

The KIR gene locus is a multigene family; however, individuals vary with respect to the number of KIR genes that they possess (see Figure 11.2). By sequencing the KIR region of 68 individuals, Uhrberg et al. found that all individuals contain a set of framework genes including KIR3DL3, KIR2DL4, and KIR3DL2. They could also discern common haplotypes with varying gene content, called haplotype A and B [27]. Although both haplotypes contained inhibitory and activating KIR genes, the A haplotype showed fewer genes, typically only one activating receptor (KIR2DS4). In contrast, the B haplotype contained the full complement of inhibitory genes, but also expressed multiple activating KIR genes [27]. These haplotypes are present in relatively equal frequencies in Caucasians [28]. A study by Cooley et al. showed that donors expressing KIR B genes and, in particular, the centromeric portion of the KIR B haplotype ("cen B/B" homozygotes) had a dramatically lower rate of myeloid leukemia relapse and improved survival in multivariate analysis [29]. Similarly to a number of other studies, these findings were found only in myeloid leukemia and not in patients with lymphoid leukemia. Thus, NK-cell-mediated graft-versus-leukemia effect (GVL) appears to be mainly myeloid leukemia specific and not all donors are equivalent with respect to GVL. It may be possible to select NK "super donors" for myeloid leukemia transplantation.

To add further complexity to the KIR system, each gene shows considerable polymorphism [27] and some are functionally important. For example, KIR3DL1*004 is not expressed on the cell surface, so it cannot recognize its respective Bw4 ligand [30]. Still further complexity is appreciated since KIR and MHC exist on separate chromosomes and do not co-segregate during meioses. This leads to a situation where some individuals express receptors for ligands they do not have [27]. Thus, KIR is clonally distributed on individual NK cells, resulting in a complex system of individual NK cells, each expressing a different combination of KIR receptors (Figure 11.2). This creates a network of NK cells capable of recognizing subtle changes in a single MHC gene on target cells. Although beyond the scope of this chapter, KIR are not restricted to NK cells and have also been observed on mainly CD8$^+$ T cells of memory phenotype and may play a role in such responses.

NKG2A

NKG2A is the other MHC-specific inhibitory receptor expressed by the majority of NK cells. For NKG2A to be presented on the cell surface, it must associate with the CD94, creating the heterodimeric protein complex CD94/NKG2A [31]. Like inhibitory KIR, NKG2A contains two ITIM regions in the cytoplasmic tail segments [31] and ligation of this complex results in strong inhibition of cytotoxicity [32]. In contrast to KIR, which recognizes the classical MHC class I proteins (HLA-A, -B, and -C), CD94/NKG2A recognizes HLA-E [33,34]. Remarkably, a small segment of the leader peptide of classical MHC class I (amino acids 3–11) is complexed within HLA-E and stabilizes HLA-E surface expression, which can bind to CD94/NKG2A [32,33]. Thus, whereas KIR *directly* assesses the MHC class I expression of the surface of target cells, CD94/NKG2A both directly assesses the HLA E expression, and also *indirectly* determines MHC class I expression since, if HLA-A, -B, and -C are not expressed, then the leader sequence is not presented in the groove of HLA-E and the expression of HLA-E is reduced.

NKG2A is one member of the NKG2 gene family found within the NK cell gene cluster [35]. Other members of this gene family have also been investigated, including NKG2C, NKG2E, and NKG2D (see below). Analogous to "long' and "short" KIR, which have similar extracellular domains but differ in the intracellular signaling domains, NKG2C shares considerable homology with the extracellular portion of NKG2A and also recognizes HLA-E. In contrast to NKG2A, both NKG2C and NKG2E lack ITIMs and mediate NK-cell activation through association with DAP12 [36]. NKG2C is of increased interest as expression increases after exposure to viruses such as hantavirus or cytomegalovirus (CMV).

NK-cell-activating receptors: NKG2D, natural cytotoxicity receptors, and DNAM-1

NKG2D

Unlike other NKG2 members (NKG2A, -C, -E, etc.) that form a heterocomplex with CD94, NKG2D exists as a homodimer [37,38]. Interaction of NKG2D with its ligands (see below) delivers signals that can override inhibitory KIR. NKG2D is not able to signal directly and uses the adaptor protein DAP10 to transmit intracellular signals (Figure 11.1] [37]. DAP10 in turn recruits the p85 subunit of the PI3 kinase protein [37]. Additionally, NKG2D triggering leads to cytokine production (TNF-α, GM-CSF, IFN-γ) [39].

NKG2D binds to two groups of self-proteins which appear to be preferentially expressed on distressed cells, such as malignant cells. The first group of NKG2D ligands are MHC class I polypeptide-related sequence A and B (MICA and MICB). These two closely related MHC-like proteins are within the MHC gene complex, approximately 46 kb centromeric to HLA-B. The promoter region of MICA contains a heat shock protein-like element, similar to HSP70, and accordingly, MICA and MICB are expressed during cell division, radiation-induced genotoxic stress, malignant transformation, and following infections (viral and bacterial) [40–43]. MICA also shows limited expression on normal cells with the highest level of expression in the gastrointestinal tract, a target tissue for graft-versus-host disease (GVHD) [40]. Like MHC class I, MICA and MICB are polymorphic with approximately 58 and 18 individual alleles, respectively. Some of these alleles show differing binding affinities for NKG2D [44], again reflecting the complexity of NK receptor–ligand interactions. The other group of ligands recognized by NKG2D is the UL16-binding proteins, ULBP1–6, which have minimal homology to the MICA/B proteins. Like MICA and MICB, ULPB1–6 are also expressed on malignant tissues and freshly isolated malignant cells [45,46].

Natural cytotoxicity receptors (NCRs)

The natural cytotoxicity receptors (NCRs) were identified by Moretta's group after raising monoclonal antibodies (mAbs) against NK clones and then screening these antibodies for the ability to trigger NK cytotoxicity. Using this approach, these investigators identified three functionally similar, but structurally separate, NK-activating receptors. Collectively, these receptors have been referred to as NCRs since they play a major role in NK-cell recognition of malignant targets. The first of these receptors, NKp46, is identified as a type I transmembrane receptor of the Ig superfamily located within the leukocyte receptor complex on chromosome 19 (19q13.4) [47]. The second receptor, NKp30, is a V-type receptor found in the TNF cluster of the MHC gene complex on chromosome 6 [48]. Both NKp30 and NKp46 are expressed on nearly all resting and activated human NK cells. The third NCR, NKp44, is found only on IL-2-activated NK cells. Similarly to NKp30, the NKp44 gene is also present within the TNF cluster of the MHC gene complex on human chromosome 6.

Like other NK cell receptors, NKp30, -44, and -46 are not able to signal directly and rely upon adaptor proteins to transmit activation signals. NKp30 and NKp46 use CD3ζ and FcεR1γ, whereas NKp44 associates DAP12. Ligation of these receptors induces Ca^{2+} flux, cytotoxicity, and cytokine release. NCRs are critically important in recognition and killing of numerous malignant targets (reviewed in [49]). Antibody blockade of a single receptor shows some attenuation in cytotoxicity; however, blockade of two or more of the NCR receptors results in a significant reduction in cytotoxicity, suggesting that these receptors cooperate with one another. Although most individuals have NK cells with strong NKp30 and NKp46 expression, some show an NCR^{dim} phenotype and this correlates with a reduction in NK cell cytotoxicity [48,50].

NKp44 and NKp46 recognize viral products, such as hemagglutinin [51]. Likewise, Chisholm and Reyburn showed that vaccinia virus-infected cells become susceptible to NK cytolysis. Using solublized receptors, they further demonstrated increased expression of NKp30, NKp44, and NKp46 ligands [52]. Whether these ligands are self-proteins induced by viral infection or viral proteins themselves remains to be determined. Such ligands may be increased on cells that have undergone malignant transformation, perhaps like the NKG2D ligands MICA and MICB. Further support for NCR recognition of self-proteins is that NKp30 interacts with immature DCs through NKp30 [53], implying that DCs express a ligand for NKp30. The HLA-B-associated transcript 3 (BAT3) is expressed on DCs and appears to be a ligand for NKp30 [54]. Additional studies suggest that MHC-related receptor B7H6 is also a ligand for NKp30.

DNAM-1

DNAX accessory molecule-1 (DNAM-1, CD226) is a receptor expressed by both NK cells and T cells and is also involved in cytotoxic triggering [55]. The ligands recognized by DNAM-1 include the polio virus receptor (PVR, CD155) and nectin-2 (CD112) and NK cell cytotoxicity of neuroblastoma and leukemia cells correlate with PVR and/or nectin-2 expression [42,56,57]. For resting NK cells to become activated, DNAM-1 cooperates with other NK-cell receptors, such as NKp46 and -2B4, to induce Ca^{2+} flux, cytokine secretion (IFN-γ and TNF-α), and cytotoxicity [58].

NK killing of leukemia

NK-cell recognition of leukemia and MDS is of particular importance to the field of allo-HCT, since understanding such processes may allow for augmentation of GVL. Most in vitro studies show that acute myeloid leukemia (AML) patient samples are more sensitive than acute lymphoblastic leukemia (ALL) blasts. Considering that NK-cell cytotoxicity is a balance between the inhibitory and activating NK-cell receptor signaling, the relatively lower cytotoxicity of ALL lines could be accounted for by either high expression of inhibitory receptor ligands (i.e., HLA class I) or low expression of activating receptor ligands, or a combination of both. In fact, compared with AML blasts, ALL patient samples express higher amounts of MHC class I (i.e., KIR-L), which negatively modulate NK killing [42]. There is a significant correlation between the NK killing of ALL and the density of HLA class I (the higher the HLA expression, the less is the killing) [59]. Moreover, when high amounts of HLA class I were present on ALL blasts and KIR-L mismatched donor NK cells were used (see below), killing increased [59].

Despite a higher amount of HLA class I on ALL blasts relative to AML blasts, most malignant cells show a *reduction* in HLA relative to normal cells, perhaps as a consequence of T-cell selective pressure. Using a large panel of HLA-A and -B allele-specific mAbs and a panel of 32 patient samples [chronic myelogenous leukemia (CML), ALL, and AML), Demanet et al. showed that 65% of samples had a reduction in one or more HLA class I relative to normal lymphocytes. Interestingly, however, when they grouped the downregulated HLA-B alleles into the Bw4 and Bw6 allospecificities, they observed downregulation of significantly more Bw6 than Bw4 alleles [60]. Considering that the inhibitory KIR receptor KIR3DL1 recognizes Bw4 but not Bw6 alleles, this might suggest that such leukemia cells were selected for escaping *both* NK-cell and T-cell surveillance. On a related note, Verheyden et al. tested whether patient KIR genes might be associated with leukemia risk. In their study of 96 patients with acute and chronic leukemia and 148 controls, they found that leukemia patients were more likely to express KIR2DL2 and be heterozygous for the HLA-C-specific KIR haplotype. Such patients would express most, if not all, inhibitory KIR (and their ligands), creating a situation of excessive NK cell restraint that might favor subsequent escape of leukemia from immune surveillance [61]. In another patient cohort, the same group found that chronic lymphoid leukemia (CLL) and AML patients were more likely to express a particular KIR and its corresponding ligand (MHC class I) compared with controls, suggesting that NK cells are involved in the surveillance of malignancy and the interaction between KIR and its corresponding MHC can inhibit responses [62]. Similar observations have been made in the progression to HIV [63].

NK cells also require signals from the activating receptors to lyse targets and their expression on malignant cells may predict sensitivity to NK killing. For example, Pende and co-workers found that the NKG2D ligands ULBP1–3 were present on T-cell ALL lines and patient samples, but were essentially absent on pre-B ALL patient samples [41,42]. Likewise, only a minority of AML samples (7/25) expressed NKG2D ligands [42]. In contrast, Salih et al. found that >50% of AML, ALL, and CML patient samples expressed NKG2D ligands, perhaps reflecting different reagents used in these assays [46]. Importantly, MICA can be shed from the cell surface and can be found in patient sera [46,64]. Soluble MICA can act as a decoy by binding to NKG2D and inhibiting receptor function and leukemia recognition. Accordingly, surface expression of NKG2D is lower in newly diagnosed AML patients [65]. The DNAM-1 ligands (PVR and nectin-2) were present on most AML patient samples and to a lesser extent on ALL samples. Using individual patient samples, the involvement of DNAM-1 in NK-cell cytotoxicity of these targets was confirmed [42].

Most individuals express significant amounts of NCRs on their NK cells, constituting an NCR^{bright} phenotype. However, a minority (~10–15%) of normal donors show low NCR expression with an NCR^{dim} phenotype [66]. This NCR^{dim} phenotype is associated with reduced in vitro natural cytotoxicity. At the time of diagnosis, >60% of individuals with AML showed an NCR^{dim} phenotype [67]. As these patients were treated and entered hematological remission, most reacquired an NCR^{bright} phenotype, suggesting that leukemia results in receptor downmodulation, perhaps owing to the release of soluble NCR ligands or the elaboration of cytokines that downmodulate NCR expression (i.e., TGF-β or IL-10). However, other investigators have observed that NKp46 was not different from controls at the time of diagnosis [65].

Methods to overcome the resistance of leukemia to NK-cell cytotoxicity have also been explored. For instance, poor baseline NK-cell killing of autologous ALL blasts could be enhanced following cytokine stimulation (IL-2, -12 and -15) and culture in the presence of lymphoblast cell lines (LCLs) that activate NK cells [68]. To redirect NK cells to ALL, Imai et al. transduced NK cells with a chimeric antigen receptor (CAR) made of the external portion of a CD19 mAb and the internal signaling domains of CD3ζ and 4-1BBL. NK cells expressing this CAR showed marked proliferation, activation, and killing of NK-resistant ALL cell lines and patient samples [69]. This approach is limited by the need to introduce the CAR into NK cells, which are inherently resistant to retroviral transduction. To overcome this, Gleason et al. used single-chain variable fragments (scFvs) which recognize CD16 and CD19 (or CD22] to redirect NK cells to B-cell malignancies [70]. These CD16 × CD19 reagents potently enhance NK-cell cytotoxicity through direct activation of CD16 (resulting in granule release and cytokine production) and perhaps by bringing NK cells in close contact with malignant B cells by simulating an IS. Other approaches that have been tested to enhance NK killing of malignant cells include the use of chemotherapy to make blasts better targets. For example, treatment of AML blasts with a cocktail of differentiation agents (5-aza-2-deoxycytidine, trichostatin, vitamin D_3, bryostatin-1, and all-*trans*-retinoic acid) led to an induction of the NKG2D ligand ULPB1, which, in turn, enhanced NK-cell killing of AML targets [71]. Similarly, the histone deacetylase inhibitor trichostatin A increased NKG2D ligands (MICA and -B) on leukemic blasts and enhanced NK-cell killing [72]. In addition, IFN-γ led to a marginal increase in NCR ligands in AML patient samples and increased cytotoxicity [45].

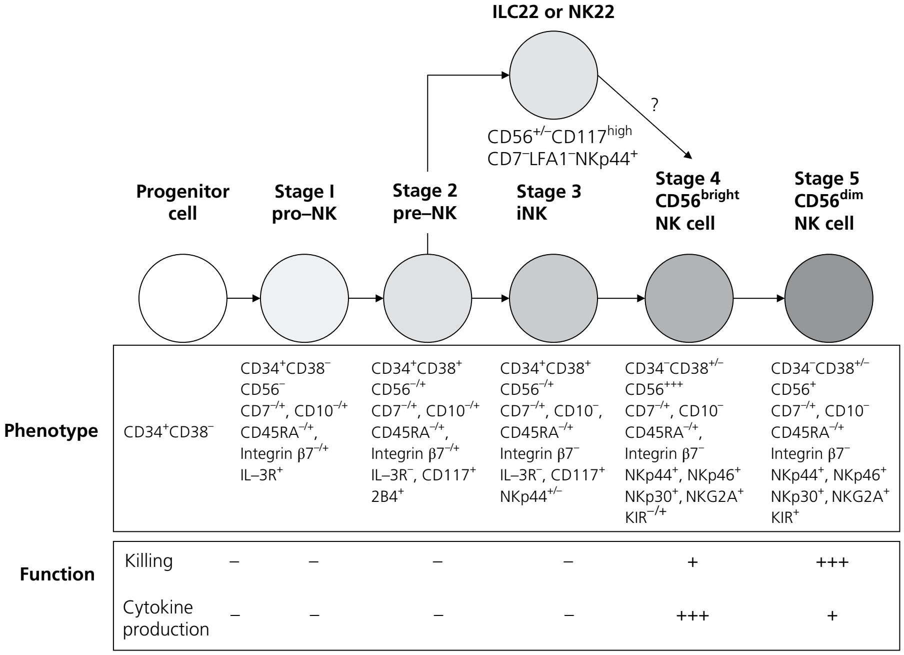
NK-cell development from hematopoietic progenitor cells

Like other hematopoietic cells, human NK cells are derived from CD34$^+$ progenitor cells. Cytokines such as IL-2, -3, -7, and -15, stem cell factor, and FLT-3L facilitate the in vitro differentiation of such progenitor cells along the NK-cell lineage [73]. Of these cytokines, only IL-15 is required for NK-cell development, since IL-15$^{-/-}$ mice are devoid of NK cells [74]. In contrast, mice engineered to overexpress IL-15 (IL-15 transgenic mice) develop uncontrolled lymphoproliferation in both the CD8 T cell and NK compartments [75]. Thus, although IL-15 does not act solely on NK cells, it plays a central role in NK-cell homeostasis. In addition, NK-cell development is critically dependent upon stromal cells. Uncommitted, CD34$^+$CD38$^-$ cells cultured in the presence of cytokines show marginal NK-cell differentiation; however, both NK-cell maturation and expansion are significantly improved if the same progenitor cells are cultured in the presence of fetal liver stromal cell lines (and cytokines) [76]. Such augmentation of NK-cell development occurs in a contact-dependent manner [76]; however, the receptor–ligand pairs involved in this process remain to be identified. Thus, both the local environment and cytokine milieu influence NK-cell development.

A recurring paradigm in hematopoietic cell development is the progressive acquisition and loss of surface receptors during development, which can be used to functionally characterize distinct developmental stages of differentiation (see Figure 11.3]. Such a schema for human NK-cell development has been proposed (reviewed in [77]). The earliest antigens expressed on CD34$^+$ progenitors that mark enhanced potential to develop into NK cells include CD7, CD10, CD45RA, CD122, and integrin β_7 [77]. Such progenitors, referred to as Stage I or pro-NK cells, show increased capacity to differentiate into NK cells under the influence of the above-mentioned cytokines, with less of a need for stromal support, suggesting that cells that bear these receptors are committed to the NK-cell lineage. Following this, NK cell progenitors transit through a pre-NK (Stage 2) and immature NK cell (Stage 3) steps, characterized by the loss of surface proteins expressed on immature progenitors (CD34 and CD38) and acquisition of cytokine receptors (IL-3R and IL-7R) and adhesion molecules (LFA-1). Finally, differentiating NK progenitors acquire additional activating receptors (NKp30, -44, and -46, and NKG2D) in addition to the inhibitory receptor CD94/NKG2A in the CD56^{bright} stage (Stage IV) of NK-cell development and eventually KIR is expressed by CD56^{dim} NK cells (Stage V). Although a similar set of differentiation steps have been identified in the murine bone marrow, the location of NK-cell differentiation in humans appears to occur in secondary lymphoid tissue such as lymph nodes and tonsils [78,79].

Innate lymphoid cells (ILCs)

Recently, it has been proposed to reclassify NK cells as part of a larger group of cells that have now been referred to as innate lymphoid cells (ILC) [82]. Although NK cells are the best described ILC cells (ILC1), other cell types within this family have also been characterized, including type 2 ILCs (ILC2, natural helper cells or nuocytes [80]) and ILCs that express the retinoic acid receptor-related orphan receptor-γt (RORγt) transcription factor (RORγt$^+$ ILCs) [81,82]. Similarly to T-cell populations, ILC cells have been defined by transcription factor expression, which dictates function. For instance, ILC1 express T-bet and produce IFN-γ and TNF-α following IL-12 and IL-18 stimulation. ILC2 cells express the transcription factor ROR-α and secrete Th2-associated cytokines (IL-5 and IL-13) following extracellular parasite infection [80,83]. As the name implies, RORγt$^+$ ILCs express the RORγt transcription factor and produce IL-22 (ILC22) and/or IL-17 in response to IL-1β and IL-23, released during bacterial infections and/or gastrointestinal tract injury [84,85]. These latter cells exist within the Stage III NK progenitor fraction (Figure 11.3) and whether these cells are a unique and stable cell lineage or they give rise to conventional NK cells is debated. Of interest to the blood and marrow transplant community, RORγt$^+$ ILC3 cells mediate lymphoid tissue development during fetal life and its regeneration in adult life [81,86]. At present, the kinetics of recovery of these cells after allo-HCT are unknown. Given their properties, adoptive transfer of RORγt$^+$ ILC cells has the potential to accelerate post-transplant immune responses against infectious challenges and malignant disease.



		Progenitor cell	Stage I pro–NK	Stage 2 pre–NK	Stage 3 iNK	Stage 4 CD56^{bright} NK cell	Stage 5 CD56^{dim} NK cell
Phenotype		CD34$^+$CD38$^-$	CD34$^+$CD38$^-$ CD56$^-$ CD7$^{-/+}$, CD10$^{-/+}$ CD45RA$^{-/+}$, Integrin β7$^{-/+}$ IL–3R$^+$	CD34$^+$CD38$^+$ CD56$^{-/+}$ CD7$^{-/+}$, CD10$^{-/+}$ CD45RA$^{-/+}$, Integrin β7$^{-/+}$ IL–3R$^-$, CD117$^+$ 2B4$^+$	CD34$^+$CD38$^+$ CD56$^{-/+}$ CD7$^{-/+}$, CD10$^-$, CD45RA$^{-/+}$ Integrin β7$^-$ IL–3R$^-$, CD117$^+$ NKp44$^{+/-}$	CD34$^-$CD38$^{+/-}$ CD56^{+++} CD7$^{-/+}$, CD10$^-$ CD45RA$^{-/+}$, Integrin β7$^-$ NKp44$^+$, NKp46$^+$ NKp30$^+$, NKG2A$^+$ KIR$^{-/+}$	CD34$^-$CD38$^{+/-}$ CD56$^+$ CD7$^{-/+}$, CD10$^-$ CD45RA$^{-/+}$ Integrin β7$^-$ NKp44$^+$, NKp46$^+$ NKp30$^+$, NKG2A$^+$ KIR$^+$
Function	Killing	–	–	–	–	+	+++
	Cytokine production	–	–	–	–	+++	+

Figure 11.3 Stages of NK-cell and innate lymphoid cell development. As NK cells develop from progenitor cells they lose and acquire surface receptors. Shown are some of the hallmark receptors and functional properties of developing NK cells. Not shown is the ontonogy of the CD94$^-$NKG2A$^+$ NK cells [89,143]. How these cells fit into the current "linear" schema of NK-cell development is unknown. Also shown is a concept that ILC cells share some phenotypic features with conventional NK cells and may give rise to conventional NK cells (denoted by the arrow with the question mark). Source: adapted from Freud and Caligiuri 2006 [77].

Development of NK-cell self-tolerance

The mechanism by which NK cells acquire self-tolerance has been referred to as "NK-cell education." Several models have been proposed that integrate inhibitory receptor signaling with the acquisition of effector functions. "Licensing" describes a terminal differentiation step by which NK cells only acquire mature function when they receive an appropriate signal via an inhibitory receptor ligating with self-MHC [87,88] Thus, human NK cells lacking inhibitory receptors are hyporesponsive [89]. In support of this, in vitro differentiation models show that NK cells acquire functionality (killing and cytokine production) only after the acquisition of CD94/NKG2A [90]. Thus, NK self-tolerance may be the result of a coordinated genetic developmental sequence during which mature NK function is synchronized with the acquisition of adequate expression of self-inhibitory molecules. However, murine HCT experiments show that transfer of mature NK cells into strains with differing MHC class I molecules rapidly results in either the activation or inhibition of cytotoxicity [91,92]. These studies have important implications for human HCT studies and suggest that licensing is not the only developmental process and that the function of mature NK cells can change depending upon the MHC class I of the recipient.

NK-cell reconstitution after allo-HCT

Reconstitution of immunity after allo-HCT can be influenced by a number of factors, including intensity of conditioning, hematopoietic cell source, graft manipulation, GVHD prophylaxis, and host factors (i.e., age and thymic function). NK cells typically recover within the first month after transplant and are therefore one of the first lymphocytes to recover after HCT, regardless of stem-cell source. Comparing the NK-cell reconstitution following reduced intensity or myeloablative conditioning, Peterson et al. found that NK cell reconstitution was rapid (by D+28) and not different [93]. Similarly rapid reconstitution has been reported for umbilical cord blood (UCB), bone marrow (BM), and haploidentical transplantation [94–96]. Regarding the function of NK cells recovering after transplantation, some have shown robust cytotoxicity [97], whereas others have found impaired killing and cytokine production [98,99]. Whether NK function early after transplant has any impact on transplant outcomes is currently unknown. One question was whether the recovering NK cells would have the same KIR repertoire as was present in the donor. This was addressed by Shilling et al. by following patients for up to 3 years after transplant [100]. Early after transplant, the majority of patients showed high expression of CD94/NKG2A and low amounts of KIR that slowly increased over time after bone marrow transplantation (BMT). Based on KIR acquisition, patients fell into three groups. The first group, which made up the majority of patients, recovered a KIR pattern similar to that of the donor within 6–9 months after transplant. A second group took longer (up to 3 years), but eventually showed a KIR distribution similar to that of the donor. The last group showed poor KIR acquisition and had multiple medical problems.

GVHD prophylaxis and treatment may also affect NK cell reconstitution and function. Vitale et al. studied the NK cells from transplant recipients before, during, and after steroid treatment for acute GVHD. They found a sharp decrease in cytoxicity, associated with a reduction in NKp30 and -46 [101]. Wang et al. cultured NK cells with physiological doses of cyclosporine (CSP) and found that it significantly inhibited the proliferation of CD56^{dim} NK cells and only marginally influenced CD56^{bright} cells [102]. After 1 week of culture in CSP, NK cells retained their cytotoxicity and their ability to secrete cytokines and were not different from controls. Such findings are interesting considering that CD56^{bright} cells predominate early after transplant [103–105], perhaps suggesting that CSP may play a role in phenotype and function of NK cells recovering early after transplant. Giebel et al. evaluated the number of NK cells in the peripheral blood of individuals at D+30 after transplant and showed that patients receiving prednisone for GVHD prophylaxis had fewer NK cells compared with those who did not receive prednisone. In contrast to prednisone-treated recipients, there was no difference in the number of NK cells in the peripheral blood of patients treated with or without antithymocyte globulin (ATG) [106], suggesting that prednisone may have more long-lasting effects on NK-cell recovery. Cooley et al. compared recipients of either T-cell-depleted or T-cell-replete transplants at D+100 with their donors and found that the percentage of NK cells did not differ. However at D+100, recipients of T-cell-replete transplants had more KIR and a higher percentage of IFN-γ-producing NK cells compared with T-cell-depleted recipients, suggesting that T cells within the graft influence NK-cell reconstitution [105]. Freud et al. also demonstrated that T cells could enhance NK-cell development from progenitor cells in vitro [79].

NK–DC interactions

DCs are professional antigen-presenting cells that are critically important in a number of immune responses. DCs exist in the circulation and peripheral tissues as immature (iDCs). Such cells are phagocytic, but poor at presenting peptide antigens to T cells. However, following activation, mature DCs (mDCs) are highly efficient at stimulating naïve T-cell responses. NK cells and DCs interact with one another both through soluble factors (i.e., cytokines) and via cell-to-cell contact.

NK–DC interaction, also known as "cross-talk," can result in a number of outcomes, including reciprocal activation of both cell types. Following co-culture with either immature or mature DCs (iDC or mDC), NK cells proliferate, express activation markers (CD25), acquire cytotoxicity, and produce IFN-γ and TNF-α [53,107]. This is driven by DC-derived cytokines including type I IFNs (α and β), IL-12, and IL-18 and by trans-presentation of IL-15 to NK cells via the IL-15α receptor on DCs [108–111]. The same conditions (NK:iDC co-culture) also lead to iDC maturation characterized by the acquisition of CD83 and CD86 and the ability to prime alloreactive T-cell responses [53,107,112]. NK-cell-induced maturation of DCs (and vice versa) occurs in part through the ligation of NKp30 (on NK cells) by its ligand (on DCs) [53,107]. Other studies showed that interactions between NKG2D on NK cells and MICA/B on DCs are also important for these interactions [112].

Paradoxically, NK cells can also kill DCs. The maturational status of DCs influences the sensitivity to NK-cell killing. Compared with mDCs, iDCs display relatively lower amounts of MHC class I on the cell surface and are more sensitive to NK-cell killing [53,107]. Higher amounts of MHC class I on mDCs result in a relative resistance to killing by autologous, but not allogeneic, NK cells. Killing of autologous mDCs by NK cells can be enhanced with blocking mAb against MHC class I, suggesting the involvement of KIR receptors [107]. Likewise, NK cells that express KIR receptors that cannot recognize MHC on the mDC show better cytotoxicity (i.e., KIR-L mismatched) [107]. Similarly to killing of tumor targets, the killing of DCs by NK cells is through NKp30, NKG2D, and DNAM-1

[53,107,112,113]. Communication between NK cells and DCs is potentially important in the setting of HCT, since recipient-derived DCs can present antigen to donor T cells and induce GVHD [114]. Studies using a murine model suggested that donor-derived NK cells can eradicate these recipient DCs and prevent GVHD [115].

NK cells, viral infections, and “NK memory”

Inherited deficiency and/or dysfunction of NK cells has been associated with severe and life-threatening viral infections, including infections with members of the herpes virus family, such as varicella zoster virus (VZV) [116,117], CMV [118], and Epstein–Barr virus (EBV) [119]. Similarly, humans with mutations in the perforin gene (in the case of hemophagocytic lymphohistiocytosis) result in fatal and uncontrolled viral infections. Following HCT, reactivation of herpes viruses also occurs and whether NK cells play a role in this process is not well defined. There is a clear association between the expression of NK-cell-activating protein Ly49H in certain mouse strains and the susceptibility to murine CMV [120]. Although NK cells have been described as innate immune cells that are devoid of immunological memory, recent studies by Lanier’s group have questioned this dogma by uncovering striking similarities between antigen specific T cells and NK cells that have previously been challenged by antigen. In particular, following mCMV infection, “memory-like” Ly49H$^+$ NK cells persist in mice. These cells rapidly respond to secondary mCMV challenge through proliferation and effector function, properties consistent with memory cells [121]. Following exposure to CMV in humans, a population of NK cells that express NKG2C emerges. Whether these are “memory NK cells” is not entirely clear, yet during acute infection, NKG2C$^+$ NK cells expand over 4 weeks and persist for the first year after transplantation [122]. These NK cells predominantly express self-KIR with robust IFNγ production after target cell exposure. However, this does not prove memory. In a second study, recipients of adult donor HCT were assessed to evaluate the role of donor/recipient CMV status on the expression and function of NKG2C$^+$ NK cells in order to determine responses to secondary CMV events. Expansion of NKG2C$^+$ NK cells was seen with clinical CMV reactivation, as expected. In addition, they also expanded in the absence of detectable CMV viremia when both the donor and recipient were CMV seropositive. Upregulation of NKG2C was detected on NK cells from CMV-seropositive recipients receiving grafts from CMV-seropositive donors. These in vivo-expanded NKG2C$^+$ NK cells were highly functional. In conclusion, the enhanced secondary exposure is consistent with NK-cell memory and shows that they are transplantable.

Clinical studies

Allogeneic HCT functions through both conditioning therapy (chemotherapy and irradiation) and an immune-mediated eradication of resistant leukemia cells. This latter point, referred to as graft-versus-leukemia (GVL), is mediated via the actions of alloreactive T cells and NK cells. NK-cell alloreactivity has attracted considerable attention over the past decade, in part due to observations that allogeneic NK cells can induce GVL without GVHD [115,123]. Considering the importance of HLA matching in HCT and the fact that each KIR receptor recognizes specific allelic determinants of HLA, such interactions are likely to be important in GVL reactions. However, such interactions are complex and not entirely understood. For instance, the expression of KIR on individual NK cells occurs through a stochastic process with each NK cell expressing a varying number of KIR receptors of differing MHC class I specificity [27]. Thus, contained within the circulation of all potential donors is a heterogeneous pool of NK cells (with regard to KIR expression, see Figure 11.2) and in any individual only a minority of these cells may have alloreactive capacity (i.e., are unrestrained by KIR–MHC interactions).

The observation that NK-cell alloreactivity is important in GVL was established by the Perugia group in the context of T-cell-depleted and CD34-selected haploidentical transplantation [115,124]. In these transplants, donors (parents or siblings) share one haplotype with the recipient, but are also mismatched at the other haplotype. This creates a situation where alloreactivity is possible, especially if donor NK cells express KIR that cannot recognize MHC on recipient leukemia cells, fulfilling the “missing self” hypothesis. As shown in Figure 11.4, following haploidentical transplantation three possible outcomes could occur: (1) NK-cell alloreactivity in the graft-versus-host direction, which would favor GVL reactions, (2) NK alloreactivity in the host-versus-graft vector, which could favor rejection, or (3) no alloreactivity owing to compatibility between the donor and recipient with respect to HLA epitopes.

In general, alloreactions between NK cells expressing receptors for HLA-C appear to be dominant over those for HLA-B [125]. This is perhaps due to the higher number of HLA-C-specific KIR receptors and possibly their greater affinity for ligand. Consequently, in the original report addressing NK cell alloreactivity after transplant, Ruggeri et al. found that the majority of alloreactive NK cell clones generated after haploidentical transplantation were reactive to differences in HLA-C [124]. Such clones could be detected in

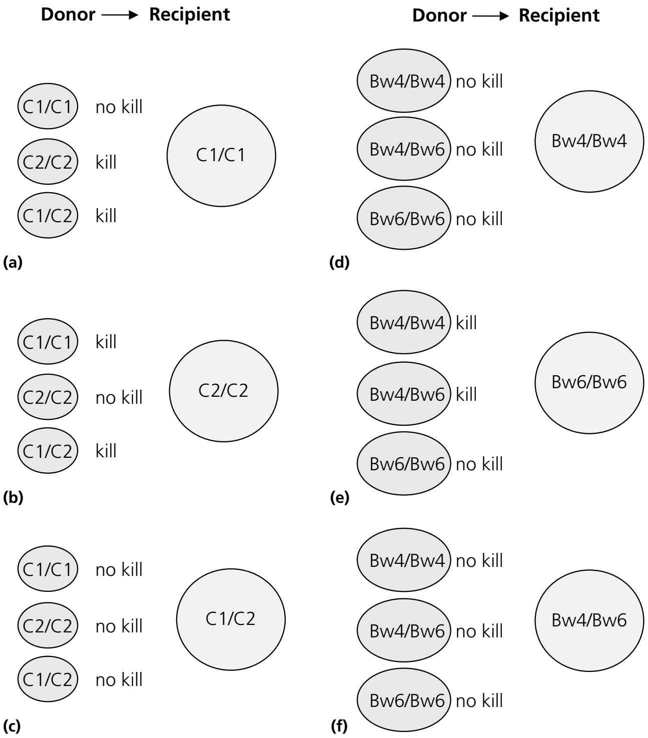


Figure 11.4 Potential outcomes following transplants depending upon donor and recipient KIR-L compatibility. Shown are the possible outcomes depending upon donor and recipient HLA-C dimorphisms (C1/2) [(a)–(c)] and HLA-B dimorphisms(Bw4/Bw6) [(d)–(f)]. Source: adapted from Wit and Christiansen 2006 [144].

patients for up to 1–3 months after transplantation. Following KIR-L mismatched transplants (see below), these anti-recipient NK cell clones could be detected in all patients, whereas they were essentially absent in KIR-L-matched transplant recipients [115]. AML patients transplanted with a KIR-L mismatched donor showed a low probability of relapse at 5 years. In contrast, a similar population of AML patients transplanted in the same manner with a KIR-L-matched donor poor showed poor outcomes, with 75% relapsing by 5 years [115]. Importantly, the effect of KIR-L mismatching was restricted to patients with myeloid leukemia and no relapse or survival benefit was observed for ALL patients transplanted with a KIR-L-mismatched donor. An updated analysis by the Perugia group showed that patients with chemotherapy relapses did not benefit from KIR-L-mismatched donors [126].

The above observations on NK alloreactivity have been referred to as the "ligand–ligand" model, where prediction of NK-cell alloreactivity is based on the presence or absence of MHC class I alleles (HLA-B or-C) in the recipient relative to the donor (i.e., whether the donor and recipient express the same or different HLA-Bw4 or HLA-C1/C2 alleles). Thus, KIR ligand mismatch analysis using the ligand–ligand model can be viewed as an analysis of donor-versus-recipient epitope similarity in the GVH direction for HLA-B and -C alleles. Determination of KIR-L mismatch status using this model can be performed using an online tool, available at http://www.ebi.ac.uk/ipd/kir/ligand.html. However, this approach may be limited since it assumes that a particular donor expresses KIR receptors for the corresponding ligand. It is well established that individuals differ in the number of KIR genes contained in their genome, hence not all individuals will express all KIR receptors [27]. Leung and co-workers took into account whether donors and recipients differ with respect to HLA-B and -C alleles, but also determined whether the donor expressed the KIR receptor for the mismatched HLA class I allele (i.e. KIR-L). They found that using their "receptor–ligand" model, patients with both AML and ALL experienced lower leukemia relapse rates if transplanted with a KIR-L-mismatched donor who expressed the appropriate KIR [127,128].

Importantly, not all investigators have been able to demonstrate a benefit for AML patients undergoing haploidentical transplantation using KIR-L-mismatched donors. Nguyen et al. transplanted 10 AML patients with a haploidentical donor (8/10 being KIR-L mismatched) using the same regimen and peripheral blood progenitor cell (PBPC) selection techniques as in the above studies. However, unlike in the above studies, they found that all patients died due to either relapse (7/10) or infection (3/10) [99]. The authors demonstrated that NK cells recovering after this procedure were immature in both phenotype and function and speculated that this might account for their clinical observations.

Whether KIR-L mismatching affects outcomes in the setting of unrelated donor transplantation has been the subject of numerous retrospective studies, with mixed results. Initially, a large registry analysis by Davies et al. showed that a KIR-L-mismatched donor had no impact on the incidence of AML relapse following T-cell-replete transplantation [129]. Shortly afterwards, Giebel et al. showed a protective effect of KIR-L mismatch for AML patients in the unrelated donor setting [130]. Interestingly, this latter study used ATG for in vivo T-cell depletion (TCD), leading to the speculation that T cells might influence NK alloreactivity. Similar results were obtained by Hsu et al. in a TCD-matched sibling cohort [131].

The use of KIR-L-mismatched donors may also affect transplant-related outcomes other than relapse. For instance, Schaffer et al. found an increase in treatment-related mortality (TRM) in KIR-L=mismatched recipients due to a higher rate of infectious complications [132]. Similarly, Kroger et al. found that KIR-L-mismatched recipients had a higher rate of TRM [133]. Examining HLA-C mismatch, De Santis et al. found that in donors and recipients who were mismatched for C1 and C2 epitopes, there was more grade II–IV GVHD if the mismatch was in the GVH vector [134]. Conversely, if the mismatch was in the rejection vector (HVG), more rejection was observed. Regardless of the vector, these investigators found that KIR-L mismatching was associated with inferior outcomes and increased TRM [134]. However, analyzing a large data set (n = 1571), Farag et al. were not able to demonstrate any impact of non-relapse mortality in KIR-L-mismatched transplants in either vector [135]. Clearly, differences in hematopoietic cell source, conditioning regimen, and GVHD prophylaxis may affect such observations.

In addition to KIR-L mismatching, some investigators have analyzed data sets based on the presence or absence of a specific KIR or the quantity of KIR receptors expressed by the donor. In the setting of fully HLA-matched transplant (HLA 8/8), KIR-L mismatch cannot occur; however, transplant recipients whose donor expressed both KIR2DS1 and KIR2DS2 had better outcomes due to a reduction in leukemia relapse [136]. In a large National Marrow Donor Program (NMDP) study, Cooley et al. showed that donors with an increase in activating KIR had lower relapse and improved disease-free survival for myeloid leukemia patients [29]. Chen et al. found that more activating KIR in the matched related donors resulted in lower TRM and better overall survival (OS) [137]. An increase in the total number of KIRs (both activating and inhibitory) contained within the donor was associated with a reduction in GVHD and an improved OS [134].

Adoptive NK-cell transfer for refractory leukemia and cancer

Another way to promote antitumor activity with alloreactive NK cells is through NK-cell adoptive transfer. This can be achieved using several strategies. One approach is based on ex vivo NK cell expansion using supraphysiologic concentrations of cytokines (i.e., high-dose IL-2]. The safety and success of this approach were established in a trial using haploidentical, related-donor NK cells that were activated overnight in IL-2 and infused into 43 patients with metastatic melanoma, metastatic renal cell carcinoma, refractory Hodgkin disease, or AML [123]. The trial, which tested three preparative chemotherapy regimens of differing intensity, confirmed that successful NK-cell expansion was see only n in the cohort who received the fully lymphodepleting chemotherapy consisting of cyclophosphamide (60 mg/kg) days –4 and –5 and daily intravenous fludarabine (25 mg/m^2) days –5 to –1. NK cells were infused on day 0, followed by six doses of subcutaneous IL-2 (10 million units) over 2 weeks. Following the lymphodepleting chemotherapy, a surge of endogenous IL-15 was observed over the first 28 days after chemotherapy. A significant inverse correlation was seen between the IL-15 levels and the absolute lymphocyte count, and high levels correlated with successful NK-cell expansion, supporting the importance of IL-15 for NK cell homeostasis. Of the 19 patients with poor-prognosis AML, five achieved complete remissions. The remission patients had significantly higher proportions of circulating NK cells which were significantly more cytotoxic against K562 targets, suggesting that clinical efficacy was mediated by the in vivo-expanded allogeneic donor NK cells. The presence of haploidentical NK cells in the circulation is transient and we speculate that these cells are likely rejected by recipient T cells. To overcome this hurdle, some investigators are now planning clinical trials with NK cells that have been expanded long term with engineered feeder cells or EBV lymphoblastoid cell lines that lead to NK-cell expansion. However, concern has been raised in both short- and long-term culture conditions where removal of the NK cells from the high-dose cytokine culture media may lead to cell death. In addition, upon activation, NK cells undergo marked changes in size and adhesion receptor expression, which may alter homing characteristics in vivo.

In addition, the use of post-infusional IL-2 also has the potential to stimulate regulatory T cells which express high levels of IL-2Rα (CD25). These regulatory T cells can impair NK-cell function and perhaps expansion [138]. To overcome this, IL-15 may be optimal to promote adoptive transfer of allogeneic NK cells because it has a minimal effect of regulatory T-cell activation.

Conclusion

NK cells recover early after transplantation. These cells kill malignant cells without prior priming, using a limited number of pattern recognition receptors that detect cells undergoing stress. By understanding these receptors and their ligands, an understanding of how NK cells function to mediate GVL after allo-HCT is emerging. However, there is still an incomplete understanding of both the receptors that they use and the ways to maximally activate NK cells. At present, there are several limitations to the therapeutic potential of adoptively transferred allogeneic NK cells. The NK-cell yield from lymphopheresis collections is limited. Although NK cells can be successfully expanded in vivo, the success is unpredictable and the expanded cell population is transient. Additionally, the homing signals required to direct NK cells to tumor sites are not fully understood. Furthermore, the alloreactivity of in vivo expanded NK-cell populations may be heterogeneous due to variable KIR repertoire expression or to differences in NK cell or accessory cell subsets. The use of mAbs that block NK-cell inhibitory receptors may increase antitumor killing [139], and more sophisticated techniques for subset selection in NK-cell products may affect the interaction between the innate and adaptive immune responses, for example by removing any regulatory T cells that can suppress NK-cell proliferation and killing, and may also improve the immune effector functions of the expanding NK cells [140]. NK-cell expansion may be improved by refining the use of lymphodepleting chemotherapy or the use of concurrent exogenous cytokine therapy. Irradiated cell lines such as NK-92, KHYG-1 and embryonic stem-cell-derived NK cells may provide an inexhaustible supply of NK cells, but the in vivo homing and survival are not well known [141,142]. Ex vivo expanded cells from any source can be genetically modified to express tumor-specific receptors. For example, the NK-92 cell line has been transfected with a chimeric antigen receptor for HER2/neu, which conferred superior cytotoxicity against HER2/neu positive targets [142]. Lastly, the antitumor activity of NK cells depends not only on the KIR ligand mismatch status, but also on tumor expression of appropriate activating ligands. Future strategies to enhance activating ligand expression on tumor cells may increase their susceptibility to NK-cell-mediated lysis. Other avenues of research for NK-cell immunotherapy include engineering NK cells with transferred genes, incorporating NK cells into DC vaccine therapies, and combination therapy with immunomodulatory drugs such as thalidomide, Toll-like receptor agonists, and mAbs to target ADCC.

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CHAPTER 12

Mechanisms of Tolerance

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Introduction

In classical experiments, Medawar and co-workers injected neonatal mice of one strain with lymphoid tissue cells from another. After the recipient mice grew to adulthood, they permanently accepted skin grafts from the donor strain but rejected the skin grafts from a third-party strain; this phenomenon was called "actively acquired tolerance of foreign cells" [1]. In order to apply this approach to adult instead of neonatal mouse recipients, approximately 20 years later, investigators at Stanford University developed the first non-myeloablative regimen (total lymphoid irradiation, TLI) that resulted in permanent acceptance of combined bone marrow (BM) and skin transplants associated with the development of stable mixed chimerism and without graft-versus-host disease (GVHD) [2]. Prior studies of BM transplantation in mice used total body irradiation (TBI) to condition recipients, and engraftment was almost always associated with the development of GVHD when multiple minor histocompatibility antigens or single major histocompatibility antigens differed between hosts and donors [3]. Transplantation immune tolerance is now defined as a physiological state, in which the immune system does not launch a destructive response against an allogeneic organ or tissue graft. It is now known that the destructive response is mediated by alloreactive TCR$\alpha\beta^+$ T cells, B cells, NK cells, and a variety of other cells, but T cells play a dominant role. This chapter focuses on mechanisms that tolerize alloreactive TCR$\alpha\beta^+$ T cells that mediate host-versus-graft (HVG) responses in organ transplantation and graft-versus-host (GVH) responses in hematopoietic cell transplantation (HCT); the former causes graft rejection and the latter can cause GVHD.

General mechanisms of alloreactive T-cell immune tolerance

Alloreactive T cells include both mature T cells that are in the peripheral lymphoid tissues before transplantation and newly developed T cells derived from hematopoietic stem and progenitor cells after transplantation. Induction of tolerance among the developing T cells in the thymus after encounter with antigens in the thymus is defined as central tolerance. Induction of tolerance among mature T cells that encounter antigen in the peripheral lymphoid tissues is called peripheral tolerance. The general mechanisms of T-cell tolerance include clonal deletion, ignorance, anergy, exhaustion, and suppression. These mechanisms can involve both central and peripheral tolerance, with clonal deletion being the major mechanism in central tolerance, and anergy, exhaustion, and suppression being the major mechanisms in peripheral tolerance.

Clonal deletion

Burnet theorized that immature T cells encountering their antigen during development are clonally deleted instead of being activated [4]. This theory was confirmed by Kappler and co-workers by showing that the T-cell subset expressing the Vβ17a$^+$ T-cell receptor (TCR) accounted for about 10% of T cells in mice that did not express an I-E molecule. In contrast, mice that express I-E molecules had a marked reduction of the Vβ17a$^+$ cells. When I-E$^+$ and I-E$^-$ strains were crossed, the F1 offspring contained few Vβ17a$^+$ cells in the periphery; this dominant elimination process was found to take place in the thymus. The deletion of T-cell clones with the Vβ-specific TCR was found to be mediated by mouse mammary tumor virus (Mtvs) superantigen, which activates T cells by interacting with both the Vβ17a TCR and the I-E molecule at the same time [5]. The deletion of immature autoreactive thymocytes was also demonstrated by transgenic T cells specific for H-Y antigens; these T cells were markedly reduced in the thymus of male mice [6]. Hence clonal deletion is a major mechanism of central tolerance via thymic negative selection.

Thymic clonal deletion of immature T cells is predominantly mediated by hematopoietic cell-derived antigen-presenting cells (APCs) [e.g., CD11c$^+$ dendritic cells (DCs) and B1 B cells] in the thymus, especially DCs [7]. Thymic medullar epithelial cells can mediate deletion of T cells that recognize tissue-specific antigens, and this process is often regulated by the autoimmune regulator (AIRE) protein [8]. Thymic DC-mediated clonal deletion usually takes place at the junction of cortex and medulla, but it can also take place in the cortex [9]. Circulating activated T cells and DCs carrying peripheral tissue-specific antigens can also enter the thymus to mediate clonal deletion [10,11].

Thymic clonal deletion takes place when the avidity of immature T-cell interaction with APC is high; the avidity is influenced by variations of TCR affinity and density, antigen–major histocompatibility complex (MHC) complex density, and the expression of costimulatory molecules by APCs. Higher avidity and costimulatory signaling augment clonal deletion [12]. Transgenic autoreactive T cells are often deleted at the transitional stage between CD4$^-$CD8$^-$ and CD4$^+$CD8$^+$ during thymocyte development; non-transgenic T cells are usually deleted at late stage of CD4$^+$CD8$^+$ thymocyte development in the cortex and the junction of cortex and medulla [9]. Early CD24^{hi}CD4$^+$CD8$^-$ T cells can be deleted in the

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medulla by interaction with APCs that express costimulatory molecules such as CD40 and B7 [13].

The activation threshold for thymic deletion is much lower than that for peripheral T-cell activation, hence the autoreactive T cells that escape from thymic deletion are usually not activated by low-avidity interacting ligands in the periphery [14]. However, clonal deletion is not only limited to immature thymocytes, since mature T cells in the periphery can also undergo clonal deletion through activation -induced cell death [15]. Mature donor reactive T cells in the periphery undergo clonal deletion early after HCT in the presence of costimulatory blockade (anti-CD40L and CTLA-4-Ig) [16]. Donor-type APCs in mixed chimeras given MHC-matched or -mismatched donor BM transplants mediate thymic deletion of donor reactive T cells and provide immune tolerance to donor-type tissue and organ transplants [17]. GVHD causes damage to the thymus, which may result in defective thymic deletion of host-reactive T cells and development of chronic GVHD [18,19].

Even under normal thymic negative selection, clonal deletion of autoreactive T cells in normal individuals or alloreactive T cells in HCT recipients in the thymus is rarely complete, and some of the auto- or alloreactive T cells can be exported into the peripheral lymphoid tissues. There, they are controlled by peripheral tolerance mechanisms, including ignorance, anergy, exhaustion, and suppression.

Ignorance

Immune ignorance is referred to as a state in which auto- or alloreactive T cells in the lymphoid tissues are not activated, because antigen levels are below the threshold level required for activation of T cells. For example, autoantigens from tumor cells can be too low to activate the antigen-specific T cells in the draining lymph nodes [20]. Allografts without contaminating passenger leukocytes allow alloantigens to stay outside organized lymphatic tissues, and can avoid immune rejection [21].

Anergy

T-cell anergy is a state in which T cells intrinsically become functionally inactivated following an antigen encounter, but remain alive for an extended period as hyporesponsive T cells [22]. The basic types of T-cell anergy fall into two categories: T-cell clonal anergy and adaptive tolerance. The former is usually induced in vitro and is principally a growth arrest state arising from incomplete T-cell activation; the latter is also known as in vivo anergy, resulting from the challenge of T cells in the periphery by APCs presenting superantigen or specific peptides in the absence of costimulatory signaling [22]. T-cell clonal anergy and adaptive tolerance have many common and distinguished features: both involve CD4$^+$ and CD8$^+$ T cells; both show little IL-2 production and hyporesponsiveness; and both can be reversed by anti-OX40 mAb (monoclonal antibody) treatment. However, although T-cell clonal anergy shows inhibition of proliferation, it does not show reduction of effector function such as production of IL-4 and IFN-γ and also cytotoxicity activity. In contrast, T-cell adaptive tolerance not only shows inhibition of proliferation but also shows reduction in IL-4 and IFN-γ production and cytotoxicity activity. Although T-cell clonal anergy does not downregulate IL-2R and can be reversed by exogenous IL-2, T-cell adaptive tolerance does block IL-2R signaling and exogenous IL-2 cannot reverse it. Finally, CTLA-4 signaling is involved in T-cell adaptive tolerance but not T-cell clonal anergy; persistence of antigen is required for maintenance of T-cell adaptive tolerance but not clonal anergy [22].

T-cell anergy is usually induced by TCR stimulation (signal 1) without concomitant CD28 and IL-2R signaling (signal 2). In the absence of signal 2, TCR signaling leads to activation of calcium influx and nuclear factor of activated T cells (NFAT), and induces a cascade of anergy proteins, including the E3 ubiquitin ligases Cbl-b, Itch, and Grail, which target proximal TCR signaling molecules for degradation [23]. Signal 2 leads to activation of the PI3–AKT–mTOR pathway and IL-2 production, which can reverse T-cell anergy [24]. Anergic T cells are blocked at the G1/S checkpoint of the cell cycle and in a metabolically anergic state characterized by a failure to upregulate nutrient transporters, such as CD71, CD98, and Glut1 [25]. Activation of the AKT–mTOR pathway is also critically required to induce a switch in the T-cell metabolic machinery from catabolism to anabolism, through stimulation of glycolysis and upregulation of key nutrient transporters [26]. This establishes the basis for the optimal activation, proliferation, and differentiation of T cells. This T-cell activation and anergy switch can also be regulated by AKT–mTOR independent pathways. Plasmacytoid DCs that express indoleamine 2,3-dioxygenase (IDO) activate the GCN2 kinase in responding T cells and mediate the T-cell anergy [27]. Additionally, TCR signaling alone has been shown to upregulate A2A adenosine receptors on T cells, and A2A receptor interaction with tissue-derived adenosine can inhibit activation of the Ras–Map–kinase pathway and result in T-cell anergy [28].

T-cell anergy is generally involved in peripheral tolerance, and donor-specific unresponsiveness has been shown in animal models and patients with organ transplantation immune tolerance, but the role of T-cell anergy in transplantation tolerance is not easily distinguished from clonal deletion, because both can result in lack of alloreactivity in the mixed lymphocyte reaction (MLR) assay. Treatment with calcineurin inhibitors (e.g., cyclosporine and tacrolimus) that block the calcineurin–NFAT pathway and the induction of T-cell anergy can prevent the induction of transplantation tolerance; in contrast, treatment with mTOR inhibitors (e.g., sirolimus) that block the PI3–AKT–mTOR pathway and augment the induction of T-cell anergy can facilitate the induction of transplantation immune tolerance [29]. Additionally, "infectious tolerance" is found to be associated with induction of anergy of alloreactive T cells by regulatory T (Treg) cells [30]. The latter finding suggests that T-cell anergy contributes importantly to transplantation immune tolerance.

Exhaustion

T-cell exhaustion is a state of T-cell dysfunction defined by (1) poor effector function such as marked reduction in production of cytokines (e.g., IFN-γ, TNF-α, IL-2) and cytotoxicity and (2) upregulation of expression of inhibitory receptors such as PD-1 and Tim-3 [31]. T-cell exhaustion usually arises in the case of chronic infection and cancer [31]. T-cell exhaustion differs from T-cell anergy in the following respects. (1) Although both can be hyporesponsive, T-cell anergy is induced at the time of first antigen stimulation and is initiated rapidly owing to lack of costimulation; but T-cell exhaustion takes place after full T-cell activation with costimulation. T-cell exhaustion is progressive, with dysfunction worsening over time. (2) Although NFAT has a role in both situations, anergy-associated genes such as Grail do not seem to be upregulated in exhausted T cells; instead, Blimp that controls the terminal differentiation of cells is overexpressed by exhausted T cells. The latter is associated with upregulated expression of many inhibitory receptors such as PD-1, Tim-3, LAG3 (CD160), and CD244 (2B4). (3) Antigen persistence is required for maintaining but not inducing anergy, but is required for inducing exhaustion. (4) Although both anergy and exhaustion involve CD4$^+$ and CD8$^+$ T cells, anergy is better studied with CD4$^+$ T cells and exhaustion is better studied with CD8$^+$ T cells [31].

The contribution of T-cell exhaustion to organ transplantation immune tolerance is unclear. The conditioning regimen of TLI that induced tolerance to combined organ and BM transplants has been shown to induce the PD-1$^+$Tim-3$^+$ exhausted phenotype among host CD8$^+$ T cells [32]. The latter host cells are unable to reject the donor marrow cells that establish chimerism and clonal deletion. In addition, alloreactive CD8$^+$ T-cell exhaustion and loss of graft-versus-tumor (GVT) effect was reported to be mediated by antigen presentation by non-hematopoietic cells in HCT recipients [33]. In vivo OX40 ligation was reported to expand CD4$^+$Foxp3$^+$ Treg cells but led to downregulated expression of Foxp3 and exhaustion of the Treg cells [34].

Suppression

Studies in the early 1980s by Strober and co-workers showed that naturally occurring immune suppressor cells existed in the neonatal spleen and adult BM of mice and rats, and that suppressor T cells contributed to allograft tolerance in recipients treated with TLI [35]. Hall et al. also showed that suppressor T cells existed in the rat recipients of cardiac allografts after cyclosporine treatment, and the suppressor T cells contributed to the long-term survival of the grafts in the absence of continuous cyclosporine therapy [36]. In the mid-1990s, Sakaguchi and co-workers found that CD25$^+$CD4$^+$ T cells regulated tissue-specific autoimmune diseases [37], and subsequently that CD25$^+$CD4$^+$ Treg cells express Foxp3 [38]. Despite the skepticism that once prevailed among immunologists, it is now widely accepted that the normal immune system harbors a variety of naturally occurring immune-regulatory cells that target a wide variety of antigenic specificities. In addition to Foxp3$^+$CD25$^+$CD4$^+$ Treg cells, there are other CD4$^+$ and CD8$^+$ Treg cells and also regulatory B (Breg) cells, dendritic cells, and myeloid cells [39]. Below is a summary of major immune suppressor cells.

CD4$^+$CD25$^+$Foxp3$^+$ Treg cells

CD4$^+$CD25$^+$Foxp3$^+$ Treg cells can be divided into natural (nTreg) cells that develop in the thymus and induced (iTreg) cells that derive from mature CD4$^+$ T cells in the peripheral lymphoid tissues during T-cell activation and differentiation. It is proposed that thymocytes with intermediate TCR avidity for autoantigens presented by thymic stromal cells are activated to express Foxp3, and become Foxp3$^+$ nTreg cells; thymocytes with low avidity for autoantigens become Foxp3$^-$ conventional T cells; and the thymocytes with high avidity TCR for autoantigens are negatively selected [40]. nTreg cells are positively selected in thymic medulla instead of cortex at the HSA^{hi}CD4$^+$ single positive stage instead of CD4$^+$CD8$^+$ double positive stage. Either thymic medullar epithelial cells or medullar hematopoietic cell-derived APCs are sufficient to mediate the positive selection, although it is unclear whether the different APCs select different nTreg cells with different antigen specificity. Intrinsic factors such as TCR signaling and costimulatory signaling mediated by CD28 and also extrinsic factors such as cytokines (e.g., IL-2 and IL-15) regulate the selection. CD28 deficiency results in defective nTreg cell development [41].

A majority of Foxp3$^+$CD4$^+$ Treg cells in the periphery are nTreg cells, and nTreg-cell survival in the periphery requires presence of self-antigens, but there are also iTreg cells that derive from conventional CD4$^+$ T cells during T-cell activation and differentiation in the secondary lymphoid organs or inflamed tissues [42,43]. During in vitro culture, in the presence of TGF-β or retinoic acid (RA), conventional T cells upregulate Foxp3 expression and become iTreg cells [44,45]. This differentiation can be shifted to Th17 by the presence of IL-6 and IL-1 [45]. In vivo, conventional T cells can differentiate into iTreg cells in a tolerogenic microenvironment such as the mucosa of the small intestine and also during acute and chronic inflammation, but iTreg cells are generated at a much lower rate than T effector cells [46]. The use of non-depleting anti-CD4 monoclonal antibodies (mAbs) that weaken coreceptor engagement and blockade of PI3–AKT–mTOR led to the generation of iTreg cells [47,48]. The common feature of nTreg and iTreg cells is the expression of one or more anti-inflammatory molecules, such as IL-10, TGF-β, or IL-35, and/or inhibitory molecules, such as CTLA-4, LAG-3, GITR, CD39, or CD73. Although in vivo-generated iTreg and thymic-generated nTreg cells have similar gene expression profiles, nTreg cells tend to have higher expression of Gzmb (granzyme B), Ikzf2 (helio), and Nrp1 (neuropillin-1) genes, and iTreg cells have higher expression of Il10 (IL-10), Ctla4 (CTLA-4), Ebi3 (a component of IL-35), and Klrg1 genes [46].

Treg cells downregulate T-cell homeostatic expansion, autoimmunity, and alloimmunity through several mechanisms, including the following. (1) Consumption of IL-2: Treg cells that express CD25 IL-2Rα with high affinity to IL-2 are more efficient than naïve or effector T cells in capturing IL-2 [49]. (2) Treg cells that express high levels of CTLA-4 that has a higher affinity than CD28 for CD80 and CD86, in particular CD80. Treg CTLA-4 interacts with APCs (e.g., DCs) with greater avidity than conventional T cells, and the interaction also activates IDO-dependent catabolism of tryptophan to immunosuppressive kynurenine in DCs, which can induce DC apoptosis or induce DC into a tolerogenic state that anergizes naïve T cells. This interaction can also activate Foxp3 in DCs and subsequently downregulate DC production of IL-6. CTLA-4 on Treg cells can also induce endocytosis and trogocytosis of CD80 and CD86 from APCs [50]. (3) Treg cells can secrete suppressive cytokines such as TGF-β, IL-10, and IL-35, and can directly kill target lymphocytes via granzyme B [51,52]. (4) nTreg and iTreg cells working together can produce optimal immune suppression. For example, in an autoimmune colitis model, full rescue of mice could be obtained only if the naïve T-cell population had been obtained from wild-type but not Foxp3-deficient mice that could generate in vivo Foxp3 iTreg cells [43].

CD8$^+$Foxp3$^+$ Treg cells

Several types of CD8$^+$ regulatory T cells have been reported in recent years, including (1) CD8$^+$CD28$^-$ T cells that were identified in a mouse experimental autoimmune encephalomyelitis (EAE) and renal transplantation patients after alemtuzumab (anti-CD52) treatment [53,54]; (2) CD8$^+$ IL-10-producing T cells identified in a renal transplantation patient with long-term graft acceptance on immunosuppressive drugs [55]; (3) FOXp3$^-$CD8$^+$122$^+$CXCR3$^+$ T cells identified in autoimmune diabetic NOD mice [56]; and (4) Foxp3$^+$CD8$^+$ T cells that were identified in systemic lupus mice after tolerogenic treatment with consensus peptide [57] and in lupus patients after autologous HCT [58], and also in multiple sclerosis patients [59].

Foxp3$^+$CD8$^+$ T cells were reported to share developmental and phenotypic features with classical CD4$^+$Foxp3$^+$ Treg cells; the Foxp3$^+$CD8$^+$ T cells can arise naturally in the thymus, although the percentage among CD8$^+$CD4$^-$ thymocytes is much lower than that of Foxp3$^+$CD4$^+$ T cells. The Foxp3$^+$CD8$^+$ T cells can be

CD28$^+$ or CD28$^-$, and express high levels of CTLA-4, CD25, GITR, and CD103 [60]. The Foxp3$^+$CD8$^+$ T cells can be converted from conventional CD8$^+$ T cells in mesenteric lymph nodes or in vitro culture with IL-2, TGF-β, and RA; the conversion was augmented by blockade of the AKT–mTOR pathway. They can suppress autoimmunity and alloimmunity in a cell–cell contact manner, kill activated T and B cells, and prevent lupus, allograft rejection, and GVHD [61–63].

Natural killer (NK) T cells

Differing from conventional T cells that recognize peptide antigens presented by polymorphic antigen-presenting molecules, MHC I and II, NKT cells recognize lipid antigens presented by the non-polymorphic antigen-presenting molecule CD1d. Thus, NKT cells represent a family of developmentally and functionally distinct T- cell subsets. NKT cells produce large amounts of Th1 (i.e., IFN-γ), Th2 (i.e., IL-4), and Th17 (i.e., IL-17) cytokines hours after TCR engagement, which can either promote cell-mediated immunity against tumor and infection organisms or suppress autoimmunity and alloimmunity [64]. NKT cells can be divided into type I and II subsets. Type I NKT cells express the invariant TCR α chain (Vα14Jα18 in mice and Vα24Jα18 in humans) coupled with a TCR β chain of limited diversity, including Vβ8.2, -7 and -2 in mice and Vβ11 in humans [64]. Type I NKT cells include subsets that are CD4$^+$ or CD4$^-$CD8$^-$ in mouse and CD4$^+$, CD8$^+$, or CD4$^-$CD8$^-$ in humans. Type I NKT cells recognize the prototypical lipid antigen glycosphingolipid α-galactosylceramide (α-GalCer); they also recognize bacterial glycolipids (e.g., α-galacturonosylceramide), and mammalian glycolipids such as isoglobotrihexosylceramide (iGb3) [64]. Type I NKT cells can be identified by staining with CD1d-αGalCer tetramer [64]. Type II NKT cells usually express diverse TCRαβ, but some subsets have a biased repertoire such as Vα3.2 or Vα8 coupled with Jα9 or Vβ8. Type II NKT cells are often CD4$^+$ or CD4$^-$CD8$^-$, and recognize sulfatide, lysophosphatidylcholine, or non-lipid small aromatic molecules [65]. They can be identified with sulfatide-loaded CD1d tetramers [65].

Studies of NKT development mainly concerned the type I invariant subset. NKT cells develop in the thymus and diverge from conventional T cells at the CD4$^+$CD8$^+$ thymocyte stage, and they are positively selected by CD1 expressed on CD4$^+$CD8$^+$ thymocytes [64]. The negative selection of NKT is not yet clear, but NKT development is abrogated by the presence of dendritic cells that transgenically overexpress CD1d, suggesting that DC may mediate its negative selection. Thymic differentiation of NKT cells involves stages of selection, proliferation, and maturation, which include three stages; stages 1 and 2 takes place in the thymus, and stage 3 is continued in the periphery [66]. The differentiation process is associated with changes of surface marker and cytokine profile. Thus, NKT cells exhibit "'memory at birth," meaning that they acquire an antigen-experienced CD44^{hi} phenotype in the mouse thymus [66].

The simultaneous production of Th1, Th2, and Th17 cytokines by NKT cells is probably due to the existence of functionally distinct subsets. For example, human type I CD4$^+$ NKT subset produces both Th1 and Th2 cytokines in vitro, whereas type I CD8$^+$ or CD4$^-$CD8$^-$ NKT cells produce mainly Th1 cytokines in vitro [65]. NK1.1$^-$ and NK1.1$^+$ type 1 NKT subsets in mouse thymus have striking differences in cytokine profile. Whereas the NK1.1$^-$ subset produce large amounts of IL-4 with little IFN-γ, the NK1.1$^+$ subset produce large amounts of IFN-γ, with small amounts of IL-4 [65]. A subset of CD4$^+$ NKT cells that express IL-25 receptor produced large amounts of IL-4 and IL-13 yet little IFN-γ in response to IL-25 stimulation [65]. Another subset of type I NKT cells is called NKT-17; they are αGalcer-tetramer$^+$NK1.1$^-$CD4$^-$; they express the nuclear transcription factor RoRγt and produce large amounts of IL-17 [65].

The paradoxical role of NKT cells in promoting and suppressing immune responses is also probably due to different NKT subsets. For example, IL-25R$^+$ but not IL-25R$^-$ type 1 NKT cells were found to mediate allergic tissue damage [67]. Type I NKT cells augmented but type II NKT cells suppressed tumor immune surveillance [68]. In addition, type II NKT cells mediated anergy of type I NKT cells and prevented inflammatory liver diseases [68]. Abnormal cytokine profiles of NKT cells were associated with type 1 diabetes and systemic lupus [69,70]. Prevention of allograft rejection and prevention of GVHD was associated with NKT cell production of IL-4 [32,71]. In humans, NKT cell levels in donor BM transplants or in donor chimeric cells in the early recovery phase after HCT are powerful predictors of acute GVHD [72].

Regulatory B cells

Although B cells play a central role in humoral immunity and also in APC function that amplifies cellular autoimmunity and chronic GVHD [73–75], the existence of B cells with a suppressive capacity was initially reported in the 1970s, since B-cell-depleted splenocytes were unable to inhibit delayed-type hypersensitivity (DTH) skin reactions in guinea pigs [76]. Subsequently, in the early 1980s, it was reported that suppressive B cells were associated with induction of antigen-specific T suppressor cells in mice [77]. It was then convincingly demonstrated, in 2002, that Breg cells that produce IL-10 and upregulate expression of CD1d can suppress chronic intestinal inflammation [78]. Currently, Breg cells have been shown to downregulate autoimmunity in a variety of autoimmune diseases in mouse models and humans such as EAE, collagen-induced arthritis (CIA), colitis, and systemic lupus [79]. Breg cells also suppress antitumor immunity, augment induction of organ transplantation immune tolerance, and prevent GVHD [79].

The origin of Breg cells is not yet clear. Although some data suggest that Breg cells develop from naïve follicular B cells, other data indicate that Breg cells arise from a common progenitor that give rise to marginal zone (MZ) and T2 transitional B cells [79]. In addition to the production of IL-10, Breg cells generally have the MZ-T2 B-cell surface phenotype of CD19$^+$CD5$^+$CD1^{hi}CD21^{hi}CD23^{hi} [79]. More recently, they have been identified as CD19$^+$TIM-1$^+$, because ~70% of IL-10-producing B cells are TIM-1$^+$; TIM-1 is also preferentially expressed by MZ-T2 B cells, and ~30% of TIM-1$^+$ MZ-T2 B cells express IL-10 [80].

The activation and expansion of Breg cells are critically associated with signaling from Toll-like receptors (TLRs), and CD40 and CD86, but it is not yet clear if BCR-signaling is required [79]. Mice lacking MyD88, TLR2, or TLR4 exclusively on B cells developed a chronic form of EAE, whereas TLR engagement on DCs or monocytes initiated proinflammatory responses. TLR engagement on B cells activated a counter-regulatory effect that damped an excessive inflammatory response. Mice with CD40 deficiency that was restricted to B cells suffered from a more severe EAE and showed a profound decrease of IL-10 production and an increase of encephalitogenic Th1 and Th17 responses. Expression of CD86 on B cells was necessary for mediating recovery of EAE [79]. Breg cells can directly suppress Th1, Th2, and Th17 and also proinflammatory macrophages via their secretion of IL-10 [78]. In addition, Breg cells can augment the induction of Tr1 CD4$^+$ suppressor T cells via their secretion of IL-10, and augment expansion of FoxP3$^+$

CD4$^+$ T cells [79]. Although Breg cells express high levels of CD1d, and CD1d^{hi} B cells were reported to produce IgM autoantibody in lupus mice [81], the influence of Breg cells on CD1d reactive NKT cells is not yet clear.

Tolerogenic dendritic cells

DCs have long been considered as the professional APCs that play crucial roles in linking innate and adaptive immunity; DCs constitute a heterogeneous population of BM-derived cells that can induce both immunity and tolerance [82]. DC tolerogenicity is not specific to a DC subset or restricted to the immature state of the APC. Tolerogenic DCs present antigen to antigen-specific T cells but deliver inadequate costimulatory signals or deliver inhibitory signals for effector T-cell activation and proliferation. This results in conventional T-cell apoptosis and anergy or generation and/or expansion of Treg cells in peripheral lymphoid tissues [83]. The major features of tolerogenic DCs include (1) the ability to acquire and present antigen to antigen-specific T cells; (2) low constitutive expression of surface MHC molecules and low net expression of costimulatory molecules (e.g., CD40, CD80, CD86, OX40L, ICOSL), but high expression of PD-L1; (3) low production of IL-12 and high production of IL-10 and IDO; and (4) resistance to maturation in response to "danger signals," such as high-mobility group box1 protein (HMGB1), TLR ligands, or CD40 ligation [83].

Tolerogenic DCs can be myeloid and plasmacytoid DCs, and the latter are generally more tolerogenic than the former [84]. Tolerogenic DCs can be natural DC subsets such as DCs in the thymus and steady-state CD103$^+$ DCs in intestinal and lung tissues, and also induced DC subsets that develop under abnormal circumstances such as in tumor environments [83]. It is important to stress that there is bidirectional feedback between tolerogenic DCs and Treg cells in mice and humans. For example, under the influence of thymic stromal lymphoietin (TSLP) produced by Hassall's corpuscles, thymic DCs, especially plasmacytoid DCs, augment the development of nTreg cells in the thymus [85]. In peripheral lymphoid tissues, tolerogenic DCs also promote the generation of Treg cells from naïve T cells, and Treg cells make DC progenitors into tolerogenic DCs [86]. An increase in DCs can lead to an increase in Treg cells, whereas the constitutive absence of DCs leads to fatal autoimmunity [79]. Tolerogenic DCs can secrete IL-10, TGF-β, and/or RA to induce naïve T cells into Treg cells; in turn, CTLA-4 on Treg cells can interact with B7 on DCs, resulting in IDO activation and tolerogenic characteristics in DCs [87].

Plasmacytoid DCs can transport peripheral antigens to the thymus to promote central tolerance [88]. Infusion of CCR9$^+$ tolerogenic plasmacytoid DCs suppressed acute GVHD [89], and infusion of dexamethasone-activated tolerogenic DCs prolonged allogeneic skin graft survival [90]. An increase in PD-L1$^+$ plasmacytoid DCs was associated with an increase in Treg cells and liver transplantation tolerance in patients [91].

Myeloid-derived suppressor cells (MDSCs)

MDSCs are defined as cells of myeloid origin with suppressor functions [92]. Although initially described in cancer patients, MDSCs are also present in other inflammatory settings such as solid organ transplantation and allogeneic HCT, in which MDSCs promote organ transplantation immune tolerance, and suppress GVHD [93,94]. Despite their heterogeneity, most MDSCs express common phenotypic markers; mouse MDSCs express CD11b and Gr-1 without MHC II, and human MDSCs express CD11b, CD33, CD34, and low levels of HLA-DR [95]. Myeloid progenitor cells with these markers are not usually suppressive in normal mice and humans, and may subsequently differentiate into macrophages, DCs, and granulocytes [95].

MDSC activation and differentiation are regulated by factors produced by other cells present in the same microenvironment, including stromal cells, activated T cells, and tumor cells [95]. Anti-inflammatory cytokines such as IL-10, TGF-β, IL-13, and IL-4 in the tumor environment can augment MDSC expansion and suppressive function via activation of Stat6 and Stat4 pathways [95]. In transplantation, the ischemic donor allograft and the surgical procedure cause tissue release of inflammatory signals that mobilize BM CD11b$^+$Gr-1$^+$ cells into circulation and infiltration of the graft [96]. The mechanisms of immune suppression by MDSCs include the following: (1) MDSCs can directly suppress proliferation and cytokine production by effector T cells, B cells, and NK cells via producing inducible nitric synthase (iNOs) and arginase 1, and this process requires IFN-γ activation of STAT1 pathway in the MDSC; (2) MDSCs can augment induction of Foxp3$^+$CD4$^+$ Treg cells and indirectly tolerize T cells; this process also requires IFN-γ activation of MDSCs and their production of IL-10 and TGF-β and upregulation of PD-L1 [96].

Other suppressor cells

Tr1 cells

Human CD4$^+$ T cells can be converted into so-called "Tr1" cells in a mixed leukocyte culture in the presence of IL-10. The Tr1 cells are Foxp3$^-$ and suppress effector T-cell proliferation via their secretion of IL-10 and TGF-β; Tr1 cells can function to inhibit GVHD and allograft rejection [97].

DNregulatory T cells

A subset of CD4$^-$CD8$^-$ (double negative, DN) Treg cells have the unique surface phenotype of $\alpha\beta$TCR$^+$CD4$^-$CD8$^-$CD25$^+$CD28$^-$NK1.1$^-$CD30$^+$CD44$^-$ and are distinguished from CD4$^-$CD8$^-$ NKT cells [98]. The DN Treg cells use a unique mechanism of suppression in which trogocytosis and the presentation of allogeneic MHC-peptide complexes are used to target and kill CD8$^+$ T cells with the same TCR specificity, and the killing is Fas–FasL dependent [98]. The DN regulatory T cells were reported to prevent graft rejection, GVHD, and autoimmune diabetes development and also to facilitate the induction of mixed chimerism in non-myeloablatively conditioned recipients [99].

Multipotent mesenchymal stromal cells (MSCs)

MSCs constitute a heterogeneous population of precursor cells, which are found in situ within all mammalian supportive stromal tissue compartments, but BM, adipose tissue, and the umbilical cord are the most commonly used cell sources [100]. The general phenotype is CD73$^+$CD90$^+$CA105$^+$ together with CD11b$^-$CD14$^-$CD34$^-$CD45$^-$CD19$^-$CD79a$^-$HLA-DR$^-$ [100]. MSCs can release a vast array of molecules (e.g., IL-10, TGF-β, galectin 1, galectin 3, and leukemia inhibitory factor) and also bioactive metabolites (e.g., nitric oxide, carbon monoxide, and prostaglandin E2) that have immunomodulating properties [100]. With these secreted factors and cell contact-dependent mechanisms, MSCs suppress CD4$^+$ and CD8$^+$ T-cell proliferation, cytokine production, and cytotoxicity and also modulate DCs and augment generation of Treg cells [100]. Infusion of MSCs has been shown to augment organ transplantation tolerance and prevention of GVHD [101].

Interactions among different suppressor cells

After organ transplantation or HCT, pro- and anti-inflammatory innate and adaptive immune responses are simultaneously activated; innate immune responses are followed by adaptive immune response. Despite the occurrence of active regulation mediated by various suppressor cells in the allograft and in the peripheral lymphoid tissues, rejection and GVHD mediated by inflammatory innate immune cells and alloreactive T cells will dominate in the absence of any other forms of immunosuppressive therapy, as proposed by Wood et al. [39]. Infusion of particular subsets of suppressor cells has been shown to augment suppression of graft rejection or prevention of GVHD, and the infused suppressor cells may interact with other suppressor cells in the recipient. Conditioning with TLI and antithymocyte globulin (ATG) or conditioning with anti-CD3 mAbs was reported to increase the percentage of both NKT and Foxp3$^+$ Treg cells and to prevent allograft rejection and GVHD [32,102]. Recent studies showed that both NKT and Treg cells are required for prevention of GVHD, and NKT cells mediate the Treg cell expansion via their secretion of IL-4 [32]. Hence the optimal effect of suppressor cells may be dependent on the interactions of a network of different types of suppressor cells.

Induction of host immune tolerance to organ allografts

Induction of immune tolerance to allogeneic organ transplants in humans remains the "holy grail" of the field, because tolerance avoids chronic immunosuppressive therapy and prevents acute and chronic rejection. Approaches for induction of immune tolerance in laboratory animal models can be divided into two major categories: non-chimeric approaches that mainly induce peripheral tolerance and chimeric approaches that induce both central and peripheral tolerance.

Major non-chimeric transplantation tolerance-inducing regimens

Many reagents, such as chemical immunosuppressants, anti-CD3, anti-CD4, anti-CD8, anti-CD52 mAbs, and ATG, have been used to prevent graft rejection, but only some of the regimens have been shown to be able to induce immune tolerance in at least mouse models. Below is a summary of the major regimens.

Costimulatory blockade for induction of transplantation tolerance

Costimulatory signals are critical for optimal T-cell activation, proliferation, and differentiation, and blockade of costimulatory signals is proposed to induce T-cell anergy and apoptosis; blockade of interactions between CD28–CD80/CD86, CD40L–CD40, or LFA-1/ICAM-1 has been reported to augment induction of transplantation tolerance in mouse models, but not yet in non-human primates or humans [103]. The challenges for translating tolerance inducing costimulatory blockade regimens from mouse to non-human primates or humans may explained by the following barriers. (1) Non-human primates and humans may have a higher frequency of alloreactive T-cell precursors, because in a mouse model naïve T cells present at high frequency appear not to require costimulation during priming, and fewer proliferation cycles are needed to generate sufficient activated T cells to mediate graft rejection [104]. (2) Non-human primates and humans may have a higher frequency of alloreactive memory T cells, which can derive from prior transplantation, blood transfusion, pregnancy or environmental exposure to pathogens. Pathogen-specific memory T cells may exhibit cross-reactivity with allogeneic peptide: MHC ligands [105], and the memory T cells can possess a lower activation threshold [106]. (3) Infection and tissue damage in the process of organ transplantation may prevent the induction of immune tolerance or break down immune tolerance induced by costimulatory blockade and donor-specific infusion [107]. Thus, a regimen that combines T-cell-reducing reagents with costimulatory blocking reagents that target not only naïve but also memory T cells may be required for induction of transplantation immune tolerance in humans.

Blockade of the CD28/CD80/CD86 pathway by CTLA4-Ig induced immune tolerance of islet or heart transplants in mouse models [108,109], but administration of CTLA-4-Ig only moderately prolonged renal graft survival in non-human primates [110]. LEA29Y, a second-generation CTLA-4-Ig with increased avidity, showed a 10-fold increase in inhibiting T-cell activation in vitro and also significantly prolonged graft survival in non-human primates compared with the original CTLA-4-Ig. LEA29Y also markedly improved graft survival when used as part of a combined immunosuppressive regimen containing either MMF and steroids or anti-IL-2R [111]. Phase II and III clinical trials showed that combination immunotherapy with LEA29Y (belatacept) is effective in reducing immunosuppressants in renal transplantation patients [112].

Administration of humanized anti-CD80/CD80 mAbs significantly prolonged renal graft survival in non-human primates [113], and was also safe in a phase I clinical trial [114], but phase II trials to evaluate efficacy have not been reported. Both antagonistic and agonistic anti-CD28 mAbs augmented the induction of transplantation tolerance in mouse models, although with different mechanisms: the former inhibited T-cell proliferation and the later augmented T-cell apoptosis [115,116]. Unfortunately, agonistic anti-CD28 caused T-cell hyperactivation and a severe cytokine storm in humans [117]. However, an antagonistic anti-CD28 mAb that specifically blocks CD28–CD80/CD86 interaction augmented CTLA-4-mediated immune regulation, expanded Treg cells and significantly prolonged cardiac graft survival when used in combination with the calcineurin inhibitor cyclosporine [118].

Blockade of CD40L–CD40 interactions via anti-CD40L mAb leads to inhibition of DC maturation and T-cell activation [119], augmentation of T-cell secretion of immunomodulatory cytokines and T-cell apoptosis [120], depletion of activated T cells [121], and augmentation of the generation of FoxP3$^+$ Treg cells from conventional CD4$^+$ T cells [122]. Administration of anti-CD40L in combination with CTLA-4-Ig induced tolerance of skin and cardiac grafts in mouse models [123] and prevented rejection of renal grafts in non-human primates [124]. Administration of humanized anti-CD154 alone with an extended course was also able to prevent acute renal allograft rejection in non-human primates [125]. Additionally, administration of CTLA-4-Ig and anti-CD154 in combination with rapamycin but not cyclosporine was able to induce immune tolerance for not only cardiac but also skin grafts in mouse models [126]. Treatment with donor-specific cell transfusions (DSTs) in combination with anti-CD40L markedly prolonged the survival of allo-skin grafts and deleted alloreactive T cells in a CTLA-4-dependent manner [127]. Combination therapy with DST, anti-CD40L, and rapamycin prolonged allo-skin graft survival in non-human primates, which was considered to be one of the most promising costimulatory blockade regimens for augmenting graft survival [128]. Unfortunately, a clinical trial using anti-CD154 mAb was halted following evidence of unanticipated thromboembolic side effects

[129]. Alternatively, humanized anti-CD40 mAbs were developed, and administration of anti-CD40 with CTLA-4-Ig (LEA29Y) significantly prolonged islet allograft survival in non-human primates [130], but no human trial has yet been reported.

The LFA-1/ICAM interaction plays an important role in formation of the immunological synapse, T-cell activation, and migration [131]. LFA-1 blockade attenuated T-cell activation, inhibited T-cell migration into inflammatory sites, and confined effector and regulatory T cells in lymph nodes [132]. Blockade of LFA-1/ICAM interaction via anti-LFA-1 and anti-ICAM-1 was initially reported to induce immune tolerance for cardiac and skin allografts in mice [133]. Anti-LFA-1 treatment had a synergistic effect with anti-CD40L in inducing immune tolerance for hepatocyte allografts [134]. Humanized anti-LFA-1 mAb was able to prolong significantly the survival of cardiac grafts in rhesus monkeys when used with immunosuppressants [135]. More recent reports showed that anti-LFA-1 mAb could target memory T cells and a combination therapy of anti-LFA-1 and CTLA-4-Ig or a combination therapy of anti-LFA-1, anti-IL-2Rα, and sirolimus resulted in long-term survival of islet allografts in rhesus monkeys, although single use of those reagents did not have an effect [136]. The CD2-specific fusion protein alefacept (LFA-3-Ig) that specially eliminates memory T cells in combination with CTLA-4-Ig prevented renal allograft rejection and alloantibody formation in non-human primates [137]. Hence a costimulatory blockade regimen that can both eliminate memory T cells and induce naïve T-cell tolerance may represent a novel approach for induction of organ transplantation tolerance.

Anti-CD4 mAb treatment for induction of transplantation tolerance

Administration of non-depleting anti-CD4 and anti-CD8 mAbs in combination with donor BM and T-cell-depleted spleen cells was initially shown to induce immune tolerance for skin grafts from MHC-matched donors in mouse models [138]. Subsequently, the $CD4^+$ T cells from the primary tolerant recipients were found to be able to tolerize naïve T cells in the adoptive recipients, and this phenomenon was called "infectious tolerance" [139]. The suppressor $CD4^+$ T cells were finally found to be $CD25^{hi}FoxP3^+CD4^+$ Treg cells [47]. On the other hand, administration of depleting anti-CD4 mAb that markedly reduced but not completely eliminated $CD4^+$ T cells in combination with DST was reported to induce immune tolerance for cardiac allografts in fully MHC-mismatched recipients, and the tolerance was associated with the presence of suppressive $CD4^+$ T cells in the recipients [140]. The suppressor $CD4^+$ T cells in the tolerant recipients were also subsequently found to be thymus-derived nTreg cells [141]. Anti-CD4 mAb could selectively expand alloantigen-specific $Foxp3^+$ Treg cells [142]. Clinical translation of either depleting or non-depleting anti-CD4 regimens has not been reported.

Other regimens that can potentially augment induction of transplantation tolerance

Treatment with a non-FcR-binding and non-depleting anti-CD3 mAb was reported to induce immune tolerance for cardiac but not skin allografts in a rat model, and the tolerance induction was associated with induction of Th2 cells and T-cell anergy [143]. A study showed that non-depleting anti-CD3 mAbs selectively depleted activated pathogenic T cells while augmenting nTreg cells with expression of Helio and preserving nTreg cells [144]. Administration of non-depleting anti-CD3 at an appropriate time (~7 days) after transplantation was able to induce immune tolerance for islet allografts [145]. Simultaneous T-cell depletion with ATG and costimulatory blockade with CTLA-4-Ig in combination with rapamycin markedly prolonged skin allograft survival in mouse models, in which effector T cells were reduced and Treg cells were expanded [146]. Treatment of renal transplantation patients with alemtuzumab (anti-CD52, Campath-1H) that depletes T, B, NK, and monocytes in combination with low-dose calcineurin inhibitors could achieve graft acceptance with reduced immunosuppression, which was associated with increased Breg and $Foxp3^+CD4^+$ Treg cells [1,46,147,148].

Although conventional T cells can be induced to express Foxp3 transiently and then become pathogenic T cells in the inflammatory environment, "true" Treg cells that have DNA demethylation of the Foxp3 locus do not become pathogenic T cells [149]. Ex vivo expanded alloantigen-specific nTreg cells are powerful in suppressing rejection of heart allografts in rat models [150], and a multicenter phase I/II clinical trial of cell therapy including expanded nTreg cells, Tr1 cells, myeloid suppressor cells, and tolerogenic DCs in living-donor kidney transplantation is ongoing [151].

Clinical regimens for induction of chimerism and transplantation tolerance

Chimerism is established after donor hematopoietic stem cells (HSCs) and other progenitors engraft in an allogeneic recipient and develop into lympho-hematopoietic cells that persist in the recipient. When a donor stem-cell-derived lympho-hematopoietic system totally replaces that of the host, it is called complete chimerism; and when donor- and host-type lympho-hematopoietic systems coexist in a recipient, it is called mixed chimerism. The major obstacle for donor stem-cell engraftment is the rejection mediated by host T, B, NK, and other cells, and alloreactive host T cells play a dominant role. Mixed and complete chimerism can be induced via allogeneic HCT after myeloablative or non-myeloablative conditioning of the recipient.

Classical allogeneic HCT was designed for the treatment of patients with hematologic malignancies, in which recipients were usually conditioned with myeloablative or non-myeloablative TBI or high-dose chemotherapy. The patients usually developed complete chimerism, and the pre-existing donor-reactive host-type T cells were eliminated by the conditioning TBI and/or the infused donor T cells in the transplants. The de novo development of donor-reactive T cells after HCT is negatively selected by donor-type APCs in the thymus in the absence of GVHD. The infused donor T cells in transplants can mediate GVHD and graft-versus-leukemia effects (GVLEs). HCT regimens have been used not only for the treatment of malignancies in humans, but also for the induction of organ transplantation immune tolerance. Clinical protocols using this approach are summarized below.

Stanford clinical tolerance induction regimen (mixed chimerism)

TLI was originally used as a curative therapy for Hodgkin lymphoma by Kaplan and colleagues at Stanford University Medical Center. In the late 1970s, the non-myeloablative TLI procedure was adapted to laboratory animal studies by Stanford investigators as a conditioning regimen for BM transplantation to achieve mixed chimerism in MHC-mismatched mice and rats. The animals developed mixed chimerism, showed no signs of GVHD, and were tolerant to donor-type skin and heart allografts [152]. This was the first demonstration that stable mixed chimerism could be established in adult laboratory animals.

In order to accommodate the clinical situation of deceased donor organ transplantation, in which timing of the availability of the graft is not predictable, the conditioning regimen was changed to perform the TLI and administration of ATG after organ transplantation was in place [153–156]. Accordingly, the rats or mice were given TLI and ATG right after organ transplantation and an injection of donor BM cells immediately after conditioning[153–156]. The resultant mixed chimeras were tolerant to donor skin and cardiac grafts and showed no signs of GVHD[153–156]. The recipients conditioned with TLI and ATG were highly resistant to GVHD induction when donor spleen cells were added to donor BM cells to induce complete chimerism, as compared with recipients conditioned with TBI [157]. The prevention of GVHD was associated with the enrichment of residual host-type NKT cells that suppress GVHD via their secretion of IL-4 that activated donor-type Treg cells [157]. In addition to central thymic deletion due to the engraftment of donor APCs in the thymus, NKT and Treg cells in the periphery also contribute to donor graft immune tolerance, because NKT-deficient CD1d$^{-/-}$ or Ja18$^{-/-}$ recipients with mixed chimerism still reject donor cardiac grafts [158], and deletion of Treg cells with anti-CD25 mAb also caused donor-graft rejection [159]. Tolerance is dependent on the interaction between the host NKT cells and host Treg cells such that the Treg cells are polarized to IL-10 secretion after exposure to NKT cell derived IL-4 [160]. Similarly, myeloid derived suppressor cells (MDSCs) are required for tolerance induction after interaction with NKT cells in an IL-4 dependent manner [161]. Thus, a network of regulatory cells contributes to prevention of both GVHD and graft rejection in this protocol (Figure 12.1).

The TLI and ATG conditioning regimen was first used in the context of HLA-matched HCT to treat leukemia and lymphoma patients to induce complete chimerism. Consistent with observations with mouse models, the conditioning regimen significantly reduced GVHD but retained anti-tumor (GVT) effects in these patients [162]. The regimen was modified for induction of mixed chimerism in HLA-matched renal transplantation patients. The recipients were given TLI post-transplantation and ATG, starting with an intra-operational infusion. Thereafter, the recipients were infused with granulocyte colony-stimulating factor (G-CSF) mobilized donor CD34^{+} progenitor cells and CD3^{+} T cells. It was found that 17/22 patients developed multilineage mixed chimerism without signs of GVHD; 18 chimeric recipients were withdrawn from immunosuppressive drugs without subsequent rejection episodes. The tolerance was associated with early high ratios of CD4^{+}CD25^{+} Treg and NKT cells versus conventional naïve CD4^{+} T cells; and the T cells from the tolerant recipients showed specific upresponsiveness to donor alloantigens [163,164]. These studies demonstrate that TLI and ATG conditioning can promote the development of persistent mixed chimerism and transplantation immune tolerance in HLA-matched humans. Ongoing studies are applying the approach to haplo-matched or total HLA-mismatched recipients.

MGH clinical tolerance induction regimen (mixed chimerism)

In the early 1980s, Investigators at Massachusetts General Hospital (MGH) used a mixture of T-cell-depleted (TCD) BM cells from both donor and host to induce mixed chimerism in myeloablative TBI-conditioned allogeneic or xenogeneic rodent recipients, and the mixed-chimeric recipients showed specific immune tolerance to the donor-type skin grafts [165]. To avoid the toxicity of myeloablative TBI, the investigators developed a non-myeloablative conditioning regimen consisting of 3 cGy TBI, 700 cGy thymic irradiation, and depleting anti-CD4 and anti-CD8 mAbs; with this conditioning regimen, infusion of donor whole BM cells was able to induce

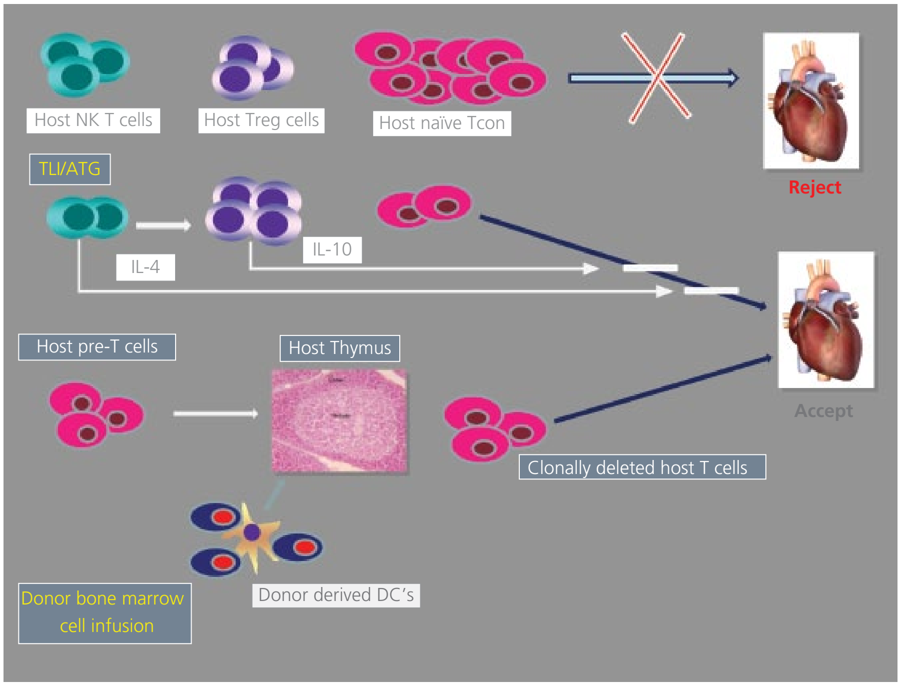

Figure 12.1 Cellular interactions that promote tolerance to combined hematopoietic cell and organ transplants after conditioning with total lymphoid irradiation and antithymocyte globulin. The conditioning regimen alters the balance of T-cell subsets to reduce the number of naïve T cells compared with NKT cells and Treg cells due to differences in the radiosensitivity of the subsets. After conditioning, the host NKT cells secrete IL-4 that activates host Treg cells to secrete IL-10. The NKT cells and the Treg cells and their secretion of these cytokines are required to suppress the ability of the host naïve T cells to reject the donor hematopoietic cells and organ graft. Once the donor hematopoietic cells engraft and establish mixed chimerism, the host T cells and precursors are negatively selected, resulting in clonal deletion against donor alloantigens. Tolerance is promoted by a combination of immune regulation and clonal deletion.

mixed chimerism in fully MHC-mismatched allogeneic mouse recipients, and the recipients developed tolerance to donor skin graft [166]. Intra-thymic deletion was the major mechanism maintaining donor-specific tolerance in this model [167,168]. The investigators developed an even less toxic conditioning regimen in mice, using costimulatory blockade of anti-CD40L and CTLA-4-Ig to replace thymic irradiation and depleting antibodies [16]. In this regimen, costimulatory blockade and infusion of donor BM cells mediated early tolerization of the pre-existing donor-reactive $CD4^+$ and $CD8^+$ T cells to allow the establishment of mixed chimerism [169,170]. The long-term tolerance was also maintained by the intrathymic deletion in the mixed chimeras. Other studies found that host-type Treg cells also contributed to induction and maintenance of tolerance in the recipients with mixed chimerism established by costimulatory blockade regimens [171].

The modified laboratory animal conditioning regimen was applied to humans for induction of mixed chimerism and renal transplantation immune tolerance. First, patients with multiple myeloma and end-stage renal disease were conditioned with cyclophosphamide, equine ATG, and thymic irradiation, and then transplanted with BM and kidney from HLA-matched donors. Sixty days after transplantation, the patients were given donor lymphocyte infusion. Seven patients developed chimerism, and three patients had long-term normal or near- normal renal function after withdrawal of immunosuppressants; the mixed chimeras showed no signs of GVHD but the complete chimeras showed signs of chronic GVHD that required immunosuppressive drug therapy [172]. The conditioning regimen was further modified for induction of renal transplantation tolerance in non-tumor HLA haplotype-matched patients with end-stage renal disease. Patients were conditioned with cyclophosphamide, anti-CD2 mAb and thymic irradiation, and then transplanted with donor BM and renal grafts. Despite only transient mixed chimerism in the first 5 patients, kidney graft acceptance without immunosuppression was observed in four patients and one patient had early graft loss due to humoral rejection. The graft acceptance was associated with donor-specific unresponsiveness in proliferative and cytotoxic assays in addition to an increased proportion of $Foxp3^+CD4^+$ Treg cells [173,174]. However, a follow-up study with a total of 10 patients showed 2/10 with early graft loss and another with chronic rejection, and 9/10 patients with engraftment syndromes with marked transient graft dysfunction. Although immunosuppressive drugs were discontinues in seven patients, graft injury limits this approach [175].

Northwestern clinical tolerance induction regimen (complete chimerism)

Complete chimerism induced in NOD mice with myeloablative conditioning and transplantation of MHC-mismatched donor T cell-depleted BM or BM stem cells provided immune tolerance to donor islet grafts without causing GVHD [176]. Complete chimerism using a non-myeloablative conditioning regimen has been reported to induce renal transplant acceptance without causing GVHD in five HLA-mismatched patients who were conditioned with two doses of cyclophosphamide, 200 cGy TBI, and three doses of fludarabine, followed by injection of donor BM, facilitating cells, and low-dose donor T cells [177]. Post-transplantation immunosuppression was with tacrolimus and mycophenolate mofetil. The patients with complete chimerism were completely withdrawn from immunosuppressive drugs without subsequent rejection [177]. The stable chimerism was induced in about half of the patients, and the rest lost chimerism [178,179]. However, there is concern about the immune function of full HLA-mismatched complete chimeras, as one of the five recipients suffered a near-lethal infectious complication associated with loss of renal graft [177].

In addition, a complete replacement of the host-type lymphohematopoietic system with the donor-type cells is associated with a considerable risk of acute or chronic GVHD. The acceptance of donor organ transplants by complete donor chimeras is not an example of host-versus-donor immune tolerance, since host immune cells are not present. Mixed chimeras can still generate donor-reactive host-type T cells in the thymus, and mixed chimerism can mediate both central and peripheral tolerance of donor-reactive T cells. The donor-derived APCs mediate thymic negative selection of the newly developed donor-reactive host-type T cells after transplantation, and Treg and NKT cells can control the residual donor-reactive T cells in the periphery [17,158]. In the mixed chimeras established with myeloablative TBI conditioning and infusion of both donor- and host-type TCD BM cells, thymic deletion is sufficient for prevention of rejection of donor organ transplants [168]. In the mixed chimeras established with non-myeloablative conditioning regimen such as TLI or costimulatory blockade, in addition to central deletion, host- and donor-type regulatory T cells such as NKT and Treg cells in the periphery are also required to prevent residual host peripheral T cells from rejecting the donor organ transplants [158,180]. This deletional and regulatory tolerance provided by mixed chimerism is particularly robust and allows acceptance of highly immunogenic primary skin or small bowel grafts across the most extensive histocompatibility barriers [17].

Radiation-free regimen for induction of mixed chimerism

Low-dose TBI, TLI, or thymic irradiation was used in the Stanford, MGH, and Northwestern conditioning regimens for induction of chimerism and tolerance as discussed above. Investigators are also searching for a radiation-free conditioning regimen to reduce side effects for induction of mixed chimerism. Below is a summary of major radiation-free conditioning regimens:

1. *Costimulatory blockade-based regimens:* Wekerle et al. reported that injection of mega-doses of donor BM cells and administration of anti-CD40L and CTLA-4-Ig were able to induce stable mixed chimerism and donor-specific immune tolerance in MHC-mismatched non-autoimmune mouse recipients without cytoreductive conditioning. Infusion of host-type $FoxP3^+CD4^+$ Treg cells was able to augment donor cell engraftment and reduce the required donor BM cell dose [181,182]. This regimen has not been attempted in autoimmune mice or large animal models.
2. *Anti-CD3/CD8 mAb-based conditioning regimens:* Pre-existing alloreactive memory T cells in autoimmune mice and also in laboratory large animals and humans are resistant to tolerance induction by costimulatory blockade and remain a major obstacle for induction of mixed chimerism. Donor $CD8^+$ T cells play a major role in facilitating donor stem-cell engraftment and are less powerful in inducing GVHD than $CD4^+$ T cells [74,183,184]. In addition, $CD4^+$ T cells can interfere with donor stem-cell engraftment [184]. Tissue damage caused by TBI- or high-dose chemotherapy-conditioning plays a major role in initiating GVHD [185,186]. Therefore, investigators at the City of Hope National Medical Center designed a radiation-free anti-CD3/CD8 mAb conditioning regimen followed by the infusion of donor BM and $CD4^+$ T-depleted spleen cells for induction of chimerism in autoimmune mice.

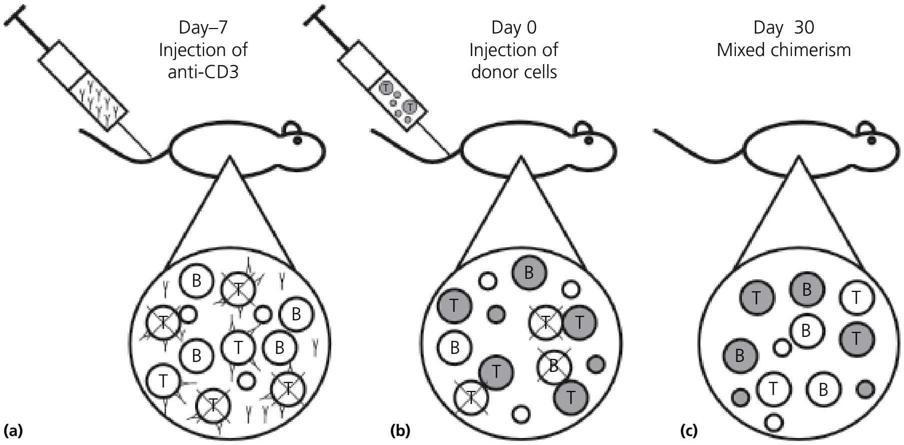

Figure 12.2 Anti-CD3/CD8 conditioning for induction of mixed chimerism. Recipient mice are injected with anti-CD3 and anti-CD8 mAbs to deplete a majority of recipient T cells, and the remainder of surviving T cells downregulate their expression of the T-cell receptor (a). Seven days after conditioning, the injected antibodies have been cleared from the system and donor T cells and stem cells (shaded cells) are injected into recipient mice. The donor T cells are activated and kill the disarmed residual host T cells that express little TCRs to prevent rejection of donor stem cells and facilitate engraftment without inducing (b). By 30 days post-HCT, the newly developed donor- and host-type T cells from progenitors begin to repopulate the system and establish mixed chimerism (c).

As depicted in Figure 12.2, recipient mice were conditioned with anti-CD3/CD8 mAbs to deplete host T cells partially and downregulate the TCR–CD3 complex on residual host T cells. The host T cells did not re-express their TCRs until ~10 days after antibody injection. Infusion of donor BM and CD4$^+$ T-depleted spleen cells (CD8$^+$ T cells) at 7 days after antibody injection avoided the negative impact of anti-CD3/CD8 mAb on donor CD8$^+$ T cells, because antibodies were undetectable at that time. The latter cells can kill the disarmed host T cells to facilitate donor cell engraftment. Under this radiation-free ant-CD3/CD8-conditioning regimen, both non-autoimmune and autoimmune mice developed mixed and complete chimerism depending on the dose of infused donor CD4$^+$ T-depleted spleen cells, and neither mixed nor complete chimeras showed any signs of GVHD [102,187]. The mixed chimerism with MHC-mismatched donor BM was able not only to tolerize alloimmunity and provide long-term donor-specific tolerance in non-autoimmune recipients but also to tolerize autoreactive T cells and reversed autoimmunity in mice with type 1 diabetes and also in mice with systemic lupus [188,189].The GVHD prevention by the radiation-free anti-CD3/CD8 conditioning regimen is explained by multiple mechanisms, including (1) avoidance of tissue damage caused by TBI or high-dose chemotherapy; (2) depletion of CCR7$^+$ DCs in draining lymph nodes that imprint donor T-cell tissue homing and chemokine receptors and also reduction of GVHD target tissue release of chemokines, such that donor T cells were prevented from migration into GVHD target tissues [190,191]; (3) maintenance of a high percentage of NKT and Treg cells among residual host T cells that can prevent GVHD [102]; (4) maintenance of tissue expression of protective molecules such as B7-H1 (PD-L1), so that infiltrating T cells can be tolerized [192]; and (5) conversion of injected CD8$^+$ T cells into regulatory T cells such as FoxP3$^+$CD8$^+$ T cells in the long-term chimeras [187].

The major obstacle for translating this radiation-free anti-CD3/CD8 mAb conditioning regimen into clinical application is the cytokine storm triggered by FcR-binding intact anti-CD3 [193]. Although conditioning with FcR-non-binding anti-CD3 mAbs that do not cause a cytokine storm failed to facilitate induction of mixed chimerism, sequential injection of FcR-non-binding and FcR-binding anti-CD3 was able to avoid a cytokine storm and facilitate the induction of mixed chimerism in late-stage diabetic non-obese diabetic (NOD) mice and re-establish central and peripheral tolerance [194–197].

Summary and future directions

Seventy years after Medawar and colleagues' first report about induction of transplantation tolerance in mice, induction of chimerism and transplantation immune tolerance in adult humans has finally been realized in small numbers of renal transplantation patients who received donor hematopoietic cell grafts [163,164,173,174,177,178]. In order to promote the wider application of induction of chimerism and transplantation tolerance, much more work needs to be done, including expansion to all live or deceased donor transplants of a variety of organs. Simpler and less toxic procedures such as radiation-free clinical tolerance regimens and also those that do not require chimerism are likely to be tested in the future.

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CHAPTER 13

The Pathophysiology of Graft-versus-Host Disease

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Acute GVHD pathophysiology: a three-step model

Acute graft-versus-host disease (GVHD) results from complex interactions between donor T cells and host tissues in an inflammatory milieu. The pathophysiology of acute GVHD can be considered as a three-step process involving both the innate and adaptive immune systems (Figure 13.1). The three steps are (1) tissue damage in the recipient by the conditioning regimen, (2) donor T-cell activation and clonal expansion, and (3) cellular and inflammatory factors. Intrinsic to this schema is the role of mononuclear phagocytes and other accessory cells in complex interactions with cytokines secreted by activated donor T cells.

In *step 1*, the conditioning regimen leads to damage and activation of host tissues throughout the body. The inflammatory cytokines and chemokines enhance donor T-cell recognition of host alloantigens by increasing the expression of major histocompatibility complex (MHC) antigens, adhesion receptors, and other molecules on host antigen-presenting cells (APCs). An important aspect of the gastrointestinal (GI) mucosal injury is the resultant leakage of endotoxins and pathogen-associated molecular products (PAMPs) into the circulation, resulting in activation of innate immune cells through Toll-like receptors (TLRs).

In *step 2*, host and most likely donor APCs present alloantigen in the form of a peptide–human leukocyte antigen (HLA) complex to the resting T cells. Costimulatory signals are required for T-cell activation, and these signals further activate APCs and enhance T-cell stimulation. T-cell activation is characterized by cellular proliferation and the secretion of cytokines. Regulatory T (Treg) cells that can act as a powerful brake on the graft-versus-host (GVH) reaction also expand at this time.

In *step 3*, effector functions of mononuclear phagocytes, natural killer (NK) cells, and neutrophils are triggered through a secondary signal provided by mediators, such as lipopolysaccharide (LPS) that leak through the damaged intestinal mucosa. Release of inflammatory cytokines stimulates host tissues to produce inflammatory chemokines that direct effector cells into target organs. This mechanism amplifies local tissue injury and promotes an inflammatory response which leads to target tissue destruction.

Step 1: effects of conditioning

Prior to hematopoietic cell transplantation (HCT), a patient's tissues have been damaged by underlying disease and its treatment, infection, and transplant conditioning. Conditioning regimens activate host APCs that are critical to the stimulation of donor T cells infused in the stem-cell inoculum. This process helps explain a number of unique and seemingly unrelated aspects of GVHD. For example, the risk of GVHD increases with advanced-stage leukemia, certain intensive conditioning regimens, and histories of viral infections [1]. Total body irradiation (TBI) is particularly important in this process because it activates host tissues to secrete cytokines, such as tumor necrosis factor- α (TNF-α) and interleukin-1 (IL-1) [2], and it induces endothelial and epithelial cell damage in the GI tract [3]. Further damage by GVHD effectors amplifies GI tract and systemic GVHD by allowing the translocation of LPS and PAMPs into the systemic circulation. The relationship between conditioning intensity, inflammatory cytokines, and GVHD severity has been confirmed by animal models [4] and clinical observations [1].

Step 2: Donor T-cell activation and cytokine secretion

Donor T-cell activation

Murine studies have demonstrated that host APCs alone are both necessary and sufficient to stimulate donor T cells [5]. Donor T cells migrate to secondary lymphoid organs, such as Peyer's patches, lymph nodes, and the spleen, where they encounter host APCs [6]. Immediately after HCT, both host- and donor-derived APCs are present in secondary lymphoid organs. Donor T-cell receptors (TCRs) can recognize alloantigens on either host APCs (direct presentation) or donor APCs (indirect presentation). During direct presentation, donor T cells recognize either the minor histocompatibility antigens (mHAs) bound to allogeneic MHC molecules or the foreign MHC molecules themselves. During indirect presentation, T cells respond to allogeneic peptides generated by degradation of host proteins that are then presented by donor MHCs [7]. In GVHD to mHAs, direct presentation is dominant because APCs derived from the host rather than from the donor are critical [8]. Several laboratories have shown that naïve ($CD62L^+$) T cells cause experimental GVHD whereas memory ($CD62L^-$) T cells do not, although memory stem cells may be involved [9].

TCR signaling after antigen recognition induces a conformational change in adhesion molecules, resulting in higher-affinity binding to the APCs [10]. Full T-cell activation also requires costimulatory signals provided by APCs. Currently, there are four known CD28

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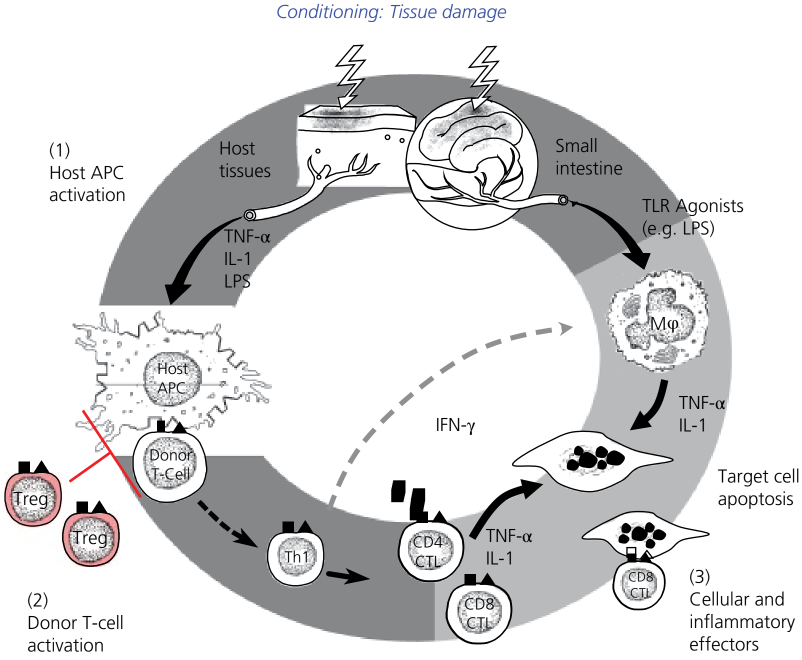

Figure 13.1 The pathophysiology of a acute graft-versus-host disease (GVHD). GVHD pathophysiology can be summarized in a three-step process.

superfamily members expressed on T cells: CD28, cytotoxic T-lymphocyte antigen 4 (CTLA-4), inducible costimulator, and programmed death-1 (PD-1). In addition, there are four TNF receptor family members: CD40 ligand (CD154), 4–1BB (CD137), OX40, and herpes simplex virus glycoprotein D or herpes virus entry mediator. The best-characterized costimulatory molecules, CD80 and CD86, deliver positive signals through CD28 that lower the threshold for T-cell activation and promote T-cell differentiation and survival, whereas signaling through CTLA-4 is inhibitory [11].

The most potent APCs are dendritic cells (DCs); however, the relative contribution to the development of GVHD of DCs and other semiprofessional APCs, such as monocytes/macrophages and B cells, remains to be elucidated. Donor APCs may also amplify GVHD, but in the skin, Langerhans cells of host origin appear to be essential for the activation of donor T cells [11]. Signaling through TLRs and through other innate immune receptors such as non-obese diabetic (NOD) receptors may act as "danger signals" and activate host APCs, amplifying the donor T-cell response [12]. Release of damage-associated molecular patterns (DAMPs) activates the inflammasome, a multicomponent multimer that leads to the activation of the caspase 1 cascade. Agents that mediate epigenetic changes, such as the histone deacetylase inhibitor vorinostat, may also suppress T-cell activation through reducing the DC response [13].

Cytokine secretion by donor T cells

Several factors influence the ability of DCs to instruct naïve CD4$^+$ T cells to secrete cytokines. These factors include the type and duration of DC activation along with the DC:T-cell ratio and the proportions of DC subsets present during T-cell interactions [14]. IL-2 has a pivotal role in both controlling and amplifying the immune response against alloantigens. The addition of low doses of IL-2 during the first week after allogeneic bone marrow transplantation can enhance the severity and mortality of GVHD [15]. Monoclonal antibodies (mAbs) against IL-2 or its receptor can prevent GVHD when administered shortly after the infusion of T cells [16], but this strategy was only moderately successful in reducing the incidence of severe GVHD [17]. IL-15 is another critical cytokine in initiating allogeneic T-cell division in vivo [18], and elevated serum levels of IL-15 are associated with acute GVHD in humans [19].

Increased levels of IFN-γ are associated with acute GVHD [20], and a large proportion of T-cell clones isolated from GVHD patients produce IFN-γ [21]. Cytotoxic T-lymphocytes (CTLs) that are specific for mHAs and produce IFN-γ also correlate with the severity of GVH reaction in a skin-explant assay of human disease [22]. IFN-γ enhances antigen presentation and recruits effector cells into target organs [23]. Concomitantly, IFN-γ alters target cells so that they are more vulnerable to damage during GVHD. IFN-γ also mediates GVHD-associated immunosuppression seen in several experimental HCT systems in part by the induction of nitric oxide (NO) [24]. At early time points after HCT, IFN-γ may paradoxically reduce GVHD by enhancing Fas-mediated apoptosis of activated donor T cells [25]. High doses of exogenous IL-2 early after bone marrow transplantation protect animals from GVHD mortality [26]. It has been suggested that IL-2 mediates its protective effect via inhibition of IFN-γ [20]. Such paradoxes may be explained by the complex dynamics of donor T-cell activation, expansion, and contraction. The complex role of various cytokines in mediating GVHD damage has recently been reviewed [27].

Regulatory T cells

In both humans and mice, Treg deficiency results in immune dysregulation and autoimmunity (see Chapter 14]. The IPEX syndrome in humans results from loss of function mutations of *FOXP3* [28], and scurfy mice lack the transcription factor FOXP3 [29], resulting in an analogous syndrome. *FOXP3* appears to function as a master control gene for the development and function of natural Treg cell, which normally constitute approximately 5–10% of the circulating CD4$^+$ T-cell population. Treg cell suppress both innate and adaptive immune functions [30] by producing inhibitory cytokines (IL-10 and transforming growth factor-beta (TGF-β) and also by cell contact-dependent inhibition of APC function [30]. Addition of purified CD4$^+$CD25$^+$ Treg populations can suppress the proliferation of conventional T cells and prevent GVHD [31]. The ability of extracorporeal photopheresis to reduce experimental GVHD depends on its induction of Treg cell [32]. The use of calcineurin inhibitors and thymic injury from GVHD may interfere with the development of adequate numbers of Treg cells that can control GVHD [33]. Another regulatory population consists of NK1.1$^+$ T cells. Both peripheral blood and marrow NK1.1$^+$ T cells can prevent GVHD by their IL-4 secretion [34].

Step 3: cellular and inflammatory effectors

The pathophysiology of acute GVHD culminates in the generation of multiple cytotoxic effectors that contribute to target tissue injury. Extensive experimental and clinical data suggest that soluble inflammatory mediators act in conjunction with direct cell-mediated cytolysis by CTL and NK cells to cause the full spectrum of damage. As such, the effector phase of GVHD involves aspects of both the innate and adaptive immune response, and the synergistic interactions of components generated during steps 1 and 2.

Cellular effectors

Following recognition of a target cell through TCR–MHC interaction, CTLs insert perforin into the target cell membrane, forming “perforin pores” that allow granzymes to enter the cell and induce apoptosis through various downstream effector pathways [35]. Ligation of Fas results in the formation of the death-inducing signaling complex and the subsequent activation of caspases [36]. A number of ligands on T cells also possess the capability to trimerize TNF-α receptor (TNFR)-like death receptors (DRs) on their targets, such as TNF-related apoptosis-inducing ligand (TRAIL:DR4,5 ligand) and TNF-like weak inducer of apoptosis (TWEAK:DR3 ligand) [37].

Lethal GVHD occurs even in the *absence* of perforin-dependent killing, demonstrating that the perforin/granzyme pathway plays a significant, but not exclusive, role in target organ damage. CD4$^+$ CTLs preferentially use the Fas/FasL pathway during acute GVHD, whereas CD8$^+$ CTLs primarily use the perforin/granzyme pathway, consistent with other conditions involving cell-mediated cytolysis [11].

Inflammatory cytokines such as IFN-γ and TNF-α increase the expression of Fas, a TNF-receptor family member, during GVHD [38]. FasL expression on donor T cells is also increased during GVHD [39]. Elevated serum levels of soluble FasL and Fas have also been observed in some patients with acute GVHD [40]. FasL-defective donor T cells cause markedly reduced experimental GVHD in liver, skin, and lymphoid organs [41]. The Fas/FasL pathway is particularly important in hepatic GVHD [42].

Inflammatory effectors

A central role for inflammatory cytokines, the effector phase, in acute GVHD was confirmed by murine studies using bone marrow chimeras in which either MHC class I or class II alloantigens were not expressed on target epithelium but on APCs alone [5]. GVHD target organ injury was induced in these chimeras even in the absence of epithelial alloantigens, and mortality and target organ injury were prevented by the neutralization of TNF-α and IL-1.

Severe GVHD is associated with high levels of TNF-α messenger RNA in target tissues [43]. Target organ damage could be inhibited by infusion of anti-TNF-α mAbs, and mortality could be reduced by the administration of the soluble form of the TNF-α receptor [2]. Extensive experimental data further suggest that TNF-α is an important effector molecule of GVHD [5,43,44].

Secretion of IL-1 appears to occur predominantly during the effector phase of GVHD in the spleen and skin, two major GVHD target organs. However, use of IL-1 receptor antagonist shortly after conditioning was not successful in preventing acute GVHD in a randomized trial [45]. It is likely that redundancy of inflammatory pathways bypasses the direct inhibition of IL-1 or TNF-α, reducing the clinical benefit of targeting single cytokines to prevent clinical acute GVHD.

Finally, macrophages can also produce significant amounts of NO that contribute to target tissue damage and to GVHD-induced immunosuppression [24]. NO inhibits the proliferation of epithelial stem cells in the gut and skin, and the development of GVHD is preceded by an increase in NO oxidation products in the serum [46].

Toll-like receptors (TLRs) and innate immunity

TLRs on primed mononuclear cells may be stimulated to secrete cytokines by LPS and PAMPs that leak through an intestinal mucosa damaged by conditioning regimens during step 1. The simultaneous release of DAMPs from conditioning- related injury also probably intensifies the inflammatory signals. Damage to the GI tract during the effector phase can lead to a positive feedback loop wherein increased translocation results in further cytokine production and progressive intestinal injury, propagating the “cytokine storm” characteristic of acute GVHD. Evidence suggests that damage to the GI tract during acute GVHD plays a major role in the amplification of systemic disease [47].

This conceptual framework underscores the role of the GI microbiome in the development of acute GVHD [48]. LPS is a major structural component of Gram-negative bacteria and is a potent stimulator of cellular activation and can elicit a broad range of inflammatory responses. In particular, LPS may stimulate gut-associated lymphocytes and macrophages. Following allogeneic HCT, LPS accumulates in both the liver and spleen of animals with GVHD prior to its appearance in the systemic circulation. Elevated serum levels of LPS have been shown to correlate directly with the degree of intestinal histopathology occurring after allogeneic HCT [49]. Death from GVHD can be prevented if transplanted mice are given antibiotics to decontaminate the gut or housed in a germ-free environment [50]. Molecules that act as competitive inhibitors to LPS at the cell surface and block nuclear factor-kappa B (NFκB) activation and nuclear translocation block the biologic response to LPS and result in decreased inflammatory cytokine release and reduced GVHD severity while preserving potent graft-versus-leukemia effects (GVL) and improved leukemia-free survival [51].

Traffic of cellular effectors

Regulation of effector cell migration into GVHD target tissues occurs in a complex milieu of chemotactic signals that recruit effector cells, such as T cells, neutrophils, and monocytes [52]. Upon stimulation, T cells can rapidly switch chemokine receptor expression, acquiring new migratory capacity [53]. Macrophage inhibitory protein-1 alpha recruits CCR5$^+$CD8$^+$ T cells into the liver, lung, and spleen during GVHD [54], and levels of several chemokines are elevated in GVHD-associated lung injury [55]. After T-cell activation by DCs, a profile of adhesion molecules and chemokine receptors direct the cells back to the organ of DC origin. For example, activated T cells that leave a lymph node in the skin express cutaneous lymphocyte antigen [56]. T cells expressing cutaneous lymphocyte antigen home only to the skin, where they may mediate cutaneous GVHD. The addition of the

CCR5 antagonist maraviroc to standard GVHD prophylaxis dramatically reduced GI GVHD in a small phase II study [57]. Similarly, α4β7 is necessary for homing to GI tract- and gut-associated lymphoid tissues [58]. Thus, cells bearing the appropriate cell surface adhesion receptors for the GI tract (e.g. LPAM-1) and chemokine receptors (e.g. CCR25) undergo firm adhesion followed by transmigration through the blood vessel into the appropriate organ. Mesenchymal stromal cells also appear to reduce severe GI GVHD, but their mechanism of action remains unclear [59].

GVHD prevention: from animal models to clinical practice

The development of strategies for GVHD prophylaxis and therapy has suffered from a limited understanding of the underlying pathophysiology of GVHD. This conceptual limitation led to approaches that were based on an "oncologic" model of GVHD, i.e. the notion that since GVHD is dangerous and life threatening, management requires the utilization of high-dose immunosuppressant regimens. High doses of multiple immunosuppressive agents have been used much like we use combination chemotherapy to treat malignancies. Although there is often reasonable control of the clinical manifestations of GVHD, this "shotgun" approach lacks elegance and is usually associated with opportunistic infections, lymphoproliferative disorders, or relapse of the underlying malignancy. Indeed, it is often infection while GVHD is in remission that results in mortality. The objective should be to reduce the non-specific tissue damage associated with GVHD while allowing effective immunologic recovery.

The three-phase model described above can be simply summarized. GVHD is an exaggerated and dysregulated response of a normal immune system (that of the donor) to tissue damage that is intrinsic to transplantation. The donor's immune system reacts as if there is a massive and uncontrolled infection, and its efforts to deal with this injury result in the clinical manifestations of GVHD. It is possible that the tissue injury intrinsic to the administration of high-dose chemoradiotherapy initiates the breakdown of mucosal barriers, allowing LPS and PAMPs into the tissues and also DAMPs that stimulate the inflammasome. TLRs on DCs bind to endotoxin and activate signal transduction pathways that lead to DC maturation and induce inflammation [60]. The upregulation of costimulatory molecules, MHC molecules, adhesion molecules, cytokines, chemokines, prostanoids, and other inflammatory mediators prime and trigger the attack on target tissues, fueling the fire. Thus, it is likely that components of cytokine storm [61], the danger hypothesis [12], and more traditional notions of adaptive and innate immunity [62] all apply. Moreover, with our improved understanding of inhibitory pathways that regulate and suppress inflammation, it may be possible to enhance regulatory mechanisms rather than attempt to engender immunologic paralysis. Some examples of the targeting of the various stages of GVHD are shown in Table 13.1.

Table 13.1 Examples of approaches to prevent or treat GVHD

Step	Potential intervention[a]
Step 1: Effects of conditioning	Reduced intensity conditioning Use of mAbs or other non-cytotoxic agents
Step 2: Donor T-cell activation and cytokine production	Gut decontamination and antiviral prophylaxis Corticosteroids Calcineurin inhibitors Mycophenolate, methotrexate, cyclophosphamide MTOR inhibition (sirolimus) Proteasome inhibition (bortezomib) Inhibition of costimulation Clinical use of Treg cell (low-dose IL-2) HDAC inhibitors (vorinostat) Extracorporeal photopheresis
Step 3: Cellular and inflammatory effectors	T-cell depletion (in vivo and in vitro) Cytokine inhibition (etanercept, infliximab, anti-BAFF) CCR5 inhibition (maraviroc) Tyrosine kinase inhibitors (e.g., PDGFRα inhibition)

[a] Many of the interventions listed span steps 2 and 3.

Step 1: reduced-intensity conditioning regimens

The three cardinal organs affected by GVHD are the skin, intestinal tract, and liver. The common thread would seem to be exposure to the environment. Skin and gut have very obvious barrier functions and a well-developed reticuloendothelial system. DCs resident in these organs present antigens to naïve T cells, which then have alterations in cell surface characteristics that cause them to return to the organ that originated the specific DC. Similarly, the liver is the first line of defense downstream of the gut. One would expect the lung to be a similar target; however, its less intense exposure to organisms, particularly Gram-negative rods, probably reduces its primacy as a target organ. Nevertheless, recent studies clearly demonstrate pulmonary injury that is likely to reflect GVHD. All of these organs are rich in DCs, a probable prerequisite for generating the injurious cytokines in addition to directing the attack of the adaptive immune system. They are all subject to injury from conditioning, and breaches of the barrier will allow organisms or endotoxin into the circulation.

One prediction of this model is that less-intense conditioning regimens would be associated with less GVHD. This effect was initially observed after donor lymphocyte infusions. The T cells were administered *without* pharmacologic GVHD prophylaxis, and the resultant GVHD was less frequent and severe than would be expected in the absence of immunosuppressive drugs. It could be inferred that reduced-intensity conditioning regimens might similarly be associated with less severe GVHD, both through less cellular injury per se and through a reduction in endotoxin exposure by virtue of less mucosal injury. Available data suggest that the rate of GVHD after reduced-intensity conditioning regimens is similar to the rate seen after conventional dose conditioning [63]. Interestingly, the onset of GVHD is delayed and an overlap syndrome of acute and chronic graft-versus-host disease (cGVHD) is more prevalent. This observation suggests that the initial tissue injury of high-dose regimens speeds the development of GVHD and reduced-intensity procedures seem to have little effect on cGVHD. Thus, while data on both reducing colonization with bacteria and reducing tissue injury with less-intense regimens are suggestive and support the basic concepts described above, the effect is insufficient to control GVHD adequately in human transplantation.

Step 2: modulation of donor T cells

Reduced T-cell numbers

The advent of monoclonal technology in the 1980s led to the logical conclusion that if a pharmacologic reduction in T-cell numbers could partially control GVHD, albeit at the risk of drug-induced toxicity, then eliminating the cells with mAbs, immunotoxins, lectins, CD34 columns, or physical techniques might be more effective [64]. Furthermore, the ability to identify and target specific T-cell subsets opened up the possibility that the graft could be engineered to control GVHD while allowing immunologic recovery as well as GVL. A typical unmanipulated marrow transplant entails the infusion of about 10^7 T cells/kg of recipient. Establishment of a clear dose–response relationship is difficult since the risk of acute GVHD depends on factors beyond simple dosing, for example, minor histocompatibility disparities, virus exposure, disease and stage, donor age, and recipient age. Studies of counterflow elutriation showed that 10^6 T cells/kg plus cyclosporine (CSP) resulted in a GVHD rate similar to that of CSP alone, but 5×10^5 T cells/kg plus CSP resulted in an overall rate of 22%, and only limited skin involvement was observed [65]. After lectin depletion of T cells in the absence of supplemental immunosuppression, a T-cell dose of around 10^5/kg was associated with complete control of GVHD [66]. More recently, the combination of very high stem cell numbers and CD3 T-cell numbers of less than 3×10^4/kg allowed haploidentical transplantation without GVHD [67].

Global T-cell depletion (TCD) using a broad panel of mAbs has largely been abandoned because of the risk of graft failure [68]. However, it is clear that even depletion of T-cell subsets must be undertaken with caution. Depletion of $CD4^+$ or $CD8^+$ T cells alone can be associated with a high graft failure rate [69]. However, depletion of $CD5^+$ or $CD6^+$ T cells seems to be associated with a low graft failure rate [70]. The presumption is that radioresistant T cells that survive the initial conditioning are responsible for graft rejection. When the stem-cell source is rich in T cells, the GVH reaction will further reduce the residual population capable of alloreactivity, thus decreasing graft rejection. To some extent, the higher graft failure rates may be controlled by increasing the intensity of the conditioning regimen [71], adding back T cells [65], or with additional immunosuppressants [72]. Additional problems associated with TCD include a higher incidence of Epstein–Barr virus-induced lymphoproliferative disorders, loss of the GVL with a consequent increase in relapses, and slower immunologic recovery due to reduced passive transfer of immunocompetent donor T cells. These concerns also have been approached by increasing the conditioning intensity, delayed T-cell add-back [73], rituximab [74], or the infusion of specific T-cell lines [75]. However, in no case has there been an improvement in survival that can be definitively attributed to TCD. A large multicenter study of unrelated donor HCT similarly showed no improvement in outcomes after TCD [76]. Although not confirmed in a randomized trial, in early-remission acute leukemia, CD34 selection and an intensified conditioning regimen appear to result in similar survival to conventional GVHD prophylaxis with less cGVHD [77,78]. Both antithymocyte globulin (ATG) and alemtuzumab deplete T cells in vivo. ATG appeared in some studies to reduce both acute and cGVHD [79]. Alemtuzumab also reduces acute and cGVHD but at the expense of high relapse rates and graft failure [80]. An observational database analysis of the role of in vivo TCD demonstrated inferior outcomes in reduced-intensity conditioning regimens [81]. However, a randomized trial in high-intensity conditioning HCT reported similar survival but substantially reduced cGVHD in patients treated with ATG [82,83]. Hence the role of TCD, whether in vivo or in vitro, remains to be fully elucidated.

Reduced T-cell activation

Calcineurin inhibition with either CSP or tacrolimus is the current standard for GVHD prevention. Both drugs bind to immunophilins: CSP binds cyclophilin and tacrolimus binds FKBP12. The complex of drug and binding protein, calcium, calmodulin, and calcineurin is then formed, and the phosphatase activity of calcineurin is inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines such as IL-2 and IFN-γ. The net result is the inhibition of T-cell activation. As a single agent, CSP was about as effective as methotrexate (MTX) [84]. However, in combination with MTX, there was a significant reduction in the incidence of GVHD and an improvement in survival [85]. Subsequent trials of tacrolimus and MTX compared with CSP and MTX showed no advantage for either combination [86]. DCs appear to be critical to the development of acute GVHD [8]; therefore, interference with DC function would probably prevent GVHD. The goal would be to inhibit function selectively or temporarily until transplanted DC precursors had engrafted and become functional. Alemtuzumab is a promiscuous antibody, targeting B cells, T cells, monocytes, and DCs. When the drug was administered prior to HCT, host DCs were selectively depleted and the donor DCs repopulated the recipient [87]. These observations are difficult to separate from concomitant TCD, but they may be consistent with the theme that facilitation of DC turnover may ameliorate GVHD.

Reduced T-cell proliferation

The first generally prescribed GVHD preventive regimen was the administration of intermittent low-dose MTX as developed in a dog model by Thomas and colleagues [88]. Another regimen developed by Santos et al., based on cyclophosphamide (CTX) [89], was never widely adopted. The principle of this approach is to administer a cell-cycle-specific chemotherapeutic agent intermittently immediately after the transplant, when the T cells will have started to divide after exposure to allogeneic antigens. This was an effective approach compared with no prophylaxis [90]; however, GVHD control was incomplete. Subsequently, the addition of ATG or prednisone, or both, resulted in an incremental improvement in the GVHD rate but no improvement in survival [91]. Ultimately, the course of MTX was abbreviated and combined with CSP or tacrolimus, resulting in the current gold standard for GVHD prevention. More recently, it has been shown that high-dose CTX can be substituted for MTX after stem-cell infusion. Stem cells are insensitive to CTX but activated T cells are eradicated. This approach seems to be effective in combination with tacrolimus and microphenolate mofetil (MMF) and appears to facilitate HCT across HLA barriers [92]. The use of CTX may also reduce cGVHD [92,93].

MMF is another useful agent that may prevent GVHD but with less mucositis and myelotoxicity than MTX. It is the prodrug of mycophenolic acid (MPA), a selective inhibitor of inosine monophosphate dehydrogenase, and inhibits the de novo pathway of guanosine nucleotide synthesis. Since T-lymphocytes are dependent on the de novo synthesis of purines, MPA inhibits the proliferative responses of T cells to allogeneic stimulation. Myeloid and mucosal cells can utilize salvage pathways, so the drug is less toxic to mucosa and myeloid recovery. MPA also prevents the glycosylation of glycoproteins that are involved in adhesion to endothelium and may reduce the recruitment of leukocytes into inflammatory sites. MPA does not inhibit the activation of T cells as such, but blocks the coupling of activation to DNA synthesis and proliferation [94]. Many trials of the combination of MMF with CSP or tacrolimus have shown reliable but

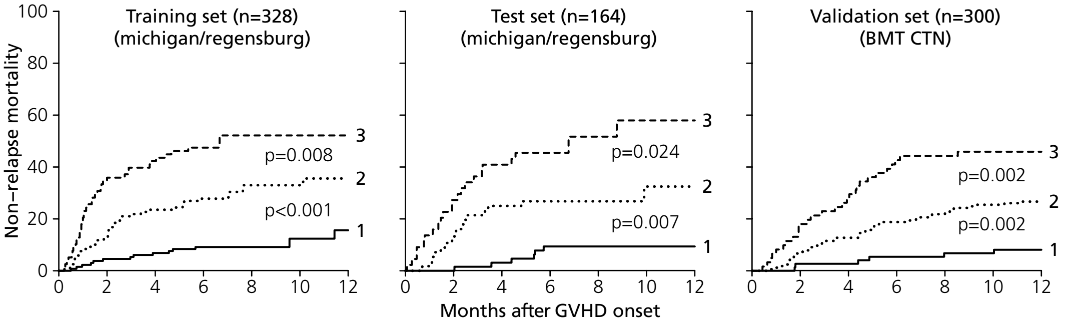

Figure 13.2 Non-relapse mortality (NRM) by Ann Arbor score at GVHD onset. Cumulative incidence of non-relapse mortality for each Ann Arbor score of 1, 2, or 3 is shown for the 328-patient training set from the Universities of Michigan and Regensburg [1 (solid) vs 2 (dots), $p < 0.001$; 2 vs 3 (dashes), $p = 0.008$], the 164-patient test set (1 vs 2, $p = 0.007$; 2 vs 3, $p = 0.02$), and the 300-patient multicenter validation set from the Bone Marrow Transplant Clinical Trials Network (Ann Arbor 1 vs 2, $p = 0.002$; 2 vs 3, $p = 0.002$). Source: adapted from Levine et al. 2012 [100].

incomplete control of GVHD when used in conjunction with a calcineurin inhibitor.

Sirolimus is a macrocyclic lactone that is similar in structure to tacrolimus and CSP. All three drugs bind to immunophilins; however, sirolimus complexed with FKBP12 inhibits T-cell proliferation by interfering with signal transduction and cell-cycle progression. Sirolimus affects lymphocyte activation at a later stage than either CSP or tacrolimus. The sirolimus–FKBP12 complex binds to mammalian target of rapamycin (MTOR), which blocks IL-2-mediated signal transduction pathways that prevent G_1/S phase transition. The drug has similar effects on the proliferation of T cells induced by IL-2, IL-4, IL-7, IL-12, and IL-15. Interestingly, it may also interfere with costimulation [95] by inhibiting CD28-mediated blocking of inhibitor of NF-κB and translocation of c-Rel to the nucleus [96]. A potential advantage of sirolimus is that it inhibits Treg cell less than calcineurin inhibitors. Since it acts through a separate mechanism, sirolimus is synergistic with both tacrolimus and CSP. Since the sirolimus–MTOR complex does not bind to calcineurin, sirolimus also is free of nephrotoxicity and neurotoxicity, making combination therapy appealing. The proteasome inhibitor bortezomib has useful effects on cytokine signaling and NF-κB activation. It can deplete alloreactive T cells while sparing Treg cell. It inhibits APCs by interfering with TLR4 activation, and it reduces T-cell differentiation induced by IL-6 [97].

Step 3: blockade of inflammatory stimulation and effectors

Reduced exposure to organisms

Another hypothesis that flows from this model is that reduction of intestinal colonization with bacteria could prevent GVHD. The first indication that elimination of exposure to microorganisms could prevent GVHD was in germ-free experiments in mice, where GVHD was not observed until the mice were colonized with Gram-negative organisms. Later, gut decontamination and use of a laminar airflow environment were associated with less GVHD and better survival in patients with severe aplastic anemia. Similarly, studies of intestinal decontamination in patients with malignancies have shown less GVHD in some but not all studies. However, despite the power of a large number of patients in registry analyses [98], it is possible that the heterogeneity of care in the large number of participating centers obscured an important effect. Viral infections, particularly herpes viruses, may also be associated with GVHD.

Blockade of effectors

Titers of several inflammatory cytokines can be used as both biomarkers for acute GVHD and to suggest targets for therapy. Recent studies have identified Regenerative 3 alpha as biomarkers specific for GVHD of the GI tract, the target organ that is least responsive to therapy [99]. Also, a new Ann Arbor scoring system uses a panel of three bioarkers [TNFR1, Regenerative 3 alpha and ST2 (a soluble IL-33 receptor)] to assign patients to a risk category at the onset of GVHD has been validated in a multicenter setting to predict non-relapse mortality, allowing for tailored therapy from the onset of symptoms (Figure 13.2) [100].Therapy directed at TNF-α produced partial responses either as therapy for steroid-resistant GVHD or as prophylaxis [101]. However, as noted above for IL-1 inhibition, redundant pathways may preclude effective therapy directed at a single cytokine. Blockade of chemokines may prevent the recruitment, localization, and activation of lymphocytes during GVHD. Prevention of lymphocyte entry into secondary lymphoid organs, acquisition of appropriate homing and adhesion molecules, or modification of their entry into target tissues could all reduce GVHD [102].

Chronic GVHD

It is important to recognize that cGVHD was originally defined temporally rather than clinically or pathophysiologically. The initial clinical reports of cGVHD used descriptions of clinical problems occurring at least 150 days after HCT [103]. By convention, many transplant clinicians and scientists use day 100 after hematopoietic cell infusion as a convenient divider between acute and cGVHD. However, it should be recognized that acute manifestations of GVHD may occur after day 100, and problems typically associated with cGVHD may occur before day 100. Therefore, it is preferable to consider the symptoms and signs per se rather than the timing of the onset of clinical findings. The National Institutes of Health consensus conference on cGVHD recognizes four categories of GVHD (Table 13.2) [104] (see Chapter 85).

The dearth of animal models that reproduce the critical aspects of cGVHD has limited our ability to address the pathophysiology of this complication, although a more recent model seems to be more representative of the human disease [105]. cGVHD can be considered to have both alloimmune and autoimmune components [105,106]. Indeed, autoantibody production is commonly observed after HCT, and some of the clinical manifestations of cGVHD are similar to those of scleroderma, lichen planus, and other autoimmune

Table 13.2 National Institutes of Health consensus conference categories of graft-versus-host disease

	Time	Acute features	Chronic features
Acute			
Classic acute	<100 days	Yes	No
Persistent, recurrent, or late onset	≥100 days	Yes	No
Chronic			
Classic chronic	No limit	No	Yes
Overlap	No limit	Yes	Yes

diseases. T cells from animals with cGVHD produce patterns of cytokines, such as IL-4, or IFN-α in the absence of IL-2 that are consistent with cGVHD reflecting in part Th2 T cell-induced injury [107]. There is often an eosinophilia as an early manifestation of cGVHD, further supporting the notion that cells with a Th2 phenotype are important mediators of this problem [108].

One mechanism to induce autoimmune attack might be failure of normal regulatory mechanisms in the setting of thymic damage or immunosuppressants, or both. Patients with cGVHD have fewer circulating Treg cell, thymic generation of naive Treg cell is impaired, and reconstituting Treg cell had a predominantly activated/memory phenotype. In response to CD4$^+$ lymphopenia after HCT, Treg cell proliferate more than conventional T cells, but with increased susceptibility to Fas-mediated apoptosis. Treg telomere length is shorter whereas Treg telomerase activity and expression of BCL-2 were each inversely associated with severity of cGVHD [109,110]. Failure of Treg cell results in autoimmune phenomena increasingly implicated in both acute and cGVHD [111]. Moreover, Treg cell are implicated in preventing autoantibody production [112].

Another explanation of autoimmunity may be that the massive apoptosis of T cells early in acute GVHD overwhelms the macrophage/DC system, resulting in autoantigen presentation [113]. The autoreactive cells of cGVHD are associated with a damaged thymus, which may be injured by acute GVHD or by the conditioning regimen, or be subject to age-related involution and atrophy. Therefore, the normal ability of the thymus to delete autoreactive T cells and to induce tolerance may be impaired.

Finally, data are accumulating to support the concept that B cells contribute to cGVHD. In a mouse model of cGVHD, fibrosis was demonstrated in the lung and liver and was associated with CD4$^+$ T cells and B220$^+$ B-cell infiltration and alloantibody deposition. Donor bone marrow obtained from mice incapable of secreting IgG alloantibody resulted in less cGVHD, and blockade of germinal center formation suppressed cGVHD [106]. The anti-CD20 mAb rituximab has been used clinically to treat severe autoimmune disorders including autoimmune hemolytic anemia and idiopathic thrombocytopenic purpura, and now several series indicate that anti-B-cell therapy is an interesting approach to treating cGVHD [114]. Alloantibodies can facilitate the cross-presentation of antigens to effector T cells, thereby amplifying T-cell responses to mHAs [115]. Donor B cells are capable of generating antibody responses specific for recipient mHAs, and cGVHD is significantly associated with the presence of high-titer mHA antibodies [116]. The presence of antibodies to mHAs also suggests that donor B cells have productive interactions with donor CD4 cells [116]. B cells can act directly as APCs in their ability to process and present limiting amounts of antigen for presentation to T cells. The presence of alloantibodies and high plasma B cell-activating factor (BAFF) levels in patients with cGVHD suggest that B cells play a role in disease pathogenesis. BAFF promotes survival and differentiation of activated B cells. Patients with cGVHD have significantly higher BAFF/B-cell ratios than patients without cGVHD or healthy donors. High BAFF concentrations are associated with activated B cells capable of IgG production without requiring additional antigen stimulation [117,118]. It has also recently been demonstrated in female-to-male transplants that donor-derived T cells can be isolated that recognize donor and recipient nonpolymorphic X chromosome-encoded proteins (e.g. DBX) [119] and also the production of antibodies to epitopes on the polymorphic Y chromosome-encoded proteins, DBY [116]. In this context, the allogeneic reaction may inactivate donor APCs that are essential for establishing cGVHD, through cross-presentation of host antigens to donor T cells [120]. Thus, during the course of cGVHD, a loss of tolerance to self may result in donor T-cell recognition of both self- and recipient antigens [121].

Conclusion

Complications of HCT, particularly GVHD, remain major barriers to the wider application of allogeneic HCT for a variety of diseases. Recent advances in the understanding of cytokine networks, and also the direct mediators of cellular cytotoxicity, have led to improved understanding of this complex disease process. GVHD can be considered to be an exaggerated, undesirable manifestation of a normal inflammatory mechanism in which donor lymphocytes encounter foreign antigens in a milieu that fosters inflammation. Tissue injury related to the conditioning regimen or infection is then amplified by direct cytotoxicity via perforin/granzyme and Fas/FasL pathways, through direct cytokine-induced damage, and by recruitment of secondary effectors such as granulocytes and monocytes. Thymic injury and loss of Treg function may enhance donor cell recognition of histocompatibility antigens by both T and B cells. Moreover, the production of auto- and alloantibodies may further result in tissue injury in addition to enhancing T-cell-related cellular injury. The net effects of this complex system are the severe inflammatory manifestations that we recognize as clinical GVHD.

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CHAPTER 14

Immune Regulation in Hematopoietic Cell Transplantation

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Introduction

Immune regulation is critical to health and disease and many disease states are caused or exacerbated by ineffective or excessive immune responses. Nowhere is this clearer than following allogeneic hematopoietic cell transplantation (HCT), where functional immune reactions can eradicate residual disease such as leukemia and other hematologic malignancies termed the graft-versus-tumor (GVT) effect, whereas dysfunctional immune responses can result in immune-mediated destruction of host tissues in graft-versus-host disease (GVHD). A deeper understanding and manipulation of immune regulatory mechanisms to control dysregulated immune responses have enormous potential for improving outcomes following allogeneic HCT and also could be extended to a variety of other fields, including induction of solid organ transplantation tolerance and treatment of autoimmune disorders. Therefore, the study of the mechanisms of immune regulation is of critical importance in many clinical settings. In this chapter, we review new approaches to the modulation of immune regulatory mechanisms to reduce GVHD risk and enhance immune recovery in allogeneic HCT. We focus on the cellular aspects of immune regulation, and strategies to modulate various cell populations that play a role in immune regulation for clinical benefit.

In the context of allogeneic HCT, it is important to note that all aspects of immune regulation must be re-established as the recipient's immune system is replaced by immune cells from the stem-cell donor. Critically, the transition from host to donor hematopoiesis often occurs rapidly as effector cells in the stem-cell graft recognize and respond to widely expressed immunogenic alloantigens in the recipient. This is a unique situation, which sets allogeneic stem-cell transplantation apart from other disorders of immune regulation and also provides unique opportunities for establishing the role of different immune regulatory cells and the mechanisms that these cells utilize in the establishment of immune tolerance. This unique setting also provides the opportunity to develop novel approaches to modulate this process for clinical benefit.

Regulation of immune function is a complex process involving a variety of important mechanisms. Given the critical importance of these mechanisms in maintaining self-tolerance and preventing autoimmunity, these mechanisms are often partially redundant and context dependent. Critical to our understanding is the compartmentalization of immune responses that is characteristic of response to infection and tissue injury. In the setting of allogeneic HCT, this compartmentalization is lost as immune effector cells, primarily donor T cells, respond to recipient alloantigens resulting in GVHD pathophysiology. Through the use of murine models, it has been determined that there are multiple priming sites for T-cell activation resulting in proliferation of alloreactive T cells present in the donor inoculum which become activated, expand, and infiltrate GVHD target tissues, primarily the gastrointestinal tract, skin, and liver [1,2]. At these sites, donor T cells cause tissue damage, resulting in organ dysfunction and increased risk of infection. Control of normal immune reactions also involves both pro- and anti-inflammatory cytokines that are critically important to immune regulation [3]. In addition, various cellular populations have been identified that play important roles in immune regulation termed regulatory T cells (Treg cells). Further work has also identified the potential for other regulatory cell populations such as natural killer T (NKT) cells, myeloid derived suppressor cells (MDSCs), B cells, mesenchymal stromal cells (MSCs), and antigen-presenting cells (APCs). Owing to their potential clinical applicability, we focus on cellular populations of regulatory cells including Treg cells, NKT cells, and MSCs and approaches to modulate these populations in vitro and in vivo. Chapter 12 also describes many of the properties of these different cell populations and their proposed roles in tolerance induction to solid organ transplantation.

Regulatory cell populations

A large body of evidence has demonstrated that a number of different cell populations are capable of controlling immune responses (see Table 14.1). The concept of suppressor cells was initially identified through in vitro assays measuring suppression of the mixed lymphocyte reaction (MLR). In this assay, irradiated stimulator cells are admixed with responder cells followed by the addition of [^{3}H]thymidine. The responding cells proliferate and incorporate [^{3}H]thymidine that can be quantified by harvesting the cells and measuring the incorporation of radioactivity. The addition of different cell populations capable of suppressing the MLR was critical to the identification of specific regulatory cell populations and remains a key in vitro assay for measuring the functional activity of regulatory cells. A defined population of CD4$^+$ T cells that co-expressed the interleukin-2 (IL-2) receptor α chain (CD25) was found to suppress the MLR and these cells were termed regulatory

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Table 14.1 Immune regulatory populations in allogeneic stem-cell transplantation

Population	Characteristic phenotype	Additional surface markers
CD4 regulatory T cells (CD4 Treg cells)	$CD3^+$ $CD4^+$ $FoxP3^+$ $CD25^+$ $CD127^{low}$	$CD152(CTLA\text{-}4)^+$ $GITR^+$ $CD223(LAG3)^+$
Natural killer T cells (NKT cells)	$CD3^+$ $CD56^+$	$CD161^+$ $V\alpha24i^+$ $CD4^{+/-}$ $CD8^{+/-}$
Mesenchymal stromal cells (MSCs)	$CD73^+$ $CD90^+$ $CD105^+$ $CD34^-$ $CD45^-$ $H\ LA\text{-}DR^-$	$CD11b^-$ $CD14^-$ $CD19^-$ $CD29^+$ $CD44^+$ $CD71^+$ $CD79a^-$ $CD166^+$
Myeloid-derived suppressor cells (MDSCs)	$CD11b^+$ $CD14^-$ $CD15^+$ $CD33^+$	$CD66b^+$ $CD124^+$ $VEGFR1^+$ $HLA\text{-}DR^-$

T cells (Treg cells) [4,5]. Later, it was determined that these Treg cells expressed the forkhead box P3 (FoxP3) transcription factor which is characteristic of naturally occurring Treg (nTreg) cells [6]. Interestingly, introduction of FoxP3 into conventional T cells resulted in acquisition of functional regulatory properties, establishing the central role for FoxP3 expression in Treg function. Further, genetic mutations that inactivate FoxP3 cause severe autoimmune disease in both animal models and in humans [7]. Although the majority of Treg cells are derived from the thymus, it has also been shown that they can be generated in vivo through the conversion of conventional CD4 T cells into Treg cells. These cells, termed induced Treg (iTreg) cells, acquire regulatory functions coincident with the expression of FoxP3 and maintain regulatory function as long as FoxP3 gene is expressed [8–10].

Although the exact mechanisms by which Treg cells suppress the MLR and autoimmune responses in vivo have not been fully elucidated, it is recognized that cell contact is important and a variety of different cytokines and cell-surface molecules play important roles [11]. It is also evident that Treg cells are capable of suppressing a variety of immune cells in addition to CD4 and CD8 T cells. These include dendritic cells, B cells, macrophages, natural killer (NK) cells and NKT cells [11,12].

The role of natural regulatory T cells in GVHD suppression

The recognized ability of nTreg cells to suppress autoimmunity led to several studies that examined the potential role of these regulatory cells in GVHD pathophysiology. Murine models have been essential for demonstrating the biological function of regulatory cell populations, initially focusing on nTreg cells and more recently on a variety of other cell populations. Initial studies focused on depleting $CD25^+$ T cells from the donor splenocyte inoculum to study the role of these cells in animal models of GVHD. These studies demonstrated that depletion of $CD25^+$ cells, typically approximately 10% of the $CD4^+$ T cells, resulted in more potent induction of GVHD compared with infusion of undepleted donor T cells [13]. Using different animal models across both major and minor histocompatibility mismatches, it was found that the addition of nTreg cells initially in a 1:1 ratio with conventional CD4 and CD8 cells resulted in suppression of GVHD [13–16]. Typically, these studies were performed by adoptively transferring highly purified $CD4^+CD25^+FoxP3^+$ nTreg and Tcon cell populations the day after transplantation with bone marrow-derived cells with or without T cell depletion to result in hematopoietic recovery. Similar studies were performed with human cells, assessing the ability of CD25-depleted stem cells to cause xenogeneic GVHD following transplantation into immune-deficient murine recipients [17]. These studies firmly demonstrated the potential for nTreg suppression of GVHD and provided the initial motivation for translation of these findings into the clinic. Further work demonstrated that nTreg cells selectively suppress conventional T (Tcon) cells, which actively replicate during GVHD induction. In these studies, it was also observed that nTreg cells rapidly proliferate in the allogeneic setting, especially in irradiated recipients, which is contrary to what was observed in vitro, where Treg cells typically do not expand in the MLR [18]. This finding suggested that the addition of Treg prior to Tcon cells would allow the former to proliferate and expand in vivo and this could be utilized to decrease the dose of Treg cells required for effective GVHD suppression. Since nTreg cells are relatively rare, obtaining sufficient numbers of cells for in vivo function is difficult, hence in vivo proliferation may be critically important to translate these concepts to the clinic. In these studies, addition of the nTreg cells 2 days prior to Tcon cells allowed for the reduction in nTreg cells by 5–10-fold, while still maintaining suppressive function of GVHD [18]. An alternative approach that is also clinically viable has been to expand the nTreg population ex vivo prior to adoptive therapy (see below).

An important component of these studies has been to examine the impact of nTreg cells on GVT reactions. In several reports, it was demonstrated that GVT reactions were preserved in some but not all tumor models [4,15,19]. To explain how nTreg cells can selectively suppress GVHD and not affect GVT, it was determined that nTreg cells selectively suppress Tcon-cell proliferation in the setting of allogeneic HCT, thereby suppressing the pathologic impact of Tcon cells in inducing GVHD. However, where there is a high T-cell precursor frequency for alloantigens, T-cell proliferation is not essential for GVT responses because the number of T cells capable of recognizing these alloantigens is relatively high and sufficient for tumor eradication. Therefore, suppression of Tcon-cell proliferation results in effective suppression of GVHD, yet still allows for GVT, especially in the setting where there is a relatively low tumor burden, as is typical in animal models, and also clinical treatments for patients undergoing allogeneic HCT due to the impact of the preparative regimen on tumor burden. The critical role of perforin and granzymes was essential for GVT function. It remains to be determined whether these predictions will hold true in clinical transplantation; however, the results of these in vivo models provided further encouragement for the translation of these concepts to the clinic.

Another concern was whether infusion or expansion of nTreg cells in vivo would result in severe global immunosuppression in recipients. In several studies, it was demonstrated that animals that received nTreg cells actually had improved immune reconstitution, as demonstrated by recovery of immune effector cell populations, and also following challenge with infectious agents such as murine cytomegalovirus (mCMV) [4,20,21]. These findings were thought to be due to the ability of the nTreg cells to suppress GVHD-induced tissue injury to secondary lymphoid tissues such as lymph nodes and thymus, allowing for more effective immune recovery in the post-transplant setting. Taken together, these studies set the stage for various approaches to expand or enhance selectively nTreg cells after allogeneic HCT in clinical trials that are detailed below.

Ex vivo expansion of Treg cells

Owing to the relative paucity of nTreg cells, it was speculated that expansion of this cell population would be important for clinical translation. Several groups have demonstrated that nTreg cells can be expanded in vitro in both murine and human systems [14,22–24]. To induce expansion, activation is required, typically through

CD3/CD28 stimulation in the presence of TGFβ and APCs. With this approach, significant expansion of nTreg cells can be achieved over a 14–21-day culture period, resulting in relatively large populations of cells, which can then be utilized for studies of in vivo biological function. Similar expansion of nTreg cells has been accomplished in humans. However, in all of these studies, a major limitation has been that although Treg cells proliferate in vitro, conventional T cells often outgrow these cultures and appear to expand preferentially [25]. A variety of strategies have been utilized to suppress Tcon-cell proliferation, including the use of rapamycin to suppress selectively Tcon cells and exhaustive depletion of Tcon cells from the Treg-cell inoculum [26]. This strategy has also been extended to the clinic, as described below. Isolation of $CD45RA^+CD4^+CD25^+$ cells prior to expansion has also been shown to result in robust expansion of suppressive populations of polyclonal Treg cells [27]. Other approaches have also resulted in robust Treg-cell expansion in clinically relevant strategies [28].

Critical requirements for Treg-cell function

Although many aspects of Treg-cell function, regulation, and homeostasis have not been fully elucidated, a large number of studies in many laboratories have greatly improved our understanding of the biology of Treg cells and the important role that these cells play in regulating immune responses in vivo [8–11]. For example, several studies demonstrating that expression of CD62L, a molecule required for lymph node entry, is essential for in vivo Treg-cell function, have established that nodal migration is required for in vivo function of these cells. These studies demonstrated that ~50% of nTreg cells express CD62L whereas 50% typically are $CD62L^-$. Both cell populations suppress the MLR yet only the $CD62L^+$ nTreg cells function in vivo [29,30]. Many other studies have been performed to identify the essential molecules required for nTreg-cell function in GVHD models [31,32]. Among these molecules, several studies have identified PD-1 and PD-L1 as playing important roles in the interaction of Treg cells with effector T cells [33,34]. Although it is not yet clear how Treg cells function in controlling GVHD reactions and also other immune responses, several cytokines, including IL-10, TGF-β, FLT3-ligand, IFNγ, and IL-35 have been implicated [35–40]. Treg cells are not able to secrete IL-2, but the constitutive expression of large numbers of high-affinity receptors for IL-2 allows these cells to sequester preferentially IL-2 and deprive other T cells of this critical growth factor [41–43]. Other cellular interactions, including alteration of APC function and direct cytotoxicity, have also been proposed as important mechanisms for Treg-cell function [11,31,42,44,45]. A critical question when extrapolating these findings to the clinic is how relevant these studies are for human Treg-cell function. It is very difficult to study the role of specific molecules in Treg-cell function in humans apart from in vitro studies assessing whether a particular interaction is critical for maintenance of the ability of Treg cells to suppress the MLR or xenogenic GVHD in immune-suppressed animal model systems. Determining whether modulation of these structures occurs during the isolation process and/or ex vivo expansion utilized in the clinical protocol is of clear importance. These studies require further investigation to elucidate better the critical requirement of different cell-surface molecules in Treg-cell function in the clinic.

Treg-cell homeostasis in vivo

Like conventional T cells, Treg cells are derived from lymphoid precursor cells that mature in the thymus and are subject to negative and positive selection. This results in mature CD4 Treg cells that have a highly diverse αβ T-cell receptor repertoire that is exported to the peripheral blood and circulate to secondary lymphoid organs and peripheral tissues [46]. In healthy individuals, Treg cells represent approximately 1.5–10% of the CD4 T-cell pool and human peripheral blood contains approximately 10–80 CD4 Treg cells/μL. Like other T-cell populations, peripheral Treg cells are maintained at constant levels through continuous production of naïve Treg cells in the thymus. Mature Treg cells also undergo homeostatic proliferation and both thymic production and homeostatic proliferation are counterbalanced by apoptotic cell death to maintain normal levels of peripheral Treg cells. Although all T-cell populations are subject to homeostatic control, recent studies have demonstrated that distinct control mechanisms are utilized for different T-cell populations. This is particularly evident for Treg cells, where this T-cell subset is uniquely dependent on IL-2 and constitutively proliferates at a higher rate that Tcon cells [47–49]. This high level of proliferation is counterbalanced by increased susceptibility to apoptosis, mediated in part through increased expression of CD95 [47]. As a consequence of this high level of endogenous proliferation, Treg cells have significantly shortened telomeres compared with Tcon cells, and this further promotes cell senescence and increased susceptibility to apoptosis [48].

In the setting of allogeneic HCT, thymic function is markedly impaired, especially in adults, and thymic generation of naïve Treg cells is significantly reduced [47]. Maintenance of normal Treg cell numbers after HCT therefore relies primarily on proliferation of memory Treg cells, which is significantly increased. However, highly proliferative Treg cells have also been shown to have markedly shortened telomeres and express higher levels of CD95 and lower levels of Bcl-2 protein compared with Tcon cells. After allogeneic HCT, Treg cells are therefore more susceptible to apoptosis than Tcon cells, and this homeostatic imbalance contributes to a relative deficiency of Treg cells. This is most evident in patients with chronic GVHD and likely contributes to the persistence of chronic tissue damage in these patients [47].

Clinical translation of Treg-cell biology

The findings that Treg cells suppress GVHD yet allow for GVT responses and more effective immune reconstitution have made the clinical translation of this cell population of high importance. Further, many animal models of autoimmunity have been described where Treg cells have been shown to have immunologic consequences, and therefore the results of clinical translation in the HCT field have enormous implications for other clinical settings [50]. However, there are major limitations in the translation of these concepts to the clinic, including the relatively small numbers of cells that circulate in peripheral blood and the difficulty in isolating this cell population with a rather common phenotype. Although CD4 and CD8 T cells do not constitutively express CD25, human effector T cells express CD25 after antigen activation, in vitro and in vivo. Hence isolation and expansion of $CD4^+CD25^+$ cells often includes activated T cells in addition to Treg cells. Since FoxP3 is an intracellular transcription factor, FoxP3 expression cannot be used for isolation of cells; however, expression of this transcription factor can define the purity of the cell population following separation. Therefore, the search for other markers that could be clinically useful has been of high importance and many studies have revealed a number of potential markers, with lack of expression of CD127 (IL-7 receptor) emerging as the most useful other cell-surface marker identified to date [51,52]. These studies demonstrated that the cell-surface phenotype of $CD3^+CD4^+CD25^+CD127^{low}$ in human

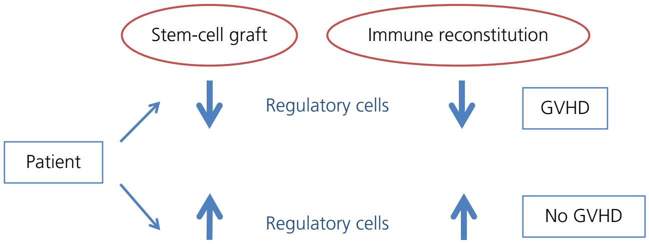

Figure 14.1 The role of regulatory cells in the development of graft-versus-host disease.

samples reliably distinguishes these cells from conventional CD4 T cells ($CD3^+CD4^+CD25^-CD127^{high}$) and CD8 T cells. Isolation of Treg cells based on these markers results in a relatively pure population of polyclonal Treg cells with relatively low contamination by effector T cells.

Role of Treg cells in human GVHD

The role of Treg cells in human GVHD pathophysiology has been assessed in a number of different ways (Figure 14.1). Several studies have attempted to correlate the subsequent clinical development and severity of acute or chronic GVHD with the number of Treg cells in the stem-cell graft. Although not all results in clinical reports have been consistent, most studies have found that increased numbers or increased frequency of Treg cells in the stem-cell products correlated with reduced incidence of GVHD after engraftment [53–55].

Several studies also quantified Treg cells in peripheral blood at various times after transplant and correlated these results with the presence or absence of GVHD. Although the results of these studies have also not been entirely consistent, they generally support the concept that lower numbers of Treg cells are associated with greater risk of acute and chronic GVHD [47,56–65]. Differences in the methods used to detect and quantify Treg cells may help explain some of these discrepancies, but it is also likely that many factors, unrelated to GVHD, influence the number of Treg cells circulating in the blood at various times after transplant. In one study, the relative number of Treg cells early in the post-transplant period correlated with GVHD risk, where patients with fewer Treg cells had higher risk [66]. In a separate study, effective treatment of chronic GVHD resulted in improvement in the number of Treg cells, further supporting the concept that Treg cells are of central importance in the biology of human GVHD [57]. All of these studies have a number of caveats, including the facts that the range of Treg cells in the donor inoculum is relatively narrow and that sampling is usually performed in the blood rather than nodal or GVHD target tissues owing to the difficulty of accessing the latter tissues. Some studies have been able to assess the tissue content of Treg cells at sites of GVHD, further supporting the concept that Treg cells are important in the underlying biology of alloreactivity after stem-cell transplantation [56,67]. Nonetheless, these studies are correlations and do not conclusively define a role for Treg cells in GVHD pathophysiology. Therefore, clinical studies assessing the effects of adoptive transfer of purified populations of cells were eagerly awaited.

Adoptive transfer of Treg cells in allogeneic HCT

Two clinical studies have been published that demonstrate that Treg cells play a major role in controlling GVHD. The first study, at the University of Perugia, was carried out in patients with high-risk leukemia who did not have HLA-matched related or unrelated donors [68]. Patients received ablative conditioning followed by selected $CD34^+$ hematopoietic stem cells from haploidentical donors. Four days prior to stem-cell transplantation, patients received a single dose of highly purified donor Treg cells. Following stem-cell infusion, patients also received a defined dose of conventional donor T cells. Previous studies from this group had demonstrated that haploidentical transplantation is possible without any immunosuppression and with minimal risk of GVHD [69]. However, these patients suffered from very slow immunologic recovery, relapse, and opportunistic infections. The addition of conventional T cells could reduce the risk of infection and perhaps improve GVT reactions, but infusion of donor T cells has been limited by GVHD risk. Specifically, it was found that infusion of as few as 5×10^4 $CD3^+$ donor T cells/kg can result in severe and often lethal GVHD.

A subsequent Perugia study employed a two-step isolation process to purify Treg cells from the haploidentical donor prior to hematopoietic stem-cell mobilization [68]. Briefly, the donor apheresis product was first depleted of CD19 B cells and CD8 T cells and subsequently $CD25^+$ cell selection was used to isolate a population of Treg cells with ~50–75% $FoxP3^+$ expression. All manipulations utilized the CliniMACS separation system (Miltenyi Biotec) and clinical-grade reagents. The infusion of donor Treg cells prior to donor Tcon cells in these studies was based on the hypothesis that the addition of Treg cells prior to the addition of Tcon cells would make it possible to increase the number of Tcon cells that could be safely infused. In these studies, up to $2–4 \times 10^6$ Treg cells/kg were infused without significant clinical toxicity. An equal number of Tcon cells were infused with limited risk of acute GVHD, clearly demonstrating the ability of the Treg-cell population to suppress alloreactivity in vivo. In this first trial, 28 patients were treated and 26 engrafted. Of these 26 evaluable patients, only two developed grade II–IV GVHD [68]. Importantly, patients who received donor Treg and Tcon cells appeared to have improved immune recovery and low risk of disease recurrence. However, transplant-related mortality was still significant, mainly due to infection. Therefore, further improvements are necessary to improve these clinical results. Nevertheless, this study provides clear evidence that Treg cells have potent biological function in vivo in that relatively few of these patients developed significant GVHD despite the infusion of an otherwise unacceptable dose of Tcon cells.

The second study, from the University of Minnesota, used expanded Treg cells from umbilical cord blood (UCB) in an effort to reduce GVHD risk in the double cord blood transplant setting. Given the relatively small number of hematopoietic stem cells in UCB products, two UCB products were used, primarily in adult patients, to enhance engraftment [70]. The use of two UCB donors increases the risk of GVHD, and the Minnesota group performed a clinical trial to examine whether the infusion of third-party ex vivo expanded Treg cells could reduce that risk [71]. In this study, Treg cells were purified from a third partially HLA-mismatched cord blood product using the CliniMACS system to isolate $CD25^+$ cells. Isolated cells were expanded after stimulation with anti-CD3/anti-CD28 Dynabeads and IL-2 for at least 14 days. In most cases, in vitro expansion was extended to provide cells for a second Treg-cell infusion. Seventeen of 23 (74%) patients received the Treg-cell infusion at the target cell dose level. Only five patients received a Treg-cell dose less than the prescribed cell dose because of insufficient culture expansion. Of the 18 patients scheduled to receive two doses, 13 received the targeted Treg-cell dose on days +1 and +15. Overall, 18 patients received a total Treg-cell dose $>30 \times 10^5$ cells/kg. With these results, this study demonstrated that Treg cells could

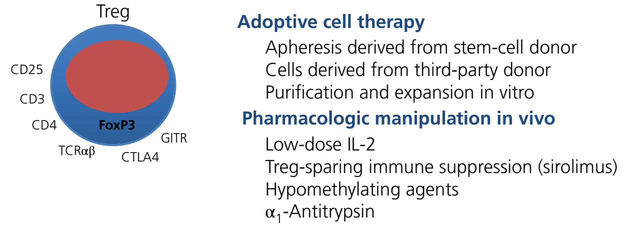
Treg
CD25
CD3
CD4
FoxP3
TCRαβ
CTLA4
GITR
Adoptive cell therapy
Apheresis derived from stem-cell donor
Cells derived from third-party donor
Purification and expansion in vitro
Pharmacologic manipulation in vivo
Low-dose IL-2
Treg-sparing immune suppression (sirolimus)
Hypomethylating agents
α1-Antitrypsin

Figure 14.2 Clinical approaches to enhance CD4 regulatory T-cell function.

be expanded from UCB on a clinical scale and there were no infusional toxicities in the 23 patients treated. After infusion, UCB Treg cells could be detected for 14 days, with the greatest proportion of circulating $CD4^+CD127^-FoxP3^+$ cells observed on day +2. Because these are third-party Treg cells, it was not expected that these cells would persist indefinitely. Although it was difficult to assess the biological function of the Treg cells in this study, it could be shown that there was an apparent reduction in GVHD risk compared with a historical control group [71].

Taken together, these two studies provide firm evidence that utilization of Treg cells on a clinical scale is feasible, infusion of the cells does not result in toxicity, and there is evidence for biological activity (Figure 14.2). These studies set the stage for further clinical applications of Treg cells in a variety of clinical settings using highly purified nTreg cells, ex vivo expanded Treg cells, and perhaps induced Treg cells to prevent and treat GVHD. Clearly, several major issues remain to be resolved in future studies. These include the required purity of infused Treg cells, activation status, cell-surface expression of important homing and functional receptors, cell dose, and ability to home to appropriate biological sites. Whether multiple infusions of Treg cells are required to maintain functional suppression of GVHD and establish long-term tolerance towards recipient tissues remains to be elucidated. Finally, whether enhancement of Treg-cell function may also suppress GVT responses must also be carefully evaluated in future studies. Nonetheless, these studies provide firm evidence that clinical translation is possible and future studies will delineate the potential clinical utility of this important population of regulatory cells.

Impact of immunosuppressive medications on Treg-cell function

A number of studies have explored the impact of conventional immunosuppressive medications on Treg-cell function. These studies revealed that calcineurin inhibitors such as cyclosporine A and tacrolimus generally inhibit Treg-cell function, but this can be partially overcome by administration of IL-2 [72,73]. Both of these immunosuppressive medications suppress IL-2 production by T cells, and therefore the reduction in production of this cytokine that Treg cells require yet do not produce is thought to be an additional mechanism by which these agents suppress Treg cells in vivo. Other immunosuppressive medications, such as rapamycin (sirolimus), appear to be more permissive for Treg-cell function [74]. This can be explained by the observation that upon T-cell receptor activation, conventional $CD4^+$ T cells signal primarily though the mammalian target of rapamycin (MTOR) pathway, whereas CD4 Treg cells mainly utilize the Stat5 pathway [73]. In more recent studies, novel agents have also been examined for their effect on Treg-cell function. For example, hypomethylating agents, such as decitabine and azacitidine, induce FoxP3 expression in Treg cells and suppress GVHD in vivo in animal models [75]. The JAK/STAT pathway also plays a role in proliferation of activated Tcon and Treg cells and methods are being developed to suppress selectively Tcon cells through modulating this pathway [32,76]. α_1-Antitrypsin is another novel agent that has been found to reduce GVHD by suppressing pro-inflammatory cytokines, selectively reduce expansion of effector T cells, and enhance recovery of Treg cells [77,78]. Thus far, most of these studies have been performed in vitro or in animal models, and to date there is no accepted immunosuppressive medication strategy avoiding calcineurin inhibitors in clinical HCT. The combination of rapamycin and mycophenolate mofetil (MMF) has been utilized clinically and resulted in higher numbers of Treg. However, this regimen was associated with excessive toxicity and no clear reduction in GVHD risk compared with historical experience with more conventional calcineurin inhibitor (CNI)-based combinations [79].

Activation and expansion of Treg cells in vivo

Although adoptive transfer experiments are clearly an effective clinical approach to modulate Treg-cell function after HCT, alternative strategies have been explored to expand Treg-cell populations in vivo. In animal models, this can be accomplished through administration of IL-2, a critical homeostatic cytokine that Treg cells require but do not produce [3,80]. In these studies, treatment with IL-2 resulted in selective expansion of Treg cells in several different animal models and in humans [81–84]. When tested in models of autoimmunity, selective expansion of Treg cells in vivo following IL-2 therapy effectively reversed disease manifestations [82]. In models of allograft rejection, IL-2 therapy promoted long-term acceptance of allografts without additional immune suppression [81]. In models of allogeneic HCT, IL-2 has been combined with rapamycin (sirolimus) to enhance suppression of conventional CD4 T cells. In this model, the combination of IL-2 plus rapamycin improved survival and reduced GVHD lethality. This was associated with selective expansion of natural Treg cells and also conversion of induced Treg cells from conventional donor CD4 T cells and reduced production of inflammatory cytokines [85].

The use of low-dose IL-2 to expand Treg cells has been explored by investigators at the Dana-Farber Cancer Institute in patients with refractory chronic GVHD [86]. Their earlier studies demonstrated that patients with chronic GVHD have low numbers of Treg cells and the administration of low-dose IL-2 by subcutaneous daily injection results in the expansion of Treg cells. Since all Treg cells constitutively express high-affinity IL-2 receptors, low concentrations of ligand are sufficient to initiate receptor-mediated signals. The studies therefore examined relatively low doses of IL-2 compared with doses commonly used for the treatment of metastatic melanoma. Three dose levels were tested and 29 patients with long-standing chronic GVHD were enrolled in the study. It was found that 1×10^6 units/m^2 was a tolerable dose of IL-2 that could be given as a daily injection over an 8-week course of therapy. Importantly, this treatment was associated with an eightfold increase in Treg cells in the peripheral blood without any increase in conventional CD4 or CD8 T cells. NK cells were also affected and increased 2.5-fold during IL-2 therapy. Of the 23 evaluable patients, 12 had major responses involving several different clinical sites of disease. In patients who had clinical responses, daily IL-2 therapy was resumed after a 4-week rest period and continued for over 1 year in several patients. Although IL-2 can also promote the expansion of effector T cells, the effect of IL-2 on Treg cells predominated and none of the patients in this trial experienced worsening of chronic GVHD while on low-dose IL-2 therapy.

In the context of this clinical trial, further studies were carried out to determine the biologic effects of IL-2 therapy [87]. Prior to starting IL-2, all patients had reduced numbers of Treg cells in peripheral blood. During the 8-week treatment period, IL-2 induced a series of sequential changes in Treg-cell homeostasis in all patients, including increased proliferation and increased thymic export of naïve Treg cells. Intracellular expression of Bcl-2 was also increased and expanded Treg cells became more resistant to apoptosis in vitro. Low-dose IL-2 had minimal effects on Tcon cells and this resulted in a fivefold increase in the Treg/Tcon cell ratio. Taken together, these findings define the mechanisms whereby low-dose IL-2 therapy restores homeostasis of CD4 T cell subsets and promotes the re-establishment of immune tolerance in patients with active chronic GVHD. These studies indicate enormous potential for expansion of Treg cells, and future studies combining adoptive transfer of Treg cells with low-dose IL-2 therapy may further enhance the potential clinical benefit of this approach [88]. Preclinical animal models have helped set the stage for various approaches focused on the modulation of CD4 Treg cells after allogeneic HCT, with additional clinical trials being organized in several different clinical settings.

Natural killer T cells

In addition to Treg cells, other cell populations have been shown to have immune modulation activity. A rare population of NK cells that co-express T-cell markers (NKT cells) have also been shown to be biologically important and have potent immunomodulatory activities primarily through the production of immune-regulating cytokines such as IL-4 [89,90]. NKT cell populations have been shown to suppress different auto- and alloimmune functions in animal modeling studies, again setting the stage for clinical translation. Invariant NKT (iNKT) cells are selectively activated by glycolipid antigens expressed by CD1d molecules. These cells express a distinct T-cell receptor (TCR) characterized by an invariant TCRα chain coupled with a variable TCRβ chain. Two major populations of iNKT cells have been described that have distinct functional activities and produce different sets of cytokines after activation [89,91]. These cells appear to play an important role in regulating immune responses after HCT [92,93]. Similarly to the studies with Treg cells, correlations have been made between the iNKT cell content of the stem-cell graft and subsequent GVHD risk [94]. In these studies, increased numbers of iNKT cells in the stem-cell graft were associated with reduced incidence of GVHD after transplant. Similarly, analysis of iNKT cell reconstitution after transplant demonstrated that early recovery of iNKT cells was predictive of a lower risk of GVHD and improved survival [95].

One approach to the translation of these concepts came from the studies of Strober and co-workers, where the use of total lymphoid irradiation (TLI) in combination with antithymocyte globulin (ATG) in murine models resulted in alteration of the ratio of conventional CD4/CD8 T cells to NKT cells and a marked reduction in GVHD risk [96]. In this model, NKT cells were shown to be relatively radioresistant such that there was less suppression following exposure to TLI altering the ratio dramatically in the tissues of the animals prepared with TLI–ATG. As a result, TLI–ATG-prepared animals could be transplanted with 1000 times the number of conventional CD4/CD8 T cells compared with TBI-conditioned animals that would otherwise cause lethal GVHD. IL-4 production was critically important, as was activation with CD1, as knockout strains of animals deficient in either IL-4 or CD1 had no such biological effect. Therefore, these studies demonstrated that altering the host conditioning could impact the ratio of NKT-cell regulatory cell populations and have profound biological effects after HCT. Further, it was demonstrated that interaction of the NKT cells with Treg cells in the donor graft was critically important for biological function in these animal models [97].

These preclinical studies set the stage for clinical translation with the TLI–ATG regimen, which was found to be tolerable on an outpatient basis and resulted in marked reductions in acute GVHD and also transplant-related mortality [98–100]. Further, in patients transplanted with TLI–ATG, it was found that there was a rise in recipient NKT cells immediately after conditioning and prior to the infusion of the donor inoculum, correlating with the animal model predictions [98]. TLI–ATG conditioning was found to be well tolerated and applicable to the treatment of older patients and patients with comorbid medical conditions, including those who had received prior autologous transplants [99]. Further, the reduction in GVHD risk allowed for the exploration of combined bone marrow and solid organ grafts for tolerance induction, initially in the setting of kidney transplants where a number of patients have been successfully tapered off all immunosuppressive medications [101] (see Chapter 12).

Adoptive transfer of NKT cells has also been explored, where it was found that these cell populations are also capable of suppressing GVHD [102,103]. Interestingly, even lower numbers of cells compared with Treg cells are functional in similar animal models [104]. Whereas Treg cells have been clearly shown to suppress the proliferation of alloreactive conventional T cells in models using bioluminescent imaging, it was demonstrated that following the infusion of NKT cells, there is no such suppression of conventional T-cell proliferation, suggesting that NKT cells function through a different mechanism of action. In addition, the use of αGalCer has been shown to activate NKT cells that also interact with Treg cells, resulting in suppression of GVHD in animal models [105]. This concept has been translated to the clinic in a phase I/II clinical trial that is ongoing. The concepts developed in animal modeling have been shown to have important biological consequences in suppressing GVHD reactions, reducing transplant-related morbidity and mortality with appropriate extension to the clinic.

Mesenchymal stromal cells

Mesenchymal stromal cells (MSCs) are a heterogeneous population of adherent cells isolated from bone marrow, adipose tissues, and other sites, which have been studied in a number of different clinical venues [106]. MSCs have been shown to have immunomodulatory and anti-inflammatory properties and have been evaluated in the treatment of both acute and chronic GVHD. Results in animal studies have demonstrated that MSCs suppress alloreactive T-cell proliferation, but results have been mixed with respect to the ability of MSCs to suppress GVHD [107,108]. In other studies, repeated injections of MSCs delayed but did not prevent GVHD [109]. Similar results were observed in a canine model of GVHD where MSCs, despite having properties similar to those of human MSCs, failed to improve the survival of dogs undergoing allogeneic HCT [110]. Despite these somewhat discouraging results in animal model systems, there have been case reports of patients with severe refractory GVHD who have responded to MSC infusions [111]. These responses prompted more extensive testing, including prospective phase II trials in patients with acute GVHD in addition to corticosteroids as front-line therapy. In one study, 32 patients received one of two doses of MSCs (2×10^6 vs. 8×10^6 MSCs/kg) within 48 hours of the first treatment with corticosteroids. A high response rate of 94% was observed independent of the dose of MSCs administered [112]. On the basis of these promising results, two prospective randomized trials were conducted comparing MSCs with placebo. Although final results are pending, they were reported not to reach their primary end-points of improvement of GVHD response rates, although a subset analysis of patients with liver GVHD may have responded favorably to MSC treatment.

Despite these setbacks, there continues to be significant interest in MSCs for the treatment of acute and chronic GVHD [113]. Overall, evaluation of the potential therapeutic role of MSC has been limited by the heterogeneity of the MSC populations that have been administered and the undefined mechanisms of action of these cells in vivo. Critical factors that contribute to the heterogeneity of MSCs include whether the cells used in clinical trials are autologous or allogeneic, what tissue they were derived from, the method of isolation, the number of in vitro cell divisions, and the cell surface phenotype after expansion [114]. Further studies are needed to address these issues and establish in vitro potency assays for the manufactured MSC product that can be used to compare different MSC products and predict the functional activity of these cells in vivo.

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) have been identified as cells that accumulate in tumor tissues and inhibit immune responses [115]. MDSCs can be cultured from bone marrow cells in the presence of granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) and have been found to inhibit in vitro immune responses. These cells have also been studied in murine models of allogeneic HCT where addition of MDSCs to stem-cell grafts suppressed GVHD and elimination of MDSCs from grafts exacerbated GVHD after transplant [116]. Following transplant, increased numbers of MDSCs were found in mice without GVHD; accumulation of MDSCs was also noted at sites of tumor relapse. However, a subset of MDSCs responsive to IL-13 has been reported to suppress GVHD without inhibiting GVT reactions [117].

Other regulatory populations

Other populations of regulatory cells, including CD8 Treg cells and regulatory B cells, have also been demonstrated to have immune regulatory functions in animal models [118–120]. CD8 Treg cells that are induced from conventional CD8 T cells have been found to suppress GVHD potently [121,122]. However, the potential clinical use of these cells is challenged by the relative paucity of these cells in peripheral blood, the variable phenotypes that have been described, and whether such populations can be induced in vitro. Although most studies have been carried out in murine models, populations of CD8 Treg cells have been described in humans [123,124]. However, it is not known whether CD8 Treg-cell populations in humans are the same as those found in murine systems, and the mechanisms by which these cells regulate other immune cells have not been well characterized. Importantly, the potential role of CD8 Treg cells in GVHD after allogeneic HCT in humans has not yet been studied.

Conclusion

Immune regulatory mechanisms play a central role in a variety of different disease settings. In acute and chronic GVHD, dysfunctional immune reactions that are improperly regulated characterize the pathophysiology of these diseases. The development of strategies to enhance immune regulatory cell number and function and also the adoptive transfer of regulatory cell populations hold significant promise for improving outcomes for patients undergoing allogeneic HCT. Clearly, many issues remain to be elucidated and technical improvements are required. However, results from the early clinical studies adapted from animal modeling have demonstrated the biological impact of these cell populations and the feasibility of clinical translation. These studies have important implications in the field of HCT but also are relevant to other clinical conditions, such as autoimmune diseases, and the induction of solid organ transplantation tolerance.

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CHAPTER 15

Immune Reconstitution Following Hematopoietic Cell Transplantation

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Introduction

Hematopoietic cell transplantation (HCT) is complicated by post-transplant immune deficiency, which is more profound after allogeneic than autologous HCT. Cytoreductive conditioning regimens eliminate tumor cells and significantly deplete the recipient's immune system, in particular cells of the lymphoid lineage (expertly reviewed in [1]), and allow for successful engraftment of hematopoietic stem cells (HSCs) in their bone marrow niche. Although this depletion of the adaptive immune system promotes HCT engraftment as it prevents host cells from attacking donor stem cells, delayed lymphoid recovery following immune depletion is associated with a high degree of morbidity and mortality, and can potentially also lead to malignant relapse [2,3]. Post-transplant immune depletion is particularly striking within the T cell compartment, which is extremely sensitive to negative regulation and remains depressed for a considerable time (Figure 15.1). Consequently, regeneration of the immune system remains a significant unmet clinical need. There are currently no US Food and Drug Administration (FDA)-approved strategies for immune reconstitution. However, several preclinical and clinical studies have identified promising therapeutic strategies for immune regeneration.

In this chapter, we review immune reconstitution following HCT, with a particular emphasis on factors that contribute to delayed immune recovery post-transplant and the clinical consequences. We also discuss current clinical and research assays to monitor post-HCT immune recovery and highlight some of the potential strategies that are currently under investigation to enhance immune recovery in HCT recipients.

Steady-state lymphopoiesis

HSCs are central throughout life for their ability to differentiate into and develop all hematopoietic lineages [4]. Branching towards myeloid and lymphoid lineages occurs at the level of hematopoietic stem and progenitor cells [5]. In fact, early hematopoietic progenitors have been phenotypically identified that are either fully committed or biased towards the lymphoid linage [6–8]. Furthermore, there is increasing evidence in animal studies that functional branching occurs at the most primitive HSCs, with both lymphoid- and myeloid-biased stem cells described [9,10]. Changes in the ratio between myeloid- and lymphoid-biased stem cells may explain the age-associated decline in lymphoid function [11,12]. Differentiation of lymphoid progenitors down the B lineage in the bone marrow (BM) or T lineage in the thymus is a complex process involving the transcriptional commitment of multipotent progenitors, egress from the BM, and entry to the thymus – in the case of T cells – and progression through a series of distinct developmental stages that are tightly controlled by their respective microenvironmental niches. This process, which is continuous for the duration of life, albeit declining significantly with age, results in the formation of mature naïve T and B cells that form the pillars of the adaptive immune system.

Progression from HSC to B cell occurs through a series of distinct developmental steps and relies on the production of specific factors by the BM niche along with downstream signaling events that promote the differentiation and development of mature B lymphocytes [13]. T-cell development is more complicated as it begins in the BM and continues through series of well-defined stages in the thymus. The thymus has traditionally been thought to contain no self-renewing cells capable of maintaining thymopoiesis [14], instead relying on the importation of progenitors that periodically egress the BM to enter the circulation and seed the thymus (comprehensively reviewed in [15,16]), although two recent studies in mouse models suggest that there are some self-renewing T-lineage progenitors resident in thymus that contribute towards thymopoiesis, at least in the setting of immune deficiency [17,18]. Although the underlying process of thymus seeding from circulating progenitors is well established, the precise identity of the circulating T-cell progenitor has remained elusive. However, it is becoming increasingly clear that there are multiple circulating cells with the capacity for T-cell differentiation that can seed the thymus, differing in their phenotype and in their lineage restriction [15,16]. Moreover, the precise mechanisms of their entry into the thymus are only just beginning to be understood (reviewed in [16]). Once in the thymus, T-lineage progenitors undergo a series of maturation and developmental steps, in addition to positive and negative selection depending on their specific T-cell receptor and its affinity for self or foreign antigen, in highly specialized microenvironmental zones that result in the formation of naïve T cells ready for export to the periphery (reviewed in [19]). Although T-cell development is predominantly restricted to the thymus, recent studies have found that there could

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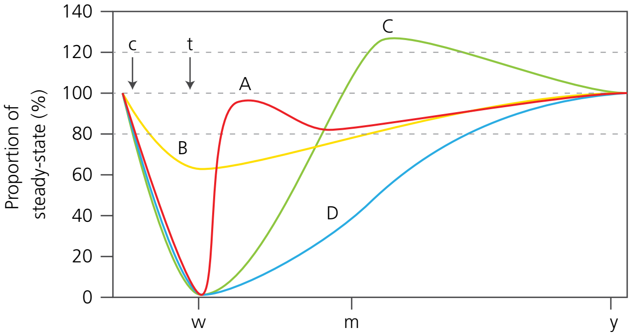

Figure 15.1 Illustrative figure showing the total number of immune cells as a proportion of steady-state levels. Displayed are approximate time frames of weeks (w), months (m), and years (y) after myeloablative conditioning (c) and HCT (t). A, Neutrophils/monocytes; B, plasma cells/dendritic cells; C, B cells/CD8$^+$ cells: D, CD4$^+$ cells/NKT cells. Source: adapted from Storek 2008 [198].

be some degree of extrathymic T-cell development, including conventional T-cell development in the human tonsil [20–22]. This is particularly interesting in light of another study suggesting that naïve T-cell output becomes negligible in human thymus with age [23]. However, despite its apparent redundancy in aged humans in the steady-state, it is likely that the thymus is of considerable benefit for reconstitution of the naïve T-cell receptor repertoire after immune depletion.

What are the causes of post-transplant immune deficiencies?

There are several factors that can influence the extent and duration of immune deficiency after HCT, including age-associated thymic involution, thymic damage due to pretransplant chemotherapy, radiation and/or antibody therapy, the source and purity of the cells transplanted, disparity of MHC and minor histocompatibility antigen (mHA) between donor and host, the use of post-transplant immune suppression, and graft-versus-host disease (GVHD).

Relationship between recipient conditioning and immune reconstitution

Successful HSC engraftment requires conditioning to (1) create space in the BM for effective engraftment of donor HSCs and (2) eliminate host-versus-graft rejection by recipient immune cells. Typically, conditioning includes cytoreductive chemotherapeutic drugs such as cyclophosphamide and busulfan, total body irradiation, and/or antibodies [such as antithymocyte globulin (ATG) or alemtuzumab] [24]. This results in severe depletion of all hematopoietic cells of the immune system. Both alkylating chemotherapeutics and irradiation target highly proliferative cells [25], including developing and naïve lymphocytes, making them particularly depleted following treatment [26]. The use of ATG (or alemtuzumab) further depletes host T cells and could impair the early development of donor-derived T cells due to the persistence of these antibodies in the patient for several weeks after administration.

Increasing the severity of conditioning can considerably increase the engraftment of unrelated unmatched HSCs, but can also increase treatment-associated toxicity and further prolong post-transplant immune deficiency [27]. Moreover, hematopoietic niches in both the BM and thymus can be significantly impacted by HCT conditioning. In the thymus, in particular, there can be significant damage to the epithelial microenvironment, leading to reduced T-cell development. The severity of thymic damage due to the conditioning regimen is dose related, affects in particular the epithelial microenvironment, and is associated with the pace of post-transplant immune recovery [28–32]. Although the epithelial compartment in the thymus of young patients can recover from these cytoreductive treatments [33,34], this regenerative ability is age dependent and older patients are less capable of thymic regeneration [35]. The thymus is also extremely sensitive to immunosuppressive strategies used as GVHD prophylaxis, and also the damage caused by GVHD itself and its treatment (such as high-dose corticosteroids) [36–40].

Relationship between graft composition and immune reconstitution

The most effective way to reduce the incidence of GVHD in HLA-unmatched or minor-mismatch HCT is to remove specifically all donor T cells from the graft, or create an inoculum with purified HSCs. Despite considerably reducing the risk of GVHD, patients receiving grafts that have been depleted of donor T cells or enriched for HSCs are also likely to undergo prolonged immune depletion [41,42] and increased morbidity and mortality from infection [43]. Moreover, in the case of patients receiving HCT for hematologic malignancies, the removal of antileukemic donor T cells diminishes the likelihood of a beneficial graft-versus-leukemia effect. Therefore, in these cases, the use of purified grafts can potentially lead to an increased incidence of malignant relapse [44,45].

It should be noted, however, that there are significant differences in the potency of graft-versus-leukemia effects based on the malignant disease being treated. For example, in patients with chronic myelogenous leukemia (CML), T-cell depletion (TCD) is associated with an increased risk of relapse [46], whereas donor lymphocyte infusion (DLI) leads to a majority of patients in the TCD group achieving complete remission and no difference in overall survival, compared with recipients of non-TCD grafts. In contrast, studies in patients with acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL) have reported comparable low rates of relapse after TCD HCT [47–49]. A recently published retrospective analysis in AML that compared 115 patients who received TCD grafts after ablative conditioning with a cohort of 181 patients who received unmodified grafts demonstrated no significant differences in the rate of relapse at 3 years between TCD and unmodified graft recipients (18% vs. 25%, $p = 0.3$) [50].

One of the most critical determinants for successful HCT is the number and quality of HSCs transplanted [51]. Although progressive deterioration of the immune system with age is a widely studied phenomenon [52,53], at least part of this degeneration can potentially be attributed to the decreased function of HSCs (reviewed in [11]). With age, HSCs exhibit a reduced engraftment and reconstitution potential [54,55], and an overall skewing away from lymphoid and towards myeloid lineages [56,57]. As a result, the age of an HSC donor can be of considerable clinical importance in determining HCT outcomes [58,59].

In addition, the source of HSCs can also have a significant impact on HCT outcomes. At present, commonly used HSC sources are BM, peripheral blood (PB), and umbilical cord blood (UCB). Homing to the BM of BM-derived HSCs is more efficient than PB-derived HSCs [60]; however, hematopoietic engraftment, as measured by platelet and neutrophil reconstitution, is significantly faster using PB [61]. This can be attributed to the fact that recipients of PB transplant receive fewer long-term HSCs and more committed multipotent progenitors (MPPs) than from adult BM [61]. Although initial studies seemed to indicate delayed immune reconstitution after HCT using UCB, postulated to have been due to the composition of UCB as comprising only primitive HSCs compared with BM or PB [62,63], later studies suggested that the use of ATG during conditioning might have been the reason for this delay [64].

Impact of GVHD on immune regeneration after HCT

Although ideally all HCT patients would receive an infusion of HLA-matched cells, giving the best chance of a successful outcome [24,65], this is not always possible. In fact, it is estimated that HLA-matched related HCT is performed in only approximately 40% of cases [66]. Several studies have identified that hematopoietic organs can be direct targets of alloreactive T cells during GVHD. Specifically, the thymus and BM microenvironmental niches, which are critical for supporting hematopoiesis in the BM and thymopoiesis in the thymus, are severely impacted during GVHD [38–40,67]. This leads to severe and prolonged immune depletion and can explain the widely reported pan-cytopenia observed in patients with GVHD.

Consequences of prolonged immune depletion

The rate and quality of hematopoietic engraftment and immune reconstitution have a significant impact on morbidity and mortality in HCT. This is particularly the case after allogeneic HCT. Deficiencies in B- and T-cell reconstitution after allogeneic HCT can persist for over 1 year, and are associated with an increased risk of infections [68–70], decreased responses to vaccines [71], recurrence of primary malignancy [72,73], and the development of secondary malignancies [44]. Despite novel regimens to prevent and treat invasive viral, fungal, and parasitic infections, opportunistic infections represent an important primary cause of post-transplant morbidity and mortality [68,74]. Not only is the overall risk of infection greater following unrelated HCT, it also extends further post transplant, due to delayed immunologic recovery [68,74].

Endogenous immune reconstitution after HCT

Reconstitution of innate immunity

Innate immunity is comprised of two fundamental components: (1) hematopoietic cells including neutrophils, macrophages, and natural killer (NK) cells and (2) the non-hematopoietic compartment, which includes physical barriers such as skin and mucosal surfaces [75,76]. Although the non-hematopoietic components can be impacted by HCT conditioning including chemo- and radiation therapy, they are rapidly restored following HCT [77]. However, in cases of GVHD, physical barriers, including skin and mucosa, are specifically targeted [78]. The first cells to engraft following HCT are monocytes followed by granulocytes, platelets, and NK cells [77]. Neutrophil counts return to normal within 2–4 weeks; however, functionally they may remain suboptimal for a short time after transplant [79]. Although macrophages are not significantly depleted as a result of transplant conditioning, they are gradually replaced by donor-derived macrophages over the first several months after HCT [80]. Functionally, monocytes may remain suboptimal for up to 1 year [81,82]. NK cells recover both numerically and functionally within the first few weeks following HCT [83]. The importance of NK cell recovery on engraftment and relapse is discussed elsewhere (Chapter 15).

Reconstitution of adaptive immunity

Despite being exquisitely sensitive to negative stimuli, lymphopoiesis has a remarkable ability for endogenous regeneration, allowing for renewal of immune competence after infection, shock, or cytoreductive therapies [36,84]. However, recovery of the adaptive immune system, and in particular T cells, is slow, reflected by prolonged depletion of naïve T cells and reduced function [85,86]. Recovery of T-cell numbers can occur via (1) peripheral expansion of (memory) T cells or (2) endogenous T-cell development. Although there is peripheral expansion of T cells in HCT recipients of a non-T-cell-depleted graft, this ultimately leads to a narrowing of the T-cell receptor repertoire and impaired adaptive immunity [26,33]. In fact, $CD8^+$ T cells recover rapidly in both young and aged patients [87]; however, these primarily expand to fill the niche by clonal expansion [88,89].

Therefore, effective reconstitution of T-cell receptor repertoire diversity and capacity requires a functional thymus [90]. This thymic dependence in generating an effective T-cell repertoire after HCT is a critical hurdle in elderly patients, where thymic atrophy is a natural occurrence of aging [91]. Although recent studies suggest that the aged human thymus does not contribute any naïve T-cell production in the steady state [23], the reported presence of T-cell receptor excision circles (TRECs) and other markers of recent thymic emigrants suggests that, after transplant, the thymus can indeed contribute towards the T-cell pool [41,92,93]. However, in comparison with children, adults whose thymus has atrophied as a consequence of age are significantly impaired in their ability to recover following chemotherapy or HCT [33,85,94]. Full immune recovery is thought to be possible up until middle age, whereas in older patients the naïve T-cell receptor repertoire is never fully restored [95], and has been linked to infectious morbidity [69]. Although not as significant as the depletion of T cells, there is also considerable delay in reconstitution of B cells [96]. Much like the T-cell receptor repertoire, the B-cell antibody repertoire is also severely diminished and suffers a prolonged recovery [97], lasting on average 6 months after autologous and 9 months after allo-HCT [98]. Following allo-HCT, this diminished B-cell reconstitution is predominantly due to GVHD and its treatment [99].

Functional analysis of T-cell reconstitution (monitoring)

A number of approaches are available to assess post-transplant immune recovery (Table 15.1). These range from relatively simple and readily available clinical parameters such as absolute lymphocyte count (ALC), measure of lymphocyte subsets ($CD4^+$ and $CD8^+$ T cells, NK cells, B cells) or antibody titers, to more complex and less routine assays of T- and B-cell repertoire.

The absolute lymphocyte count has been shown to be predictive of survival and relapse after both autologous and allogeneic HCT. In studies performed at the Mayo Clinic, early lymphocyte recovery, defined as an ALC >500 cells/μL at day 15, was an independent predictor of improved progression-free and overall survival after autologous HCT in patients with multiple myeloma, non-Hodgkin lymphoma (NHL), mantle cell lymphoma, Hodgkin lymphoma, acute myelogenous leukemia, primary systemic amyloidosis, and metastatic breast cancer [100]. The findings of these retrospective

Table 15.1 Tests for analysis of immune reconstitution

Test	Cell population	Clinical vs. investigational	Interpretation
Quantitative tests			
Hematology			
CBC + differential	Neutrophils Lymphocytes	Clinical	Neutrophil recovery Lymphocyte recovery
Flow cytometry			
Lymphocyte subsets	T, B, NK cells	Clinical	Recovery of lymphocyte subsets
T-cell subsets	T cells	Research	Naïve, memory, effector subsets and regulatory T cells
Recent thymic emigrants	T cells	Research	Thymic output
B-cell subsets	B cells	Research	Naïve, memory subsets
NK-cell subsets	NK cells	Research	NK, NKT
Molecular tests			
TRECs	T cells	Research	Thymic output
KRECs	B cells	Research	B-cell recovery
Functional assays			
Proliferative responses			
	T cells	Clinical	Response to mitogens, recall antigens, viral antigens
Antibodies			
	B cells	Clinical	Responses to recall antigens, vaccines
ELISPOT			
	T cells	Research	Responses to specific antigens (viral, tumor)
Flow cytometry			
Intracellular cytokines	T cells	Research	Responses to specific antigens (viral, tumor)
Tetramers	T cells	Research	Responses to specific antigens (viral, tumor)
Immune diversity			
Molecular tests			
Spectratyping	T cells	Research	T cell repertoire
	B cells	Research	B-cell repertoire
Next-generation sequencing			
	T cells, B cells	Research	T-cell, B-cell repertoire

studies were further confirmed in a prospective study in patients with NHL that also identified NK cells as the key lymphocyte subset at day 15 affecting survival [101]. In addition, development of lymphopenia measured by ALC post-autologous HCT was associated with a higher risk of relapse of diffuse large B-cell lymphoma (DLBCL) [102]. Similarly, studies performed in patients undergoing allogeneic HCT have also shown an association between ALC and post-transplant outcomes. In recipients of conventional grafts, higher ALC at day 21 or 30 was associated with improved overall and disease-free survival, and also lower relapse rates [45,103].

In patients undergoing partially T-cell-depleted stem-cell transplants with early T-cell add-backs, an ALC at day 30 above the median was associated with improved survival, and a lower incidence of relapse, non-relapse mortality, and acute GVHD [104]. Similar results were observed in patients with CML who underwent the same transplant approach [105]. Furthermore, in CML patients, absolute NK cell numbers at day 30 also predicted survival in addition to the rate of molecular remission. In another study of CD34^{+} selected grafts with limited T-cell add-back, ALC at day 30 was also a strong predictor of non-relapse mortality and overall survival [106]. Finally, in recipients of umbilical cord blood transplant (UCBT), ALC at day 30 also predicted progression-free and overall survival [107].

Quantification of lymphocyte populations, including T, B and NK cells, is also performed on a routine clinical basis. Early CD4^{+} T-cell recovery has been shown to be correlated with overall survival [108], non-relapse mortality [108,109], and the risk of opportunistic infections [108,109]. Progression-free survival also correlated with higher levels of CD3^{+} and CD8^{+} T cells, in addition to regulatory T cells and myeloid dendritic cells, in a prospective study of 95 patients undergoing ablative allogeneic HCT [110].

More recently, multiparameter flow cytometry has been used to define subpopulations of T, B and NK cells further, and also myeloid subsets such as dendritic cells. CD4^{+} and CD8^{+} T-cell subsets include naïve, effector, effector memory, and central memory subpopulations defined by CD45RA, CD28, CD27, CD62L, and CCR7 expression [111–113]. The combination of CD45RA and CCR7 expression distinguishes naïve (CD45RA^{+}CCR7^{+}), central memory (CD45RA^{-}CCR7^{+}), effector memory (CD45RA^{-}CCR7^{-}), and effector (CD45RA^{+}CCR7^{-}) T cells. For example, effector memory CD4^{+} and CD8^{+} T cells were identified as the predominant T-cell subset early after T-cell-depleted allogeneic major HCT [93]. Additional CD4^{+} T-cell subsets include regulatory T cells, defined by co-expression of CD4, high expression of CD25 and FoxP3 [114], and T helper 17 cells that secrete interleukin-17 [115]. In addition, thymic output can be assessed by detection of recent thymic emigrants (RTEs), identified by the CD4^{+}CD45RA^{+}CD31^{+}CD62L$^{\text{bright}}$ CD95$^{\text{dim}}$, and CD8^{+}CD103^{+}CD62L$^{\text{bright}}$CD95$^{\text{dim}}$ phenotypes [116,117]. B cells can also be divided into memory versus naïve B-cell populations by their expression of CD27, IgM, and IgD [118,119]. These markers distinguish between CD27^{-}IgD^{+} naïve B cells, CD27^{+}IgD^{+} "class able to switch" memory cell, and CD27^{+}IgD^{-} "isotype-switched" memory cells [120]. Different NK populations include NK and TCR-Valpha-24^{+} NKT cells. Finally, dendritic cells include myeloid DCs (CD123$^{\text{low/+}}$CD11c^{+}) and plasmacytoid DCs (CD123$^{\text{bright}}$CD11c$^{\text{neg}}$) [121]. Flow cytometry is also used in the identification of antigen-specific responses using either intracellular cytokine detection or tetramers (see below).

In addition to quantitative tests, a number of functional assays provide important information on post-transplant immune recovery. T-cell proliferative responses (measured by ^{3}HTdR incorporation) to mitogens (PHA and OKT3), recall (*Candida*, tetanus), viral [cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV), adenovirus], and allogeneic antigens provide an estimate of T-cell function [68,122,123].

More quantitative functional assays include the ELISPOT, intracellular cytokine secretion detected by flow cytometry, and tetramers. The first two provide functional information by detecting antigen-specific cytokine production by CD4^{+} or CD8^{+} T cells. The last assay directly measures T cells that react with particular peptide–MHC complexes, including cells that may have no functional activity. Antigen-specific cytokine secretion can be elicited by stimulation with cells, lysates, proteins, or peptides. In particular, the use of protein-spanning pools of overlapping pentadecapeptides has the advantage of being able to detect both CD4^{+} and CD8^{+} responses and not be limited by the patient's HLA [124]. These assays can be used to detect viral-specific responses including those to CMV [125,126] and Epstein–Barr virus (EBV) [127,128], and also responses to tumor antigens such as WT1 [129,130]. CMV-specific responses may predict patients who are at higher risk of CMV reactivation or recurrent viremia [125,126]. In contrast, the risk of post-transplant lymphoproliferative disorder (PTLD) did not appear to be predicted by the frequency of EBV-specific T lymphocytes [128]. These assays can also be used to track cells after adoptive transfer [131,132]. One of the advantages of flow cytometric intracellular cytokine secretion is the ability to detect polyfunctional T cells that secrete multiple cytokines.

Polyfunctional T cells have been demonstrated in a number of preclinical models of infectious diseases and also in patients infected with HIV-1 or immunized using vaccinia constructs [133,134]. These studies indicate that polyfunctional T-cell responses correlate with improved control of viral replication, suggesting that an effective vaccine should attempt to elicit these types of responses [135]. Tumor-specific polyfunctional CD8$^+$ T cells have also been detected patients with advanced melanoma immunized against gp100 and tyrosinase [136].

In addition to the use of flow cytometry to assess thymic output, quantification of TRECs, measured by real-time quantitative polymerase chain reaction (PCR) in purified CD4$^+$ and CD8$^+$ T cells according to a method developed by Douek et al. [137], can be used as markers of thymopoiesis. TRECs have shown more rapid recovery in younger recipients and in recipients of conventional grafts compared with T-cell-depleted grafts [138], whereas the occurrence of chronic GVHD significantly decreases TRECs [139]. Furthermore, low TREC values correlated strongly with severe opportunistic infections [138,140]. Levels of TRECs have also been compared between recipients of different stem-cell sources (related, unrelated donors, cord blood), and were found to be comparable by 6 months after transplantation [110]. Similarly to TRECs, the detection of kappa-deleting recombination excision circles (KRECs) reflects newly derived functional bone marrow B cells [141,142].

In addition to the quantitative and functional assays described above, a more detailed understanding of the recovery immune system is based on the analysis of T-cell receptor (TCR) and B-cell receptor (BCR) gene rearrangement diversity, which is typically performed using molecular techniques. TCR repertoire diversity within each Vbeta subset is analyzed using the CDR3 size spectratyping method [143,144]. Similarly, the immunoglobulin heavy chain (IGH) diversity is analyzed using Vh primers.

With the advent of next-generation sequencing, an increasingly detailed analysis of T- and B-cell diversity is emerging. Using this type of approach, we recently studied TCR diversity in allogeneic HCT and found significantly higher diversity in CD4$^+$ T cells than CD8$^+$ T cells, highlighting the need to study these populations separately [145]. Furthermore, a comparison of recipients of different types of HCT showed that the most rapid recovery in diversity was seen in cord blood recipients, followed by conventional grafts and T-cell-depleted grafts. After 6 months, cord blood recipients approximated the TCR diversity of healthy controls. This approach also allows the identification and monitoring of individual clonotypes, including known clonotypes specific for viral epitopes.

The study of immune reconstitution after HCT relies on a variety of quantitative and qualitative assays. With the advent of new technology such as deep sequencing and its ability to provide information not only on TCR diversity but also on the frequency of specific clonotypes over time, future prospective studies will need to use an integrated approach where several complementary methods are used simultaneously to analyze immune recovery. It will then be possible to correlate the data obtained using the different methods and also these measures of immune recovery with clinical outcomes such as survival, infections, and relapse [110,146].

Therapeutic strategies to improve immune reconstitution

Several strategies have been developed in preclinical studies that could boost immune reconstitution after HCT, although to date only a few have progressed to clinical trials. These strategies include the exogenous administration of recombinant cytokines or growth factors, hormone-based therapies, and adoptive cell therapy.

Cytokines and growth factors

Several of the current strategies for promoting immune regeneration focus on the use of physiological cytokines and growth factors. Exogenous administration of keratinocyte growth factor (KGF), interleukin (IL)-7, Flt-3 ligand (Flt3L) and growth hormone (GH) has shown promise for their regenerative potential.

One of the most promising immune-boosting therapies, and one with some positive clinical data, is the pro-lymphopoietic cytokine IL-7, which can act directly on T and B lymphoid precursors [147,148]. Treatment with IL-7 enhances thymopoiesis and recent thymic emigrants in addition to aiding peripheral T-cell function in aged mice or following allo-HCT [149,150]. The mechanism behind IL-7-induced thymic regeneration lies in its ability to enhance the proliferation of lymphocytes and lymphoid precursors. Administration of IL-7 resulted in T-cell proliferation, increased T-cell numbers, and increased TCR diversity both in animal models and in humans [149,151]. Initial clinical trials with recombinant human IL-7 (r-hIL-7) demonstrated an expansion of CD4$^+$ and CD8$^+$ T cells in patients with solid tumors or HIV infection [152–154]. A recent phase I trial of r-hIL-7 in recipients of T-cell-depleted allogeneic HCT showed that r-hIL-7 was well tolerated and induced a rapid increase in peripheral CD4$^+$ and CD8$^+$ T cells [93]. R-hIL-7 also resulted in increased TCR diversity and the generation of functional viral-specific CD8$^+$ T cells in transplants recipients.

Administration of KGF has been found to increase significantly thymic cellularity in aged mice and following radiation or chemotherapy [155–157]. KGF, which induces proliferation and expansion of thymic epithelial cells [158], can also protect TECs from GVHD-mediated thymic damage [157]. Although KGF has been approved for prophylactic treatment of mucositis in recipients of high-dose chemotherapy (including HCT), at present no clinical data exist regarding the potential of KGF to enhance T-cell reconstitution in patients.

The use of GH has also been proposed as a possible regenerative therapy. Treatment with exogenous GH regenerates the aged thymus [159] and enhances HPC function in the BM [160]. GH has also been shown to reverse irradiation-associated loss of BM function determined by colony formation [160]. The clinical experience with GH and T-cell recovery is limited to studies in HIV patients but has been demonstrated to enhance thymus function and anti-viral responses significantly [161,162].

Several other cytokines and growth factors have been evaluated for a beneficial role in regenerating the immune system. These include IGF-1, which promotes TEC expansion and enhances reconstitution following HCT [163]; IL-22, which promotes epithelial proliferation and survival in the thymus [84]; IL-15, which predominantly promotes proliferation of circulating NK and T cells [164]; and IL-12, which stimulates thymic expression of IL-7 and enhances hematopoietic engraftment after transplant [165]. IL-12 and IL-15 have also been found recently to act on regulatory lymphoid-tissue inducer cells and NK cells [166,167]. Furthermore, preclinical studies have shown that administration of Flt3L can also enhance both thymic-dependent and -independent T-cell reconstitution [168,169]. The effects of Flt3L are predominantly due to an expansion in Flt3$^+$ progenitors in the BM [170]. However, increases in T-cell reconstitution can be at the expense of B lymphopoiesis, which is significantly decreased with exogenous Flt3L administration and, in particular, its effects on BM progenitors [171].

Hormone therapies

As sex steroids have been implicated in the degeneration of thymopoiesis [172,173], B lymphopoiesis [174], and early lymphoid precursors [175], sex steroid ablation (SSA) has been investigated for its potential to enhance immune reconstitution. SSA can be achieved pharmacologically by disrupting upstream hormone signals, blocking the binding of sex steroid receptors, or inactivating the hormones themselves. SSA leads to reorganized thymic architecture and an enhanced ability to import circulating progenitors [176], and subsequently enhances thymopoiesis in aged mice and humans [35,177,178]. The effects of SSA, however, are not restricted to the thymus with enhanced B lymphopoiesis and lymphoid progenitors [179,180] also observed, in addition to enhancing overall immune recovery following autologous [181] and allogeneic [182,183] HCT and also cytoablative therapy [28,35,180]. In fact, SSA has been demonstrated to improve thymic function in prostate cancer patients, who routinely receive treatment with a luteinizing hormone-releasing hormone (LHRH) agonist, and also recipients of auto- and allo-HCT, in a dedicated phase I trial [92].

Cellular therapies

Given that some of the delay in immune reconstitution is due to the time it takes for a transplanted HSC to develop into a naïve lymphocyte, several groups have attempted to enhance immune rejuvenation by isolating and co-transplanting BM-derived lymphoid precursors [184]. However, this approach was severely limited by the supply of lymphoid progenitors. The advent of an ex vivo system for generating T cells using Notch-1 stimulation allowed the development of large numbers of T-lineage precursors that could be used for adoptive therapy [185,186]. Adoptive transfer of T-cell precursors into lethally irradiated allo-HCT recipients significantly enhanced thymopoiesis and chimerism, translating into enhanced peripheral T-cell reconstitution [185]. Moreover, recent studies have indicated that precursor T cells can develop into functional T cells even in the absence of a thymus [21,22]. In addition to their significant benefit for immune regeneration following transplant, in vitro-generated pre-T cells can also be genetically engineered for tumor specificity and subsequently used for targeted tumor immunotherapy [187]. Importantly, precursor T cells could be transferred across MHC barriers and develop into host-tolerant and fully competent T cells [187].

In addition to these therapies to boost the immune system that is already present, given the deterioration of the thymus with age and after repeated cytoreductive treatments, several strategies concentrate on building an entirely new thymus for regenerating immunity [188,189]. Although these strategies are still in their preclinical infancy, this can potentially be achieved by using endogenous thymic epithelial progenitor cells (TEPCs) that, in early development, can give rise to an entire thymus [190,191]. The identity and existence of a similar population in the adult thymus have so far remained elusive [192,193]. However, this could be moot, as a recent study has directed the reprogramming of thymic epithelial cells into functional multipotent skin stem cells [194]. A reverse of this approach, by reprogramming skin epithelium into TEPCs, would offer an excellent opportunity either to regenerate the thymus by grafting TEPCs directly or for the generation of an ex vivo transplantable thymus. Another alternative approach that has recently been achieved is to direct induced pluripotent stem cells towards a TEPC lineage. This approach has been shown to aid thymic reconstitution after allo-HCT [195].

Combination therapies

The extensive cross-talk that occurs between the thymic stromal compartment and developing hematopoietic cells [16] suggests that a combination of strategies could offer a greater regenerative boost than could be achieved by use of individual therapies alone. Preclinical studies using KGF have demonstrated that it can be used effectively in conjunction with other regenerative therapies, successfully enhancing the sole regenerative benefits of pre-T cells [185], SSA [31], and temporary inhibition of p53 [196]. However, it is unlikely that IL-7 could be used effectively in combination with KGF or SSA, as both of these strategies promote intrathymic production of IL-7 and studies in knockout mice have demonstrated that both of these regenerative strategies are dependent on IL-7 [155,182]. Another potentially useful combination strategy would be to combine therapies acting on the thymus with those acting on prethymic lymphoid progenitors. In fact, the rationale for this approach has been bolstered by recent studies that suggest that the supply of BM-derived lymphoid precursors limits thymic reconstitution after HCT [197].

Conclusion

HCT is associated with delays in immune recovery that have a significant impact on post-transplant morbidity and mortality from infections and relapse. Post-transplant immune deficiency is due to a variety of factors, including age-dependent thymic involution, conditioning-induced thymic damage, thymic GVHD, GVHD prophylaxis including in vivo or ex vivo T cell depletion, and treatment with immune-suppressive drugs. We have made significant progress in our understanding and monitoring of post-HCT immune recovery. With the introduction of new technologies such as deep sequencing, we are able to obtain information not only on TCR diversity but also on the frequency of specific clonotypes over time. Finally, new strategies are under development to enhance post-transplant T-cell recovery and several of those are now in clinical strategies, such as IL-7, KGF, SSA, and GH.

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CHAPTER 16

Biology of the Human Graft-versus-Tumor Response and How to Exploit It

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Defining characteristics of the graft-versus-tumor effect in human allogeneic hematopoietic cell transplantation

The principle that immune cells derived from the donor could contribute to the eradication of leukemia after allogeneic bone marrow transplantation (BMT) was initially established in murine models [1,2]. A graft-versus-tumor (GVT) effect operating in human allogeneic transplantation and associated with the development of acute and chronic graft-versus-host disease (GVHD) was subsequently identified in retrospective analyses of relapse rates following allogeneic hematopoietic cell transplantation (HCT) with high-intensity conditioning regimens for advanced leukemia [3,4]. Three independent lines of evidence have subsequently confirmed the existence of the GVT effect. First, complete regression of malignant disease after discontinuation of immune suppression has frequently been reported in patients whose tumors recurred after allogeneic HCT. Remissions induced by withdrawal of post-transplant immune suppression have been observed in patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphoid leukemia (CLL), Burkitt lymphoma, diffuse large B-cell and follicular non-Hodgkin lymphoma, multiple myeloma, ovarian cancer, and non-small cell lung cancer [5–7]. Second, infusions of lymphocytes harvested from the original hematopoietic cell donor have successfully been used to treat post-transplant persistence or recurrence of a comparably broad array of malignancies [8,9]. The antitumor activity of donor lymphocyte infusion (DLI) is most effective against CML that recurs in chronic phase after allogeneic HCT, but has also been observed in many patients with other kinds of leukemia, non-Hodgkin lymphoma, and multiple myeloma. Third, allogeneic HCT with reduced-intensity conditioning regimens that permit stable engraftment of donor hematopoietic cells but have little, if any, direct tumoricidal activity has produced durable complete remissions of advanced hematologic malignancies and selected solid tumors, including metastatic renal cell carcinoma, metastatic colon carcinoma, and metastatic breast carcinoma.

Three defining characteristics of the GVT effect were identified in retrospective analyses of relapse rates following HCT with bone marrow grafts from human leukocyte antigen (HLA)-identical sibling donors for early-stage or for advanced leukemia [3,4,10–14], and have subsequently been extensively confirmed in other transplant settings. By comparing the relapse rates seen in patients who developed no GVHD, acute GVHD, chronic GVHD, or both acute and chronic GVHD, it can be inferred that the GVT effect is strongly associated with the development of GVHD (Figure 16.1). However, comparison of the relapse rate observed in HLA-matched allogeneic HCT recipients who did not develop acute or chronic GVHD with that observed in recipients of HCT from syngeneic donors demonstrated that there is an antileukemic effect of allogeneic HCT per se that is independent of clinically significant GVHD. Finally, comparison of the relapse rate seen in HLA-matched allogeneic HCT recipients who received unmodified, i.e., T-replete, bone marrow grafts with that observed in recipients of allogeneic grafts from which T cells had been depleted prior to infusion revealed that the antileukemic effect associated with allogeneic HCT is impaired by removal of T cells from the bone marrow graft.

Identifying the genetic determinants, effector cells, and target molecules of the GVT effect

Autologous and syngeneic HCT

Although the term “GVT effect” as commonly used tacitly implies an antitumor effect that develops in the setting of *allogeneic* HCT and is mediated by donor-derived cells, it is possible that a GVT effect might also occur in the setting of autologous or syngeneic HCT. Identification of putative GVT activity associated with autologous or syngeneic HCT and mediated by cells contained in or derived from the autologous or syngeneic hematopoietic cell graft has been difficult owing to the lack of appropriate controls. In theory, graft-derived immune cells directed at tumor-specific or tumor-associated antigens could mediate GVT responses in the autologous or syngeneic HCT setting. Examples of potential tumor-specific or -associated antigens include the BCR/ABL fusion protein expressed in CML, the PML/RARα fusion protein expressed in acute promyelocytic leukemia, and proteins such as WT1 and proteinase 3 (PR3) that are overexpressed in both myeloid and lymphoid leukemias. The demonstration that the relapse rate after identical twin transplantation is inversely correlated with the nucleated cell dose in the syngeneic graft suggested, albeit weakly, that GVT activity may, indeed, occur after syngeneic HCT [15]. Humoral and $CD8^+$ T-cell immune responses to proteins expressed at high levels in myeloma cells were detected in one patient with multiple myeloma who achieved a complete remission following syngeneic HCT, but the antitumor contribution of these responses was unclear [16]. To date, little experimental evidence for a significant spontaneous antitumor

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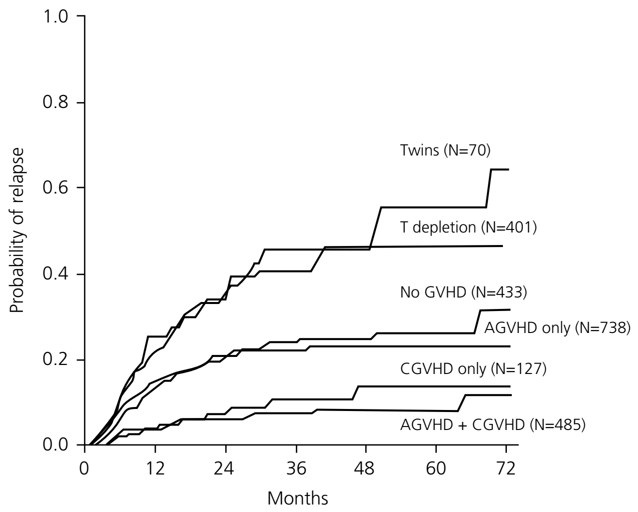

Figure 16.1 Actuarial probability of relapse among 2254 recipients of allogeneic BMT from HLA-identical sibling donors transplanted for CML in first chronic phase, ALL in first remission, or AML in first remission, according to the type of graft and the development of acute or chronic GVHD. Source: Horowitz et al. 1990 [10]. Reproduced with permission of the American Society of Hematology.

effect mediated by graft-derived immune cells after either autologous or syngeneic HCT has been reported.

Stimulation or potentiation of GVT activity in autologous or syngeneic hematopoietic cell grafts has been evaluated in numerous clinical trials. Post-transplant administration of Interleukin-2 (IL-2) without or with ex vivo-activated autologous effector cells was extensively evaluated in the autologous HCT setting [17], with inconsistent clinical results. An attempt to stimulate GVT activity in syngeneic HCT recipients based on adoptive transfer of donor lymphocytes and killed autologous leukemic cells, in order to immunize the infused donor lymphocytes against the autologous tumor, was associated with long-term disease-free survival in a small subset of treated patients [18]. The contribution of immunotherapy with donor cells and autologous tumor to the clinical outcome could not, however, be assessed. Studies in rodent models have demonstrated that administration of cyclosporine after autologous or syngeneic HCT can induce an autoimmune syndrome, termed syngeneic GVHD, with acute and chronic phases that closely resembles GVHD seen after allogeneic HCT [19]. The autoreactive effector lymphocytes in rodent syngeneic GVHD show promiscuous recognition of major histocompatibility complex (MHC) class II determinants, and can recognize and eliminate some MHC class II-expressing tumor cells. These preclinical studies consequently prompted clinical trials of cyclosporine administration after autologous or syngeneic HCT to induce syngeneic GVHD and enhance antitumor activity [20]. Although many patients in these trials did develop signs of GVHD, the induced GVHD was not associated with superior antitumor responses. In recent years, interest in potentiating GVT activity after autologous or syngeneic HCT has increasingly focused on stimulating T-cell responses against tumor-specific or tumor-associated antigens encoded by genes that are over- or aberrantly expressed in the recipient's tumor cells. In general, these antigens are also potential targets for immune therapy after allogeneic HCT, and will therefore be discussed in that context.

Allogeneic HCT

The critical genetic determinants, effector cells, and target molecules of the GVT effect associated with allogeneic HCT are strongly influenced by the degree and nature of genetic disparity between donor and recipient, the source, composition, and processing of the hematopoietic cell graft, and the recipient tumor type. Considerable evidence suggests that donor $CD4^+$ and $CD8^+$ T cells recognizing peptide–MHC complexes on the surface of recipient cells are the central mediators of GVT activity in HCT recipients who receive T-replete grafts from MHC-matched donors. Although there is no direct evidence as yet that donor natural killer (NK) cells contribute significantly to GVT in this setting, accumulating circumstantial evidence suggests the possibility of an NK contribution to GVT activity, particularly in patients with myeloid leukemias. In contrast, numerous studies suggest that donor NK cells are likely to play a central role in GVT responses that occur in recipients of hematopoietic cell grafts from MHC-mismatched donors, particularly if the degree of MHC mismatch requires the in vivo or in vitro depletion of T cells from the donor hematopoietic cell graft in order to prevent lethal GVHD. The characteristics of GVT responses in the MHC-matched and MHC-mismatched settings are therefore discussed separately, below.

GVT in haploidentical and MHC-mismatched HCT

For those patients for whom no MHC-matched donor can be identified, hematopoietic cell grafts from haploidentical related donors can be considered. To lower the risk of severe GVHD, such grafts are usually extensively depleted of T cells prior to infusion ($\sim 1 \times 10^4$ $CD3^+$ cells/kg), or the recipients are treated with agents such as cyclophosphamide or antithymocyte globulin in the early post-transplant period to deplete donor T cells in vivo. (HCT from haploidentical related donors is discussed more extensively in Chapter 46.) Pioneering work performed by the transplant group in Perugia, Italy, demonstrated that relapse after haploidentical HCT was infrequent when donor NK cells reactive with recipient malignant cells could be identified in the recipient post-transplant [21,22]. This observation led to an algorithm for predicting donor NK alloreactivity in haploidentical HCT that was based on donor–recipient MHC genotypes and whether these genotypes predicted incompatibility for a class of lymphocyte receptors encoded in the KIR (killer-cell immunoglobulin-like receptor) locus on chromosome 19q. Genes in the KIR locus encode transmembrane receptors that are expressed in NK cells and subsets of $\alpha\beta$ or $\gamma\delta$ T cells, bind to specific subsets of MHC class I alleles in a manner that is influenced by MHC-bound peptide [23,24], and participate in specific recognition of target cells by NK and T cells. The analysis performed by the Perugia team revealed that predicted donor–recipient incompatibility for the subset of KIR receptors with *inhibitory* function was strongly associated with lower risk of relapse. Functional analysis of donor NK clones from the subset of transplant pairs with incompatibility for inhibitory KIR – defined as the presence of donor NK cells that expressed as their sole inhibitory KIR a receptor for an MHC class I specificity that was expressed by the donor but absent in the recipient, termed "missing self" recognition – also revealed potent in vitro cytotoxicity against recipient lymphocytes, dendritic cells, and leukemic blasts. Moreover, the NK cytotoxicity was demonstrable primarily against lymphohematopoietic cells – including human leukemia engrafted in NOD/SCID (non-obese diabetic/severe combined immunodeficiency syndrome) mice [22] – and not against nonhematopoietic cells, which was thought to explain the potent GVT activity observed in the absence of clinically significant GVHD. A surprising finding in these groundbreaking studies was the observation that GVT activity associated with donor NK alloreactivity in haploidentical HCT was far more effective against myeloid than against lymphoid leukemia, a finding that to date has not been entirely explained. It is likely that ALL cells do not frequently express the cognate ligand for one or more *activating* receptors expressed by alloreactive donor NK cells, appropriate ligation of which is also thought to be required for the activation of NK cytotoxicity [25].

The important role of donor NK alloreactivity in T-cell-depleted haploidentical HCT has been confirmed in many studies, and has prompted the formulation of several clinical algorithms for predicting donor NK alloreactivity in this [26,27] and other [28,29] transplant settings. An alternative to the "missing self" model, the "missing ligand" model, was proposed, which more broadly defines donor–recipient KIR incompatibility as the absence in the recipient of any MHC class I specificity for which donor NK cells express inhibitory KIR, regardless of whether the donor expresses that MHC specificity [26]. This model was used with some success to predict leukemic control – the proxy for GVT activity – in HCT recipients of T-cell-depleted grafts from MHC-matched siblings [28] and in recipients of grafts from unrelated donors [29], supporting a contribution of donor NK alloreactivity to GVT responses in these settings. Although it is still unclear whether the "missing self" or "missing ligand" model more accurately predicts donor NK alloreactivity in HCT recipients who receive T-cell-depleted grafts from matched or mismatched donors, substantial clinical and laboratory evidence supports an important role for alloreactive donor NK cells in GVT responses that occur in the T-depleted, haploidentical, or multiply MHC-mismatched HCT setting.

Analysis of leukemic blasts obtained from patients with recurrent AML or myelodysplastic syndrome (MDS) after haploidentical HCT has provided compelling additional support for the notion that donor lymphocytes with specificity for products of recipient MHC genes on the unshared haplotype are the primary mediators of GVT activity in this setting. Loss of the entire recipient MHC haplotype not shared by the donor, as a result of acquired uniparental disomy of chromosome 6p [30], was observed in leukemic blasts obtained from five of 17 patients who relapsed after haploidentical HCT followed by post-transplant infusion of donor T cells. Loss of the unshared recipient MHC haplotype, once again via a mechanism that involved acquired uniparental disomy, was also observed in leukemic blasts obtained from two of three children with AML who had relapsed after haploidentical HCT [31].

It is plausible that GVT activity in HCT recipients of T-replete or partially T-depleted grafts from MHC-mismatched donors could be mediated by alloreactive donor T cells, alloreactive donor NK cells, or both. Assessment of the relative contributions of donor T and NK cells to GVT activity in this setting is limited by a dearth of relevant clinical and laboratory data. It is likely that the contributions of donor T and NK cells are in part determined by the extent of T-cell depletion and the number and identity of MHC loci at which the donor and recipient are mismatched. In particular, donor T cells are likely to play the more important role in recipients mismatched for MHC class II but not class I alleles, because the specificity of NK cell alloreactivity is determined primarily by the MHC class I, but not class II, molecules expressed on their target cells [32,33]. GVHD and GVT reactions in recipients of T-replete grafts from donors mismatched at a single MHC class I [34,35] or class II [36] locus are typically dominated by donor T cells that are heavily biased towards reactivity with the mismatched recipient allele.

GVT in MHC-matched HCT

Donor–recipient disparity at genetic loci encoded within the MHC on chromosome 6p is the single most important risk factor for the development of severe, life-threatening GVHD after allogeneic HCT [37,38]. Consequently, most patients who undergo allogeneic HCT receive hematopoietic cell grafts from donors with whom they are either genotypically or phenotypically matched at the MHC. The increased relapse rate observed in recipients of T-depleted hematopoietic cell grafts from MHC-matched donors [10,12] indicates that donor T cells play a critical role in GVT responses after MHC-matched HCT. Studies in which CD8$^+$-depleted DLI from MHC-matched donors [39,40] was administered to patients with post-transplant relapse have demonstrated that CD4$^+$ T cells can mediate GVT activity. A role for CD8$^+$ T cells in human GVT has also been suggested by the observation of in vivo expansions of CD8$^+$ T cells with in vitro reactivity against recipient tumor cells during GVT reactions after HCT [41] or DLI [42–46]. A role for NK cells in GVT activity after T-replete MHC-matched HCT has been suggested by reports that donor KIR genotype is associated with risk for relapse in both related [47,48] and unrelated [49–51] pairs, but there is limited direct evidence that NK cells contribute to GVT in this setting [52,53], and some evidence to the contrary [54].

Minor histocompatibility antigens

GVHD and GVT activity occur in the majority of allogeneic HCT recipients who receive T-replete hematopoietic cell grafts from genotypically or phenotypically MHC-matched donors. Thus, GVHD and GVT can also be triggered by donor–recipient genetic disparity at polymorphic loci outside the MHC, which are consequently referred to as minor histocompatibility (minor H) loci. Studies in both mice [55,56] and humans [57,58] have demonstrated that minor H loci encode short peptides – termed minor H antigens – that are encoded by polymorphic genes encoded outside the MHC and presented on the cell surface by MHC class I and class II molecules, where they can be recognized by donor CD8$^+$ and CD4$^+$ T lymphocytes, respectively. CD8$^+$ T cells recognize peptides that are typically 8–11 residues in length and derived from the degradation of intracellular proteins by the proteasome, transported into the endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP), and delivered to the cell surface as a trimolecular complex with MHC class I molecules and β_2-microglobulin. CD4$^+$ T cells recognize peptides that are derived from lysosomal degradation of phagocytosed exogenous proteins or proteins produced endogenously within the cell, and delivered to the cell surface as a complex with heterodimeric MHC class II molecules. Peptides associated with MHC class II molecules tend to be longer than those associated with MHC class I, due to the more "open" configuration of the ends of the peptide-binding groove in the class II heterodimer. Donor T-cell responses to minor H antigens presented on the surface of recipient cells are thought to be the central mediators of both GVHD and GVT activity.

Molecular characterization of minor H antigens

CD8$^+$ and CD4$^+$ T cell clones specific for minor H antigens presented by MHC class I and class II molecules, respectively, can be isolated from most HCT recipients of T-replete grafts [59]. Biochemical and genetic techniques have been used to identify the genes that encode human minor H antigens recognized by T-cell clones from HCT recipients, and to define the nature of the polymorphism in these genes (for a review, see [60]). Global collaborative initiatives in human genetics such as the HapMap Project [61–63] and the 1000 Genomes Project [64] have greatly accelerated the identification and characterization of minor H antigens. As of early 2013, at least 50 different genes that encode minor H antigens recognized by either CD8$^+$ or CD4$^+$ T cells have been identified (Figure 16.2). Although most of these genes are located on autosomes, with virtually every chromosome represented, at least six different genes on the Y chromosome encode male-specific minor histocompatibility (H-Y) antigens. Both sequence variation and structural variation in the human genome can give rise to minor H antigens. Many autosomally encoded minor H antigens are created by non-synonymous single nucleotide polymorphisms (nsSNPs) in protein coding sequences that give rise to immunogenic single amino acid polymorphisms. The prototypic minor H antigen HA-1, for example, is created by an nsSNP in the *HMHA1* gene that in turn creates a His⇔Arg amino acid polymorphism in its protein product [65]. A peptide derived from the antigenic allele of *HMHA1* that spans this polymorphism, VL***H***DDLLEA, binds to HLA-A*02:01 (Figure 16.3), but the homologous peptide encoded by the other allele, VL***R***DDLLEA, binds only weakly to HLA-A*02:01 and dissociates from it very rapidly [66,67]. CD8$^+$ T-cell responses specific for the VLHDDLLEA peptide presented by HLA-A*02:01 frequently occur in HLA-A*02:01$^+$ donor–recipient HCT pairs that are discordant – or "mismatched" – for the antigenic allele, that is, the recipient is positive and the donor negative. In addition to affecting the binding of peptides derived from self proteins to MHC molecules, amino acid polymorphisms created by nsSNPs can alter how such peptides are generated inside the proteasome [68], transported into the ER by TAP, the *t*ransporter associated with *a*ntigen *p*rocessing [69], or are recognized by T cells when presented on the cell surface in a complex with MHC [70].

Although most minor H antigens are created by nsSNPs in consensus protein sequences, variation in the human genome can also create minor H antigens in other ways. SNPs in autosomal genes, for example, can create minor H antigens by generating stop codons [71] or by altering mRNA splice sites [72,73]. A single nucleotide insertion–deletion polymorphism in the *P2RX5* gene that shifts reading frame was shown to create a minor H antigen [74]. Some

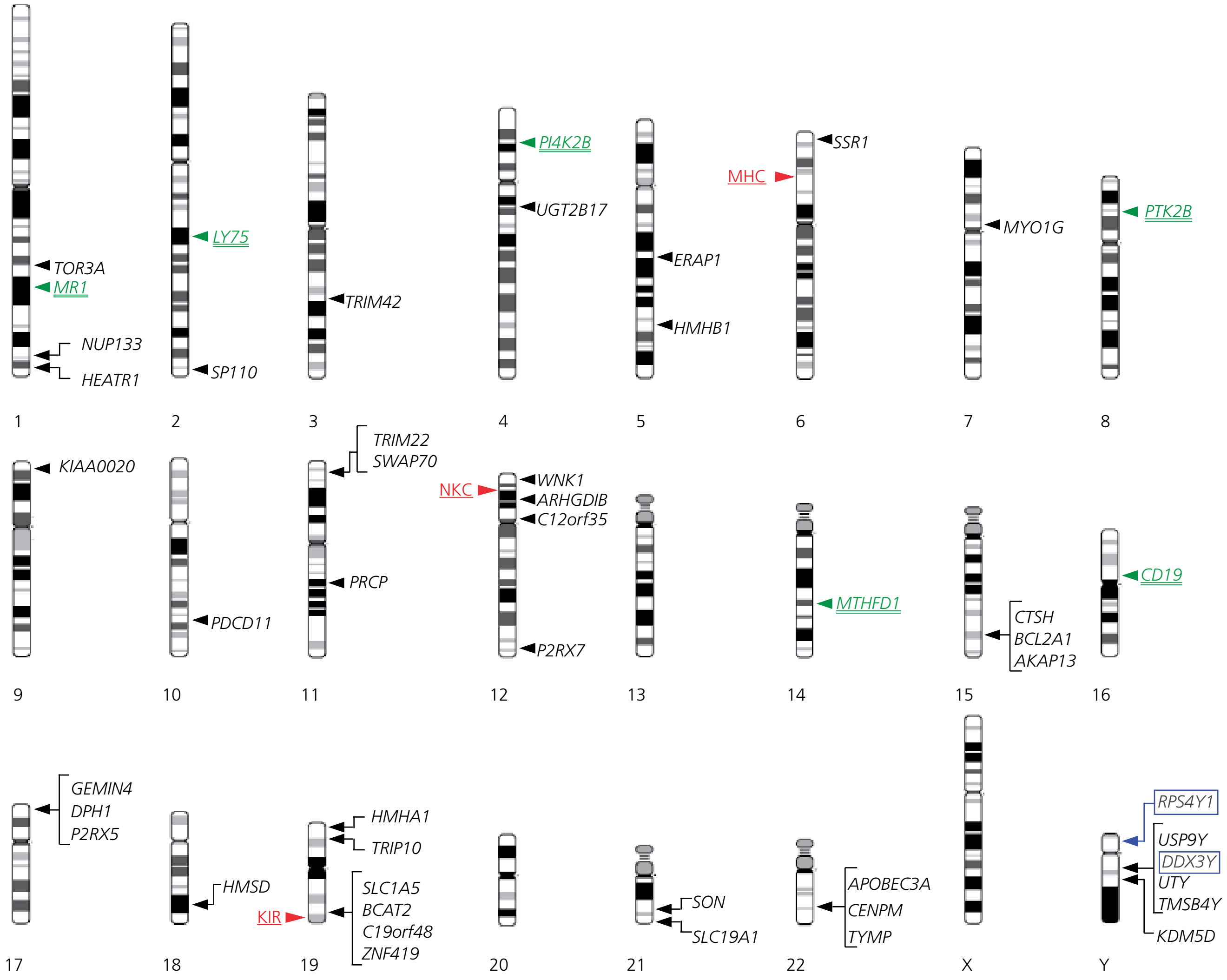
TOR3A
MR1
NUP133
HEATR1
1
LY75
SP110
2
TRIM42
3
PI4K2B
UGT2B17
4
ERAP1
HMHB1
5
MHC
SSR1
6
MYO1G
7
PTK2B
8
KIAA0020
9
PDCD11
10
TRIM22
SWAP70
PRCP
11
NKC
WNK1
ARHGDIB
C12orf35
P2RX7
12
13
MTHFD1
14
CTSH
BCL2A1
AKAP13
15
CD19
16
GEMIN4
DPH1
P2RX5
17
HMSD
18
HMHA1
TRIP10
KIR
SLC1A5
BCAT2
C19orf48
ZNF419
19
20
SON
SLC19A1
21
APOBEC3A
CENPM
TYMP
22
X
RPS4Y1
USP9Y
DDX3Y
UTY
TMSB4Y
KDM5D
Y

Figure 16.2 Map of genetic loci that can influence histocompatibility in the allogeneic HCT setting. The chromosomal location of the MHC, and of two other multigene clusters, the NKC and the *KIR* locus, are indicated by single-underlined labels and arrowheads to the left of the corresponding chromosomes. The chromosomal locations of genes that have been shown to encode T-lymphocyte-defined minor histocompatibility antigens are indicated by labels and arrowheads to the right of the corresponding chromosomes; genes that encode class I MHC-restricted minor H antigens recognized by CD8$^+$ T cells are indicated by black labels, those that encode class II MHC-restricted minor H antigens recognized by CD4$^+$ T cells are indicated by double-underlined labels, and those that encode both class I and class II MHC-restricted minor H antigens are indicated by boxed labels. Source: Warren et al. 2012 [60]. Reproduced with permission of the American Society of Hematology.

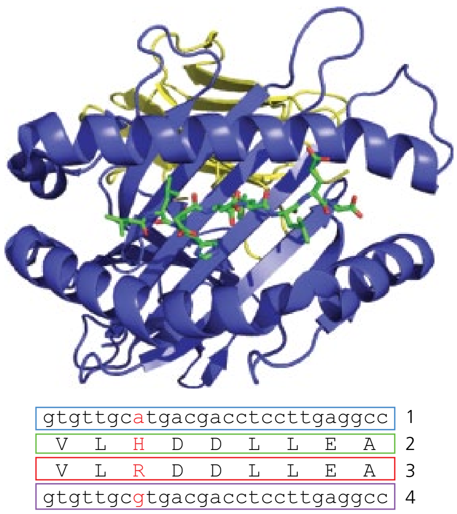

Figure 16.3 (Top) Structure of HLA-A*02:01 with the peptide VLHDDLLEA, the minor histocompatibility antigen HA-1, bound in its peptide-binding groove. The HLA-A*02:01/HA-1 peptide structures were derived from Protein Data Bank accession No. 3D25 [66], and was rendered with PyMOL [173]. (Bottom) The nucleotide sequence of the antigenic and non-antigenic alleles of the *HMHA1* gene in the interval that spans the nsSNP that creates the HA-1 minor H antigen, and their corresponding peptide sequences.

minor H antigens are encoded by SNPs that are not located in consensus protein-coding genes. Several autosomal minor H antigens, for example, are created by SNPs in cryptic, non-canonical open reading frames such as *HMHB1* [70] and *C19orf48* [75] from which no protein product has been identified. Another minor H antigen is encoded in the nominal 3′ "untranslated" region of a protein-coding gene [76]. Variation in genome structure can also give rise to minor H antigens. The *UGT2B17* gene on chromosome 4q is homozygously deleted in a fraction of every human population that has been studied to date [77,78]. Peptides derived from the UGT2B17 protein can serve as minor H antigens in donor–recipient pairs in which one member of the pair expresses the gene but the other is homozygously deleted [79]. Minor H antigens derived from UGT2B17 and presented by HLA-A*29:02, HLA-B*44:03, and HLA-A*02:06 have been identified, and it is likely that additional UGT2B17-derived peptides can bind to additional MHC alleles and serve as minor antigens.

Male-specific minor H antigens

A small class of genes on the Y chromosome has been shown to encode male-specific minor H (H-Y) antigens that are targets for female T cells in sex-mismatched transplants. The genes in this class are distinguished from most other Y chromosome genes by the fact that they have X chromosome homologues that encode proteins that are 81–99% identical in sequence with the Y-encoded isoforms, and they are expressed both in and broadly outside the testis [80,81]. This class of genes is likely derived from the ancestral autosome(s) from which the human X and Y chromosomes evolved [81]. The extensive sequence disparity between the protein products of these Y chromosome genes and the protein products of their X chromosome homologues creates a large number of male-specific peptides that could potentially serve as targets for female T cells in an MHC-matched HCT setting. Moreover, the extra-testicular expression of these genes allows the presentation of these peptides to the female system outside the immunologic sanctuary of the testis. The immunologic significance of H-Y antigens is likely enhanced by the fact that the Y chromosome is inherited as a single functional genetic element, and therefore all H-Y antigens are in complete linkage disequilibrium. More than 25 human H-Y antigens have been identified to date, and it is likely that more remain to be identified.

Expression of minor H antigens in normal tissues and leukemia

Assessment of the expression profile of minor H antigens in normal and malignant tissues has provided insight into the potential contributions of minor H antigen-specific T-cell responses to GVT activity. Many minor H antigens are expressed in both hematopoietic and non-hematopoietic cells in vitro [59,82], suggesting that T-cell responses to these antigens could contribute to both GVT and GVHD. However, a significant fraction of minor H antigens show expression that is limited to cells of hematopoietic origin [59,82,83], raising the prospect that T-cell responses against these minor antigens might contribute selectively to GVT activity. In addition, $CD4^+$ and $CD8^+$ T-cell clones specific for minor H antigens that are selectively expressed in hematopoietic cells recognize lymphoid and myeloid leukemic cells in vitro [59,83–86], and inhibit the growth of clonogenic leukemic cells in in vitro culture [85,86]. $CD8^+$ minor H antigen-specific cytotoxic T-lymphocyte (CTL) clones also inhibit the engraftment of human acute leukemia into NOD/SCID mice [87], demonstrating that leukemic stem cells express minor H antigens and can be targeted by minor H antigen-specific CTL.

Contributions of minor H antigen-specific T-cell responses to GVT

Cellular and molecular dissection of immune responses occurring in HCT recipients after HCT or DLI has provided compelling evidence that minor H antigens expressed on recipient leukemic cells are targets of GVT responses in MHC-matched HCT. Serial flow cytometric analysis with MHC tetramers complexed with minor H antigen peptides to detect and enumerate minor H antigen-specific T cells in the blood of HCT recipients has demonstrated that minor H antigen-specific T cells with in vitro reactivity against recipient leukemic cells increase in frequency in the peripheral blood before and during clinical regression of malignancy. A large in vivo expansion of $CD8^+$, MHC/peptide $tetramer^+$ T cells specific for an H-Y antigen encoded by the Y chromosome gene *KDM5D* and presented by HLA-A*02:01 [88] was observed in a male HLA-A*0201^+ patient who had received a graft from his MHC-identical sister, and this expansion was temporally correlated with achievement of molecular remission of CML in blast crisis [41]. At the peak of the response, $tetramer^+$ cells comprised 15.9% of the $CD8^+$ T cells in peripheral blood. MHC/peptide $tetramer^+$ T cells isolated and purified by flow cytometry demonstrated in vitro cytotoxicity against the recipient's leukemic blasts. Similar studies of GVT reactions occurring in patients with persistence or recurrence of malignancy after HCT who underwent DLI have identified expansions of MHC/peptide $tetramer^+$ cells specific for several other minor H antigens. In vivo expansions of $CD8^+$ T cells recognizing the HLA-A*02:01-restricted HA-1 and HA-2 minor H antigens encoded by the *HMHA1* [65] and *MYO1G* [57,89] genes, respectively, that were temporally correlated with clinical responses to DLI have been observed in HLA-A*$02:01^+$ patients with Ph^+ ALL or CML [43,45,90,91]. $CD8^+$ T-cell responses to minor H antigens encoded by *P2RX5* [74] and by *TRIP10*, *SWAP70*, *SON*, and

NUP133 [76] were demonstrated in other CML patients responding to DLI. Cloned minor H antigen-specific T cells from these patients specifically recognized $CD34^+$ leukemic progenitor cells in vitro. A $CD8^+$ T-cell response to a minor H antigen encoded by the *C12orf35* gene that coincided with achievement of remission after DLI was similarly observed in a multiple myeloma patient [92]. Minor H antigen-specific T cells may also contribute to regression of solid tumors after MHC-matched allogeneic HCT. Extensive regression of metastatic renal cell carcinoma (RCC) was observed in one patient after three separate infusions of donor lymphocytes followed by initiation of interferon-α [75,93]. $CD8^+$ CTL clones recognizing multiple distinct minor H antigens presented by RCC cells were isolated from patient peripheral blood mononuclear cells obtained during and after tumor regression, including $CD8^+$ T cells specific for the HA-1 minor antigen. The observation of HA-1 expression, which in non-malignant cells is limited to the hematopoietic lineage [82], in primary RCC cells is consistent with previous studies that demonstrated aberrant expression of HA-1 on solid tumor cell lines of epithelial origin [94,95].

Although tetramer-based analyses have demonstrated that clinical GVT activity is associated with T-cell responses against specific minor H antigens, it is likely that the GVT effect in most patients comprises simultaneous responses against multiple different target antigens – likely more than can be simultaneously monitored with tetramer-based techniques. Serial analysis of the peripheral blood T cell repertoire in CML [96] or multiple myeloma [97] patients responding to DLI with $CD4^+$-selected cells from MHC-matched sibling donors revealed the appearance of multiple distinct clonal T-cell populations that was temporally correlated with GVT activity. Moreover, complete responses observed in several CML patients after $CD4^+$-selected DLI were associated with in vivo expansion of $CD8^+$ T cells recognizing a number of novel, as yet uncharacterized, minor H antigens expressed on recipient hematopoietic cells [42]. In vivo expansion of $CD8^+$ T cells recognizing myeloma-specific antigens was observed in multiple myeloma patients responding to $CD4^+$-selected DLI [44]. Direct cloning of interferon-γ-secreting peripheral blood T cells from three multiple myeloma or CML patients responding to unselected DLI led to the identification of $CD8^+$ T-cell clones recognizing multiple distinct minor H antigens [46], and analysis of a CML patient who responded to DLI identified simultaneous $CD8^+$ T-cell responses against four different HLA-B*40:01-restricted minor antigens [76].

Numerous retrospective analyses of MHC-matched transplant outcome have attempted to evaluate the contribution of minor H antigen-specific T cells to GVT activity by correlating donor–recipient genetic disparity at specific minor H loci with post-transplant relapse or remission of disease. The studies that focused on disparity at autosomal minor H antigens generally had inconsistent results, but the largest of these showed no significant correlation between donor–recipient disparity at the loci encoding the *HA-1* [98], *HA-8* [99], or *UGT2B17* [100] loci and control of leukemia after HCT. The failure to demonstrate any such correlation is most likely attributable to the fact that the total number of minor H antigens is probably fairly large, and all donor–recipient pairs will be mismatched for scores if not hundreds of minor H antigens [60,64]. The clinical effect associated with disparity at any one locus will therefore account for a very small fraction of the total risk for relapse. In contrast, several large retrospective analyses of transplant outcome have identified a lower incidence of relapse in male recipients of female hematopoietic cell grafts (F→M HCT) compared with all other donor–recipient gender combinations, suggesting that, in the aggregate, T-cell responses to H-Y antigens have a clinically measurable antileukemic effect [101–103]. The lower incidence of relapse observed after F→M HCT is seen in patients transplanted for CML, AML, and ALL, and is apparent even after controlling for GVHD [102]. The expression of some H-Y antigens in leukemic stem cells [104] provides additional support for an H-Y contribution to GVT. The clinically measurable antileukemic effect associated with F→M HCT might, at first, seem surprising, given the lack of an identifiable antileukemic effect associated with donor–recipient disparity at any single autosomal minor H locus. However, this seeming paradox is readily resolved by the recognition that (1) the human Y chromosome contains at least 15 genes with the potential to encode minor H antigens [80,81], (2) each of these genes can potentially encode several distinct H-Y antigens, due to the extensive sequence disparity between their protein products and those of their X chromosome homologues, (3) the H-Y genes encode both MHC class I- and class II-restricted H-Y antigens, and (4) all H-Y antigens are in complete linkage disequilibrium. Therefore, in any female donor–male recipient HCT pair, the Y chromosome has the potential to stimulate a diverse repertoire of $CD8^+$ and $CD4^+$ T-cell responses to many distinct minor H antigens.

HLA-DP as a target for GVT responses in unrelated donor HCT

Alloreactive $CD4^+$ donor T cells specific for recipient HLA-DP molecules likely make a specific contribution to GVT activity in patients with $HLA\text{-}DP^+$ malignancies who receive grafts from unrelated donors. Although most related donor–recipient HCT pairs who are matched at *HLA-A*, *-B*, *-C*, *-DRB1*, and *-DQB1* are also matched at *HLA-DP* [60], several large retrospective studies have shown that most (~80%) unrelated donor–recipient pairs are mismatched for one or two HLA-DP alleles [105–107]. Donor–recipient mismatch at HLA-DP in these studies was consistently associated with a significantly reduced incidence of post-transplant relapse. Analysis of recipient-specific T-cell responses in patients with $HLA\text{-}DP^+$ malignancies who responded to DLI from HLA-DP mismatched unrelated donors has provided compelling direct evidence that donor $CD4^+$ T cells specific for the mismatched recipient HLA-DP alleles can have potent antitumor activity in this setting [108].

T-cell responses to non-polymorphic tumor-associated antigens

Accumulating evidence indicates that non-polymorphic antigens encoded by genes that are aberrantly or over-expressed in myeloid and lymphoid leukemic cells are also targets of T-cell responses that contribute to GVT after MHC-matched HCT. $CD8^+$ CTL specific for an HLA-A*0201-restricted peptide termed PR1 that can be derived from both PR3 and elastase were detected at increased frequency in the blood of several HLA-A*$02{:}01^+$ CML patients who achieved remission after MHC-matched HCT [109]. PR3, also known as myeloblastin, and elastase are both major constituents of the primary azurophil granules of normal promyelocytes and also AML and CML blasts. PR1-specific CTLs recognize myeloid leukemia cells in vitro [110] and inhibit the growth of CML colony-forming units [111]. High levels of PR3 or elastase expression in $CD34^+$ myeloid progenitor cells in patients with advanced-phase CML are also associated with improved outcome after allogeneic HCT [112].

The protein products of several other non-polymorphic genes that are selectively or exclusively expressed in malignant cells can also serve as targets for post-transplant GVT responses. CD8$^+$ T cells specific for peptides derived from the WT1 protein were detected in five out of ten ALL patients in one series [113]. WT1-specific CTL were only detected in those recipients in whom *WT1* expression was detected pre-transplant, and emergence of WT1-specific CTL post-transplant was associated with a decrease in *WT1* expression in the bone marrow. Cancer-testis antigens are occasionally expressed in hematologic malignancies, particularly in advanced multiple myeloma, and CD8$^+$ and CD4$^+$ responses to cancer-testis antigens such as NY-ESO-1 [114] and several members of the MAGE family [115] have been detected after MHC-matched HCT for multiple myeloma and also acute leukemia. Donor T-cell responses against tumor-specific antigens also develop in patients with solid tumors who undergo allogeneic HCT. A potent tumor-specific CD8$^+$ T-cell response directed against an antigenic peptide encoded by human endogenous retrovirus type E was detected in a patient with metastatic RCC who achieved a durable complete remission after non-myeloablative MHC-matched HCT [116].

NK contributions to GVT in MHC-matched HCT

Provocative indirect evidence suggests a role of donor NK cells in mediating GVT after MHC-matched HCT. Several retrospective analyses have demonstrated significant associations between the relapse rate of myeloid leukemias, donor and recipient MHC genotype, and donor KIR haplotype and gene content, in both related [28,48,117] and unrelated [29,49–51,118] HCT pairs. The KIR genes are both polymorphic and highly homologous with one another, and the gene content of the KIR locus on chromosome 19q is itself polymorphic. Two basic types of KIR haplotypes – termed A and B – distinguished primarily by their content of genes for activating receptors have been identified (Figure 16.4). The largest and most compelling studies of MHC and KIR genotype and post-transplant relapse have shown significant associations between the presence in the donor of a type B KIR haplotype, or one or more components thereof – possibly the *KIR2DS1* gene that encodes an activating NK receptor – and decreased rate of relapse in recipients undergoing unrelated HCT for AML and other myeloid neoplasms [49–51]. High levels of donor NK chimerism in the early post-transplant period have been associated with a lower rate of relapse in recipients of non-myeloablative HCT [119]. Although much remains to be learned about the cellular and molecular basis of these associations, they are consistent with the notion that alloreactive donor NK cells may mediate antitumor activity in recipients of HCT from MHC-matched donors.

B-cell contributions to GVT

Analysis of sera obtained from allogeneic HCT recipients has revealed that donor-derived humoral responses to polymorphic and non-polymorphic antigens expressed by recipient cells occur commonly in HCT recipients, and in some cases may contribute to GVT. The possibility of antibody responses to H-Y proteins was first suggested in 1979, when immunoglobulin M antibodies were found in the serum of a heavily transfused female patient with aplastic anemia that were reactive mainly, but not exclusively, with cells from male donors [120]. Analysis of males undergoing F→M HCT has subsequently demonstrated that high-titer IgG responses against Y chromosome proteins can be detected in approximately 50% of cases [121,122]. Mapping the fine specificity of H-Y-specific antibodies has demonstrated a strong bias towards regions of Y chromosome proteins where there is non-identity with the products of their X chromosome-encoded homologues [121], consistent with the antigenic specificity of H-Y T-cell responses. In a study of 121 HCT patients and 134 healthy control individuals, antibodies reactive with the protein products of one or more of five Y chromosome genes – *DBY*, *UTY*, *ZFY*, *RPS4Y*, and *EIF1AY* – were detected in 52% of male patients with female donors but only 9% of male patients with male donors, and in 41% of healthy females but only 8% of healthy males [122]. The presence of antibody responses to one or more of the five Y chromosome genes was also strongly associated with the maintenance of disease remission, suggesting that humoral responses to Y chromosome gene products may be a marker for the selective graft-versus-leukemia effect observed in male recipients of MHC-matched female hematopoietic cell grafts [102].

SEREX (SErological identification of antigens by Recombinant cDNA EXpresson cloning) analysis of allogeneic HCT recipients responding to DLI has demonstrated that GVT activity is associated with humoral responses to proteins expressed in recipient tumor cells. HCT recipients who achieve complete remission of CML [123,124] and multiple myeloma [125,126] after DLI administered for treatment of persistent or recurrent disease make high-titer IgG responses to non-polymorphic antigens that are over- or aberrantly expressed in CML or myeloma cells. Although a large fraction of the humoral response is directed at molecules that are not known to be expressed on the cell surface, antibodies to B-cell maturation antigen (BCMA), which is expressed on the cell surface, were found only in post-DLI responder sera from multiple myeloma patients [126]. Serum containing anti-BCMA antibodies was able to induce

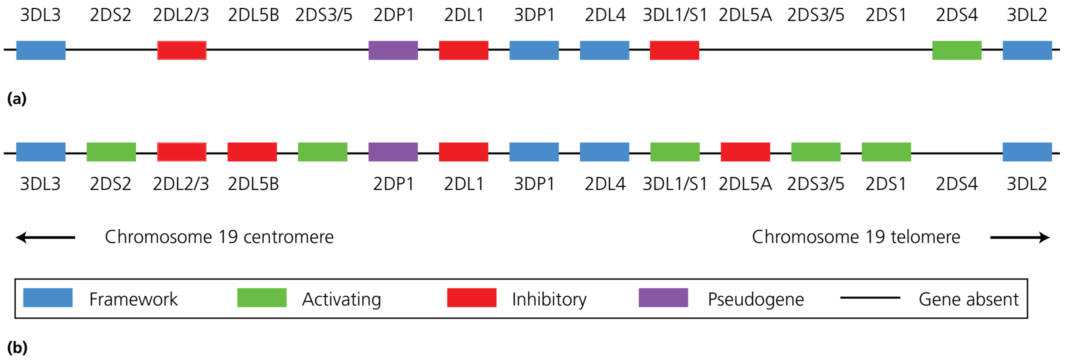

Figure 16.4 Basic organization and gene content of the human *KIR* locus on chromosome 19q. Two hypothetical haplotypes are illustrated: an A and a B haplotype. Framework genes (which can be coding genes or pseudogenes) are illustrated as boxes numbered 1, non-framework activating genes 2, inhibitory genes 3, and pseudogenes 4. Adapted from: EMBL-EBI, http://www.ebi.ac.uk/ipd/kir/sequenced_haplotypes.html, 2014. Reproduced with permission of EMBL-EBI.

complement-mediated lysis and antibody-dependent cellular cytotoxicity of a tumor cell line transfected with a *BCMA* cDNA, and also of primary myeloma cells expressing BCMA, suggesting that BCMA is a potential target of the GVT response in multiple myeloma. Antibodies to tumor-specific antigens such as NY-ESO-1 that are associated with donor NY-ESO-1-specific CD8$^+$ and CD4$^+$ T-cell responses have also been detected in myeloma patients after HCT [114]; whether these antibodies contributed to post-transplant graft-versus-myeloma activity, or simply served as a marker for it, is unknown.

Human genomic diversity and the number of minor H antigens

The advent of high-throughput DNA sequencing over the last half decade has allowed comprehensive exploration of human genetic variation in recent years. As the number of completely sequenced human genomes continues to grow at a nearly exponential rate, it is increasingly clear that genetic diversity both within and between human populations is far greater than was appreciated when the first reference genome was completed in 2000. Preliminary results of the 1000 Genomes Project, for example, have suggested that the genome of any individual will differ from the reference human genome sequence at 10,000–11,000 nsSNPs [64]; thus, by extension, the genomes of any two unrelated individuals will differ by a comparable number of nsSNPs. Concurrent advances in the detection of structural variation in the human genome (operationally defined as sequence variation of at least 50 bp in size [64]) have demonstrated that any two individual genomes will also differ at a large number of structurally variant sites. Indeed, it has been estimated that structural variants account for more heritable nucleotide sequence differences between individuals than SNPs (0.5–1% for structural variants and 0.1% for single nucleotide variants) [127].

The enormous extent of human genomic diversity has profound implications for efforts to understand how polymorphism in the genome can elicit immune responses that contribute to GVT, and also for efforts to exploit these immune responses to decrease relapse and improve HCT outcome. Extensive sequence and structural polymorphism in the genome implies that there are a very large number of protein and peptide sequences that, in the setting of MHC-matched allogeneic HCT, could potentially stimulate and serve as targets for donor T-, NK-, and B-cell responses that could mediate GVT activity. Dissection of GVT responses at the cellular and molecular level is, indeed, beginning to show that GVT in any one HCT recipient is likely to be mediated by an immune response with broad antigenic specificity that is directed at both polymorphic alloantigens and non-polymorphic over- or aberrantly expressed self antigens on recipient tumor cells. The diversity of this response is likely to be one of the keys to the remarkable potency of the GVT effect. Nonetheless, post-transplant persistence or recurrence of disease remains a major cause of treatment failure after allogeneic HCT, and developing a more comprehensive understanding of the GVT response is an urgent research priority. Recognition of the enormous diversity in the genome demonstrates that there is abundant polymorphism to be exploited, but also suggests that whole genome approaches to understanding GVT will likely provide the key to its successful exploitation.

Strategies for exploiting the GVT response

Early attempts to manipulate the GVT effect and decrease the relapse rate after allogeneic HCT were motivated by the recognition that GVT is mediated by cells contained in or derived from the hematopoietic cell graft, and that exogenous immune suppression, although it suppresses GVHD, also suppresses GVT. Complete omission of GVHD prophylaxis in the MHC-matched sibling BMT setting led to severe and hyper-acute GVHD, and the increase in GVHD-related mortality precluded the identification of any decrease in the relapse rate [128]. The prophylactic administration of donor buffy-coat cells after MHC-identical sibling BMT, with a standard course of methotrexate alone as GVHD prophylaxis, was also associated with an increased rate of severe, and often fatal, acute GVHD, and also prevented the detection of any effect on the relapse rate [129]. Characterization of T- and B-cell immune responses associated with GVT activity after HCT or DLI and identification of their antigenic targets has demonstrated that the targets of GVT and GVHD responses are not completely overlapping. Many current efforts to manipulate GVT are consequently focused on the development of therapeutic strategies for enhancing donor immune responses against defined antigens that are selectively or exclusively expressed on recipient tumor cells, and not widely expressed on tissues that are targets for GVHD. In addition, characterization of the extensive sequence and structural variation in the human genome has stimulated the search for novel polymorphic genetic loci that could potentially be exploited to enhance GVT activity.

Identifying genomic loci associated with GVT activity

The identification of extensive sequence and structural variation in the human genome suggests exciting possibilities for exploiting genomic diversity to enhance GVT, but also poses several challenging new questions. What fraction of the polymorphic loci in the genome can elicit T- or B-cell responses that can contribute to GVT? Are there specific autosomal loci at which donor–recipient disparity makes a measurable contribution to GVT activity? Are there genetic variants in the donor or the recipient that influence GVT activity in a manner that is independent of donor–recipient matching or mismatching? The advent of microarray-based genotyping technologies that permit simultaneous determination of SNP genotype and of copy number at millions of unique sites distributed throughout the genome has raised the prospect of using whole genome association analysis to address these questions in a comprehensive fashion. A genome-wide approach to the identification of loci associated with GVT could potentially facilitate the identification of polymorphic loci at which donor–recipient mismatching is significantly associated with decreased risk for relapse, and could identify genetic variants in the donor or in the recipient that, independent of donor–recipient matching or mismatching, are also associated with decreased relapse risk.

MHC genotypically identical sibling pairs represent a transplant population that is uniquely suited to the application of whole genome association analysis for the identification of loci associated with GVT activity. Full siblings share 50% of their genomes and, on average, are expected to be genetically identical for *both* alleles at 25% of their genomes. If relapse risk is in fact measurably influenced by donor–recipient disparity at specific autosomal loci, it is reasonable to hypothesize that HCT recipients who relapse post-transplant will, on average, show more genetic identity with their donors for both chromosomes at such putative "GVT loci" than recipients who do not relapse. Thus, in the case of MHC-identical siblings, where donors and recipients are selected for genetic identity for both alleles within the MHC, this hypothesis predicts that HCT recipients who relapse post-transplant will show *more* genetic identity for both alleles with their donors than the expected 25% at

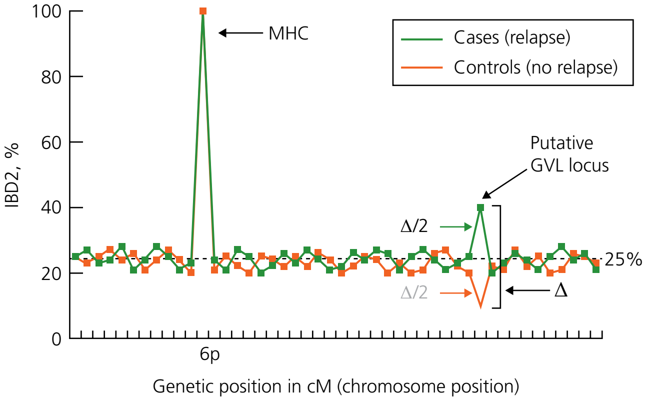

Figure 16.5 Identification of a putative "GVT locus" through assessment of donor–recipient genetic identity for both alleles at a large number of polymorphic loci distributed across the entire genome. MHC-identical sibling donor–recipient pairs are genotyped at hundreds of thousands of polymorphic loci distributed across the genome using high-density oligonucleotide arrays. Although the average fraction of donor–recipient pairs who have identical genotypes at any given polymorphic locus *outside the MHC* is expected to be 0.25, the observed fraction will fluctuate around this mean value. At a hypothetical "GVT locus," the observed fraction of donor–recipient pairs with identical genotypes in a population in which the recipient relapsed ("cases") is expected to be significantly higher than the observed fraction of pairs with identical genotypes in a population of pairs in which the recipient did not relapse ("cases"), with an effect size Δ.

GVT loci, and patients who do not relapse post-transplant will show *less* genetic identity for both alleles with their donors than the expected 25% (Figure 16.5). This hypothesis is currently being tested in a retrospective analysis of 3500 MHC-identical sibling donor–recipient pairs who underwent T-replete allogeneic HCT for the treatment of a myeloid malignancy at a single transplant center.

Donor selection

The current approach to selection of donors for patients requiring allogeneic HCT is focused on the identification of individuals who are identical with the intended recipient for sequences within exons 2 and 3 of the *HLA-A*, *-B*, and *-C* genes and also exon 2 of *HLA-DRB1* [130]. For those patients for whom several optimally matched donors can be identified, deliberate selection of a donor whose genotype at additional loci might confer an enhanced GVL benefit without an increased risk for GVHD would be desirable. For example, the results of several large retrospective studies have suggested that consideration of donor and recipient genotype at additional loci within the MHC – particularly the loci that encode the α and β chains of HLA-DP heterodimers – could potentially lead to improved transplant outcome [105–107,131]. For patients at low risk for post-transplant relapse, selection of a donor who is matched with the recipient at HLA-DP might provide benefit in the form of a lower risk for severe GVHD. In contrast, for those at high risk for relapse, deliberate selection of a donor who is mismatched with the recipient at HLA-DP could prove to be beneficial, as such mismatches are also associated with a reduced incidence of relapse [105,106,131]. Consideration of donor and recipient genotype at loci outside the MHC might also enhance the expected GVL benefit from allogeneic HCT. The lower rates of relapse observed in patients with myeloid leukemias who receive grafts from donors carrying one or more type B KIR haplotypes [49,50] – or KIR genes such as *KIR2DS1* that characterize such haplotypes [51] – suggest that prospective donor KIR typing for patients with two or more *HLA-A*, *-B*, *-C*, and *-DRB1*-matched donor candidates might identify the candidate with an optimal KIR genotype and gene content. Identification of the components of the type B KIR haplotypes (in donors) that are responsible for the lower relapse rate and superior outcome in myeloid leukemia patients – and elucidation of the associated cellular and molecular mechanisms – represent high-priority research objectives. (The subject of donor selection is reviewed more extensively in Chapter 46.)

Donor or recipient vaccination

Active immunotherapeutic approaches to enhancing GVT based on donor or recipient vaccination are being investigated at many transplant centers. Most of these efforts involve immunization of the HCT recipient, as the safety of immunizing healthy donors against minor H antigens or non-polymorphic tumor-associated or -specific antigens has, with a few notable exceptions, not yet been established. Immunogens that have been or are being evaluated in clinical trials include whole proteins, specific peptides or peptide mixtures, DNA, and whole cells, including tumor cells and dendritic cells, most of which are genetically or biochemically modified to enhance their immunogenicity; a variety of adjuvants are also being evaluated. The few published trials to date of donor vaccination have investigated administration of purified idiotype protein harvested from multiple myeloma patients to their MHC-matched sibling donors [132,133]. No significant toxicity of vaccination with purified idiotype protein was observed in these trials, and cellular and humoral idiotype-specific responses were detected in the donors and also in the sibling recipients to whom they donated bone marrow and, in one case, lymphocytes.

The majority of current vaccine studies are focused on eliciting, by post-transplant vaccination of HCT recipients, immune responses to tumor-associated antigens that are shared by many patients. Vaccination with synthetic peptides contained within the sequence of the WT1 or BCR-ABL proteins or with PR1 peptide (derived from PR3 or neutrophil elastase) has been extensively evaluated in the non-transplant setting, and to a lesser extent in the HCT setting. CML patients with post-transplant relapse have been safely vaccinated with BCR-ABL fusion peptides, and $CD4^+$ BCR-ABL fusion peptide-specific T-cell responses were subsequently detected in several patients [134]. A PR1 peptide vaccine has been administered to patients with AML, CML, and MDS in the allogeneic HCT and also non-transplant settings, and has been associated with durable complete remissions in several patients [135]. $CD8^+$ responses to vaccination with PR1 peptide, however, have frequently shown both low avidity and poor durability. A murine monoclonal antibody recognizing the PR1/HLA-A*02:01 complex has been developed, and has been used to demonstrate that this complex is unequivocally present on the surface of normal hematopoietic stem cells, myeloblasts, and monocytes [136]. Expression of this antigen on the surface of normal, non-transformed cells may in part explain why PR1 peptide vaccination often fails to generate high-avidity HLA-A*02:01-restricted PR1-specific $CD8^+$ T-cell responses in HLA-A*$02:01^+$ individuals.

Several patient-specific cellular vaccines comprising irradiated autologous tumor cells without or with other components have been administered to HCT recipients and shown to be both safe and immunogenic. A vaccine consisting of irradiated autologous leukemic blasts admixed with skin fibroblasts transduced with the *IL2* and *CD40LG* (CD40 ligand) genes was administered post-transplant to nine patients who underwent allogeneic HCT for acute leukemia [137]. Vaccination with the recipient cells did not trigger GVHD, and cellular and humoral leukemia-specific immune responses were detected in several recipients after vaccination. In another trial, a cellular vaccine composed of irradiated autologous leukemic blasts transduced with the *CSF2* (*GMCSF*) gene was administered early after transplant to patients with high-risk AML and MDS [138]. No unexpected toxicity of vaccination or aggravation of GVHD was observed, and several patients showed evidence of vaccine-induced immune responses. Although both of these vaccines were tested in single-arm, early-stage trials that were not designed to provide definitive assessment of vaccine contribution to GVL activity, the outcome of patients treated on both trials when compared with historical controls was encouraging. Further evaluation of these promising approaches to enhancing tumor-specific immunity after allogeneic HCT, therefore, appears to be warranted.

Adoptive cellular therapy – donor lymphocytes

The remarkable potency of DLI for the treatment of recurrent CML after HCT, and its frequent serious toxicities – most notably GVHD and myelosuppression [9,139,140] – have generated intense interest in the development of more selective adoptive cellular therapies that have greater antitumor efficacy, against a wider range of malignancies, with less toxicity to normal, non-malignant tissues. Strategies for exploiting GVT that are based on the adoptive transfer of donor-derived cells permit ex vivo selection and manipulation of the cellular product, and potentially of the donor from whom it is derived, prior to infusion, in addition to independent manipulation of the recipient in order to increase the safety and the efficacy of adoptive therapy. Adoptive transfer approaches also allow the genetic modification and marking of cells prior to infusion to enhance their antitumor potency, improve their safety, and facilitate precise monitoring of their in vivo persistence and trafficking after infusion.

Administration of DLI in escalating doses and selective depletion of $CD8^+$ cells from donor lymphocyte products prior to infusion have been performed in an attempt to harness the antileukemic effect of DLI while minimizing toxicity attributable to GVHD. Sequential administration of escalating doses of $CD3^+$ donor lymphocytes has proven to be an effective approach to the treatment of recurrent CML post-transplant [141,142]. Doses in the range of $1–2 \times 10^7$ $CD3^+$ cells/kg of recipient weight have been associated with high response rates and low rates of clinically significant GVHD. Patients who do not respond to an initial dose at this level often respond to subsequent higher doses, at which the risk of GVHD is greater. Based on the hypothesis that the therapeutic efficacy of DLI for CML is attributable to the activity of $CD4^+$ T cells, and that the GVHD associated with DLI is attributable to the activity of infused $CD8^+$ T cells, donor lymphocyte products selectively depleted of $CD8^+$ T cells have been used to treat recurrent CML and multiple myeloma post-transplant [39,40]. Complete responses were observed in a majority of the CML patients and many myeloma patients, with rates of GVHD that were lower than would be expected based on historical controls. Whether the lower rates of GVHD were attributable to the depletion of GVHD-causing $CD8^+$ T cells from the DLI products administered in these studies, or simply due to a lower mean dose of cells administered, cannot be determined from the available data.

A variety of strategies have been explored to enhance the antitumor activity of conventional DLI, with modest success. Administration of individual cytokines such as IL-2 or interferon-α (IFN-α did not consistently enhance the efficacy of DLI, but IFN-α combined with GM-CSF and DLI produced clinical responses in several patients who were refractory to DLI or to DLI and IFN-α) [143]. Surprisingly, however, IFN-α was associated with durable tumor regression in several patients who had persistence of metastatic RCC after reduced-intensity allogeneic HCT and DLI [93,144]. Whether the effect of IFN-α in this setting was attributable to a primary effect on activation or expansion of donor-derived effector cells in the host, or alternatively to a direct antitumor effect on recipient tumor cells, is unknown. Infusion of donor lymphocytes activated and expanded ex vivo via CD3/CD28 costimulation was used in combination with chemotherapy and conventional DLI to treat patients with a variety of aggressive malignancies whose tumors recurred or persisted post-transplant, and complete remissions were seen in eight of 18 patients [145]. Depletion of $CD4^+CD25^+$ presumptive regulatory T cells from DLI products, without or with lymphodepleting chemotherapy prior to infusion, was investigated in a pilot trial [146]. Durable GVL activity accompanied by GVHD was observed in several patients who had not responded to unmodified DLI.

The promising antitumor activity of immune checkpoint inhibitors such as ipilimumab in patients with solid tumors has suggested that these agents might be given in combination with DLI to enhance its antitumor efficacy. The safety and efficacy of a single dose of ipilimumab given as monotherapy for the treatment of post-transplant relapse has already been tested in a pilot study with 29 patients [147]. No aggravation of GVHD was seen, although organ-specific immune adverse events were noted in four patients, and three objective clinical responses (two complete, one partial response) were observed. These results suggest that ipilimumab could be combined with DLI without prohibitive toxicity. Interruption of signaling through the PD-1/PD-L1 axis with blocking monoclonal antibodies was associated with durable regression of several different types of advanced solid tumors in recent trials [148,149], and it is conceivable that interruption of PD-1/PD-L1 signaling with these agents might likewise enhance the antitumor efficacy of DLI.

Ex vivo genetic modification of donor lymphocytes with inducible suicide genes can dramatically improve the safety of DLI, by allowing in vivo ablation of adoptively transferred lymphocytes in the event that they cause GVHD or other toxicity. This approach was pioneered by a transplant group in Milan, Italy [150], who transferred donor lymphocytes transduced with a construct containing the herpes simplex virus thymidine kinase (HSV-TK) gene to recipients of T-cell-depleted grafts from haploidentical related donors. The approach was subsequently extended to the MHC-identical, T-cell-replete transplant setting [151]. The antitumor efficacy of suicide gene-transduced DLI was greater in the T-cell-depleted compared with the T-replete settings due to the immunogenicity of the transgenes, which elicit $CD8^+$ transgene-specific T-cell responses that dramatically limit the in vivo persistence of adoptively transferred, transgene-bearing cells in T-replete HCT recipients [150–152]. Although T-cell responses to transgene products have also been detected after transfer of transduced cells to recipients of T-cell-depleted HCT, GVT activity attributable to adoptively transferred cells has been observed in the majority of recipients [152]. Tumor regression was temporally correlated with the development of GVHD in most responding patients, and GVHD was largely controlled by the subsequent administration of ganciclovir. A novel suicide gene based on fusion of the human caspase 9 death domain to a modified FK506-binding protein (FKBP) domain, which can be activated by an otherwise inert drug that dimerizes the FKBP domain [153], has also been tested in the haploidentical related transplant setting [154]. Donor T cells transduced with the inducible suicide gene increased in frequency after infusion, suggesting limited immunogenicity, and were rapidly ablated with a single dose of dimerizing drug given when GVHD developed.

Adoptive cell therapy with manipulated donor lymphocytes

The low frequency of T cells in unselected donor lymphocyte products that are reactive with recipient tumor cells, combined with the presence in such products of alloreactive cells that contribute to GVHD, has stimulated interest in adoptive therapy with products manipulated ex vivo to expand selectively the subsets of tumor-reactive effector cells. Infusion of donor-derived CTL lines generated by repetitive in vitro stimulation of donor PBMC with recipient CML cells, in one of the first examples of this concept, was followed by complete remission in a CML patient with post-transplant recurrence of accelerated phase disease who had not responded to unselected DLI [155]. Although the contribution of the ex vivo-manipulated cells to elimination of residual leukemia could not be unambiguously established in this case, it did serve to highlight the many potential advantages of adoptive therapy with products enriched for tumor-reactive cells when compared with infusion of unselected donor lymphocytes.

The past decade has witnessed enormous progress in the preclinical development and clinical evaluation of adoptive cell transfer strategies for enhancing GVL activity. A wide range of cell products, in almost all cases donor derived, have been investigated, including homogeneous populations of $CD8^+$ and $CD4^+$ T cell lines or clones that are natively specific for antigens selectively or exclusively expressed on recipient tumor cells, purified natural killer cells, and donor T-lymphocytes that have been genetically modified to render them specifically reactive with molecules or antigens expressed on recipient tumor cells. Key to the successful development and evaluation of these adoptive transfer strategies has been the simultaneous development and continuing optimization of platforms for rapid ex vivo expansion of tumor-reactive cells in cGMP-compliant systems to the numbers required for clinical application. Other technical advances that have profoundly accelerated the development of adoptive cell therapy have included improvements in gene transfer techniques and transgene design.

Adoptive therapy with donor T cells that are natively reactive with antigens expressed by recipient tumor cells, including minor H antigens, non-polymorphic tumor-associated antigens, and viral antigens, has been extensively investigated in recent years. Donor-derived $CD8^+$ cytotoxic T-cell clones specific for tissue-restricted minor H antigens expressed in recipient hematopoietic cells but not recipient fibroblasts were administered to seven patients with relapse of acute leukemia or high-grade MDS after HCT from MHC-matched related donors [156]. Although five patients achieved transient complete remissions after therapy, the in vivo persistence of adoptively transferred cells was limited, and pulmonary toxicity of CTL infusion was seen in three patients. A pilot trial of adoptive therapy with donor-derived T cell lines recognizing the hematopoietic-specific HA-1 minor H antigen to treat relapse in patients with HA-1-expressing leukemias showed minimal toxicity and no GVHD but also little, if any, clinical benefit, and the persistence of transferred cells was again limited [157]. $CD8^+$ T-cell lines reactive with peptides derived from PR3, WT1, or the BCR-ABL junctional sequence were administered prophylactically to 14 CML patients after T-cell-depleted HCT without causing GVHD or other toxicity [158], and seven of the patients achieved durable molecular remission. WT1-specific $CD8^+$ T-cell clones were also given to 11 patients in another trial to prevent or treat relapse of acute leukemia or MDS. Although pulmonary toxicity of unclear etiology occurred in one patient, therapy was otherwise well tolerated. Persistence of adoptively transferred cells was fairly prolonged in some patients, and was correlated with antitumor activity [159]. T-cell lines specific for Epstein–Barr virus (EBV) antigens or the cancer-testis antigen MAGE-A4 have been used to prevent or treat post-transplant relapse of EBV+ [160,161] and EBV− [162] lymphomas, respectively, with potent antitumor activity and durable complete remissions noted in several patients.

Significant improvements in transgene design and in techniques for transferring genes into primary T-lymphocytes to redirect their specificity are actively transforming the field of adoptive cellular therapy by obviating, in most cases, the need to isolate the (usually rare] donor T cells that are natively specific for the desired target antigen. These advances have made it possible to render polyclonal donor T cells, regardless of their native antigenic specificity, capable of recognizing tumor-associated or -specific antigens through the introduction of genes encoding the α- and β-chains of T-cell receptors (TCRs) specific for tumor peptides presented by MHC molecules, or chimeric antigen receptors (CARs) [163] comprising the antigen-binding domains of immunoglobulins typically fused to cytoplasmic signaling domains from the TCR–CD3 complex and one or more costimulatory domains (for a review, see [164]). T cells expressing transgenic TCRs retain the MHC restriction of the T cell from which the receptor was derived, whereas CAR-transduced cells demonstrate MHC-unrestricted cytotoxicity against cells expressing the target antigen – generally a transmembrane molecule with an extracellular domain for which the immunoglobulin moiety of the CAR is specific. TCR- and CAR-transduced donor T cells can be expanded ex vivo with retention of transgenic receptor expression and antigenic specificity, for use in adoptive therapy. Because unselected polyclonal donor lymphocyte populations could contain T cells whose native specificity might include minor H antigens expressed on recipient cells, current strategies for generating TCR- or CAR-transduced donor T cells for use after allogeneic HCT entail isolation of donor T cells specific for common viral pathogens, often herpes viruses such as EBV or cytomegalovirus (CMV), followed by genetic modification with the desired TCR or CAR transgenes. Genetic modification of T cells that are natively virus specific – which are generally far more common in the blood of healthy donors than tumor-specific T cells – should, in principle, create a population of effector cells with potent and specific antitumor activity, by virtue of their transgenic TCRs or CARs, which will not cause GVHD after adoptive transfer, because their native TCRs are specific for viral antigens. Adoptive cellular therapy with ex vivo-expanded TCR- or CAR-transduced T cells has already been extensively tested in the autologous setting, and donor-derived CD19-specific CAR-transduced T cells have been administered to one patient with progressive CLL after allogeneic HCT who subsequently experienced dramatic disease regression [165]. A promising alternative approach to minimizing the risk of GVHD and other potential toxicities associated with adoptive transfer of polyclonal TCR- or CAR-transduced donor T cells is based on the use of zinc finger nucleases to disrupt the endogenous TCR α- and β-chain loci in the T cells [166], which completely abrogates surface expression of their natively encoded TCRs without impairing the specificity or function of the transgenic receptor.

Adoptive therapy with regulatory T cells

Procedures for ex vivo enrichment and expansion of $CD4^+CD25^+$ regulatory T cells that can suppress the in vitro activity or function of effector $CD8^+$ or $CD4^+$ T cells or of NK cells have been described, and there is compelling evidence that these cells can regulate immune responses in vivo (for a review, see [167], and also Chapter 12). The role of such cells in GVHD and GVT after allogeneic HCT in humans remains poorly defined. Studies in murine systems, however, have suggested that adoptive transfer of $CD4^+CD25^+$ regulatory T cells can inhibit GVHD after MHC-mismatched HCT [168–170] without impairing GVT [171]. These studies have suggested that analogous strategies based on adoptive transfer of T cells with regulatory function to preserve GVT while inhibiting GVHD in human HCT could be developed. A pilot clinical trial of adoptive therapy with regulatory T cells expanded ex vivo from umbilical cord blood demonstrated that infusion of the cells was safe and well tolerated [172], suggesting that additional trials to assess the potential of this therapy for preventing GVHD while preserving GVHD are warranted.

Strategies for improving the safety and efficacy of adoptive cell therapy

Despite the considerable progress made in the past decade in adoptive cell therapies for enhancing GVT after allogeneic HCT, much work remains to be done. For example, in vivo persistence of adoptively transferred GVT effector cells will likely be required for optimal antitumor efficacy, but persistence of adoptively transferred cells in many studies has been limited. Several different strategies for improving persistence and establishing durable antitumor immunity, including lymphodepletion of the recipient before adoptive transfer, concurrent administration of T-cell cytokines, and genetic modification of cells for enhanced persistence, are currently being explored. Many recent studies also suggest that the intrinsic differentiation state of adoptively transferred T cells, regardless of whether they are natively tumor reactive or have been rendered so by genetic modification, is an important determinant of the success of adoptive therapy. A growing body of evidence suggests that the differentiation state of $CD8^+$ T cells is inversely correlated with their capacity for proliferation and persistence after adoptive transfer. Improved understanding of the relationship between differentiation state and in vivo persistence and function will likely provide valuable insight into the requirements for establishing sustained GVT activity via adoptive transfer. Genetic modification of cells for adoptive therapy to confer resistance to immune suppressive agents commonly used in the allogeneic HCT setting, such as corticosteroids and mycophenolate mofetil, is also an important and active area of investigation, and such studies, if successful, will facilitate the use of adoptive cell therapy in patients suffering from or at risk for GVHD. Further development of T-cell-depleted transplant platforms in which alloreactive donor T cells are selectively removed from the hematopoietic cell graft prior to infusion will likewise facilitate the development of adoptive therapy in transplant settings where there is a high prevalence of GVHD with unmodified, T-replete grafts. It is anticipated that the development of T-cell-depletion strategies for selectively removing alloreactive donor cells that might potentially contribute to GVHD will alleviate, if not completely eliminate, the problem of poor immune reconstitution observed in recipients of non-selectively T-depleted grafts.

Conclusion

The GVT effect associated with allogeneic HCT is the clearest example of effective immunotherapy in humans. Genetic disparity between donor and recipient is the key to the therapeutic efficacy of HCT, but is also the root of GVHD, its primary limitation. Cellular and molecular dissection of GVT responses has demonstrated that GVT after MHC-matched HCT is likely initiated by donor $CD8^+$ and $CD4^+$ T cells that recognize recipient minor H antigens encoded by polymorphic genes, and that this alloresponse likely recruits additional effector cells recognizing tumor-specific or tumor-associated antigens encoded by non-polymorphic genes that are over- or aberrantly expressed in recipient tumor cells. Accumulating evidence also suggests that donor NK and B cells also contribute to GVT activity. The GVT response in most HCT recipients is therefore likely to comprise a broadly focused immune response directed at a large number of polymorphic and non-polymorphic target antigens. Selective expression of some GVT target antigens in recipient hematopoietic cells and recipient tumor cells, with limited expression in non-hematopoietic tissues, may allow the development of therapeutic strategies for enhancing GVT activity without inducing or aggravating GVHD. Characterization of the extensive sequence and structural variation in the human genome suggests that there are likely to be a large number of polymorphic protein and peptide sequences at which immunotherapy to augment GVT could potentially be directed. It is likely, however, that the extent to which alloimmunity can be exploited to enhance GVT without aggravating GVHD will be limited. Interventions that enhance specific antitumor immunity rather than alloimmunity after allogeneic HCT will therefore have the greatest impact on the outcome of allogeneic HCT.

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CHAPTER 17

Dendritic Cells in Hematopoietic Cell Transplantation

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Introduction: the dendritic cell network

Dendritic cells (DCs) are hematopoietic cells that belong to the antigen-presenting cell (APC) family, which also includes B cells and macrophages. The two main DC subsets are conventional dendritic cells (DCs) and plasmacytoid dendritic cells (PDCs). Although Langerhans cells (LCs) in the skin were described in 1868, their role as APCs was not appreciated until 1973, when Steinman and Cohn identified DCs in mouse spleen as potent stimulators of the primary immune response (reviewed in [1]). Shortly thereafter, several groups reported the presence of DCs in non-lymphoid tissues of rodents and humans, and demonstrated early evidence that these cells contribute to heart and kidney transplant rejection (reviewed in [2]). However, the low number of DCs in vivo, the paucity of markers that distinguish them from monocytes/macrophages, and the problems involved in purifying these cells made for slow progress in the understanding of DC biology. In the 1990s, the development of methods to isolate DCs from blood [3,4] and to generate DC-like cells from bone marrow (BM) or monocytes in vitro [5] led to an explosive growth of the DC field. Forty years after their discovery, the DC's exquisite ability to mount adaptive immune responses to foreign antigens is undisputable, and their contribution to the induction of tolerance to self-antigens is also becoming increasingly evident. Consequently, the potential therapeutic benefits of modulating DCs for vaccines and suppressive therapies against pathogens, tumors, and/or autoimmune diseases are now being pursued in both the academic and industrial worlds. In recognition of the importance of the discovery of the DC system, Ralph Steinman was awarded several prestigious prizes, including the Gairdner Award in 2003, the Lasker Award in 2007, and the Nobel Prize in Physiology or Medicine in 2011 [6]

Classical or conventional DCs (DCs) (Figure 17.1)

Classical DCs refer to all DCs other than PDCs. DCs form a small subset of tissue hematopoietic cells that populate most lymphoid and non-lymphoid tissues. DCs have an enhanced ability to sense tissue injuries, capture environmental and cell-associated antigens, and process extracellular antigens into major histocompatibility complex (MHC) I and MHC II peptides [7–9]. DCs also have a unique ability to migrate loaded with tissue antigens to the T-cell zone of lymph nodes in the steady state and inflamed state [10] and to prime naïve T-cell responses [11]. DCs constitutively express on the cell surface the hematopoietic markers CD45, MHC II, and CD11c and lack T-cell, natural killer (NK)-cell, B- ell, granulocyte, and erythrocyte lineage markers. This definition, however, is much too limited and should not be used in isolation to define DCs, especially in non-lymphoid tissues. Indeed, CD11c expression is evident on several macrophage populations – particularly lung and intestinal macrophages – but also on DC precursors and other leukocytes. Additional markers have now been shown to facilitate the delineation of DCs and macrophages. For example, expression of the tyrosine kinase receptor fms-like tyrosine kinase 3 (Flt3), also called fetal liver kinase 2 (Flk2) or CD135, is an excellent marker with which to distinguish DCs from macrophages.

DCs in non-lymphoid tissues (Figures 17.2 and 17.3)

DCs represent 1–5% of tissue cells depending on the organ and include two major subsets, the CD103$^+$CD11b$^-$ and the CD11b$^+$ DC subsets.

The CD103$^+$CD11b$^-$DC subset

The CD103$^+$CD11b$^-$ DC subset (referred to here as CD103$^+$ DCs) shares its origin and function including a superior ability to cross-present cell-associated antigens with lymphoid tissue CD8$^+$ DCs [12,13]. CD103$^+$ DCs populate most connective tissues. The proportion of CD103$^+$ DCs among total DCs rarely exceeds 20%. In the intestine, CD103$^+$CD11b$^-$ DCs are enriched in the Peyer's patches, and coexpress the marker CD8 on the cell surface, express low levels of MHC II and represent lymphoid tissue-resident CD8$^+$ DCs, whereas most lamina propria CD103$^+$ DCs express CD11b and are discussed in the CD11b$^+$ DC section below [14,15]. CD103$^+$ DCs lack the macrophage markers CD11b, CD115, CD172a, F4/80, and CX$_3$CR1. They express higher Flt3 levels than CD11b$^+$ DCs, proliferate in response to Flt3L and are markedly reduced in Flt3L$^{-/-}$ mice [16]. CD103$^+$ DCs commonly express the C-type lectin receptor langerin, except for intestinal and pancreatic CD103$^+$ DCs [16,17]. CD103 expression is dependent on the tissue environment and is regulated by local production of the cytokine Csf-2 [18–21].

The CD11b$^+$ DC subset

The CD11b$^+$ DC subset is a heterogeneous population of tissue antigen-presenting cells and consists of a mixture of tissue DCs and macrophages, providing a major source of confusion on the exact contribution of DCs and macrophages to tissue immunity. CD11b$^+$ DCs most often lack the integrin CD103 and express the integrin CD11b. In the muscle and lamina propria, CD11b$^+$ DCs arise from DC-restricted precursors and monocytes, but markers that distinguish the two ontogenically distinct subsets differ between tissues.

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For instance, in the muscle, expression of FcgRI helps distinguish between these two subpopulations [22], whereas expression of CD103 helps distinguishes between the two CD11b$^+$ DC subsets in the lamina propria [14,23].

Epidermal Langerhans cells (LCs)

Epidermal LCs are the DCs that populate the epidermal layer of the skin [17]. LCs account for 3–5% of epidermal cells, with approximately 700 LCs/mm^2 [17]. LCs stand apart from other tissue DCs through their unique ontogeny and homeostatic properties [24]. Phenotypically, LCs express lower MHC II, intermediate CD11c levels, and very high levels of the C-type lectinlangerin (CD207) compared with dermal DCs. Langerin is involved in the formation of the intracytoplasmic Birbeck granules, which for a long time represented a conspicuous but pathognomonic marker for LCs. Murine LCs are uniformly CD11b$^+$ and F4/80$^+$ and lack CX_3CR1 expression [17]. In contrast to most DCs, LCs develop independently of Flt3 and Flt3L [16], but require Csf-1 and Csf-1R for their development [25].

Tissue migratory DCs

Tissue migratory DCs are located in the peripheral lymph nodes (LNs), having migrated to the tissue-draining LNs through the lymphatics, in contrast to the blood-borne lymphoid resident DCs [26]. The nature of migratory DCs depends on the site of LN drainage [27,28].

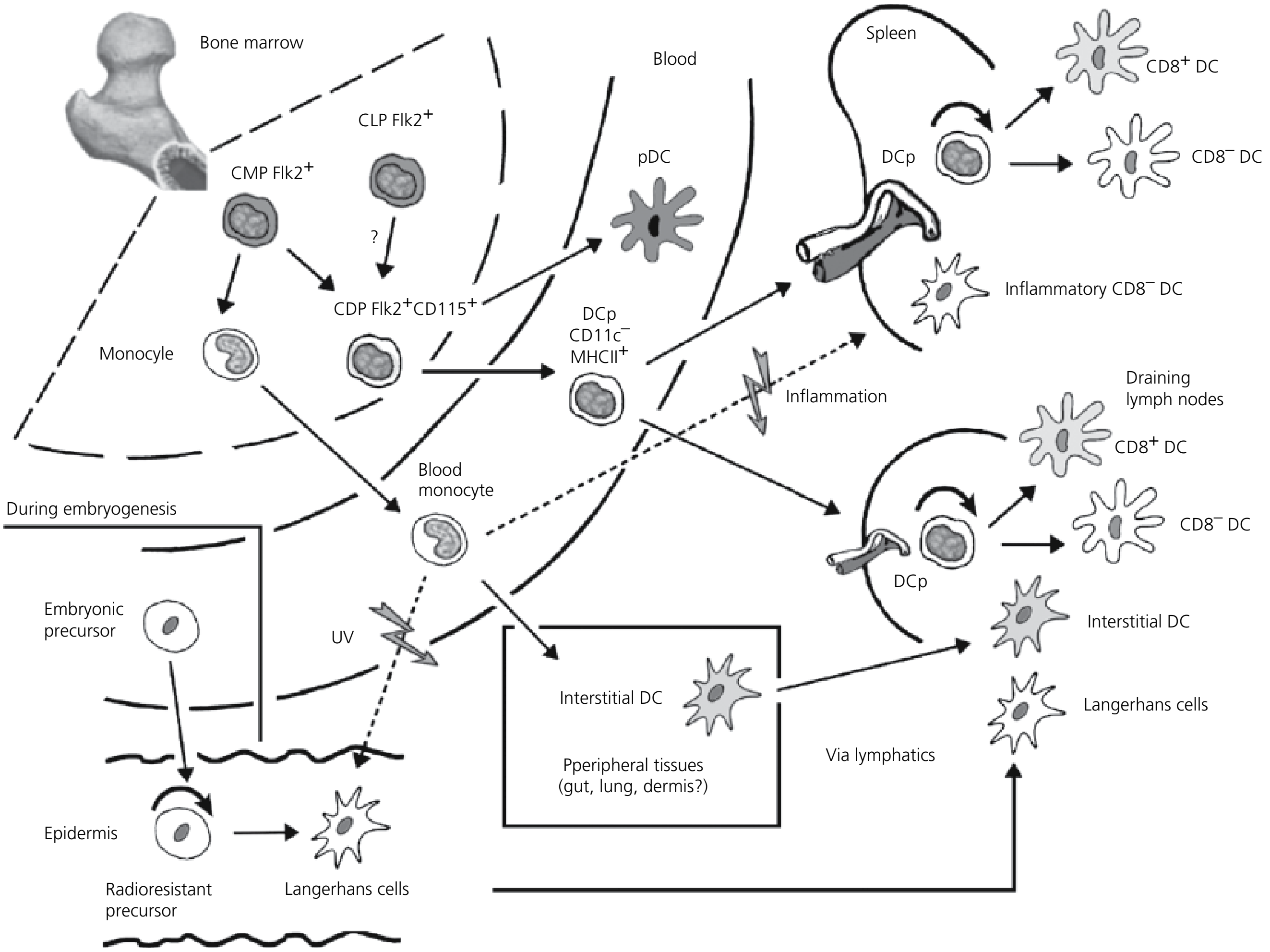
Bone marrow
Blood
Spleen
CD8$^+$ DC
CLP Flk2$^+$
pDC
DCp
CD8$^-$ DC
CMP Flk2$^+$
?
Inflammatory CD8$^-$ DC
CDP Flk2$^+$CD115$^+$
DCp
CD11c$^-$
MHCII$^+$
Monocyle
Inflammation
Draining lymph nodes
CD8$^+$ DC
Blood monocyte
During embryogenesis
CD8$^-$ DC
DCp
Embryonic precursor
UV
Interstitial DC
Interstitial DC
Langerhans cells
Pperipheral tissues (gut, lung, dermis?)
Via lymphatics
Epidermis
Radioresistant precursor
Langerhans cells

Figure 17.1 Origin of dendritic cells (DCs) in mice. This cartoon illustrates a hypothetical view of DC differentiation pathways in mice. Embryonic hematopoietic precursors give rise to Langerhans cells (LCs) in the skin. LCs are maintained by radioresistant hematopoietic progenitors that have taken residence in the skin during steady-state conditions. After severe skin injury, local LC progenitors are eliminated and LCs are repopulated by circulating monocytes. Common myeloid precursor (CMP) and common lymphoid precursor (CLP) give rise to DCs, but the DC differentiation potential is restricted to the Flk2 (fms-like tyrosine kinase 2, the receptor for Flt3L)-positive populations in mice and humans. A common DC/plasmacytoid DC precursor (CDP) has been identified in the bone marrow as an Flk2$^+$CD115$^+$MHC II$^+$ cell. This precursor likely derives from Flk2$^+$ CMP and Flk2$^+$ CLP, although this has still not been confirmed. CDPs give rise to PDCs and to DC precursors (DCps) in the blood (MHC II$^+$CD11c$^-$) that can further differentiate in lymphoid organs into CD8$^+$ and CD8$^-$ DCs. Lymphoid organ DCps divide in situ for three or four cycles. In contrast to lymphoid organ DCs, it is still unclear if CDPs participate in DC homeostasis in non-lymphoid tissues. On the other hand, monocytes have been shown to give rise to DCs in non-lymphoid tissue including the gut and the lung, and potentially the dermis, while they do not give rise to splenic DCs in the steady state. However, under inflamed conditions, monocytes can be induced to differentiate into splenic DCs and epidermal LCs. Lymph nodes (LNs) and mucosa-associated lymphoid tissue contain DCs that originate from the blood (i.e. are CDP progeny) in addition to DCs migrating from peripheral non-lymphoid tissue. For example, skin-draining LNs but not the mesenteric LNs contain LC-derived DCs, while both LNs contain CD8$^+$ DCs. MHC, major histocompatibility complex; UV, ultraviolet.

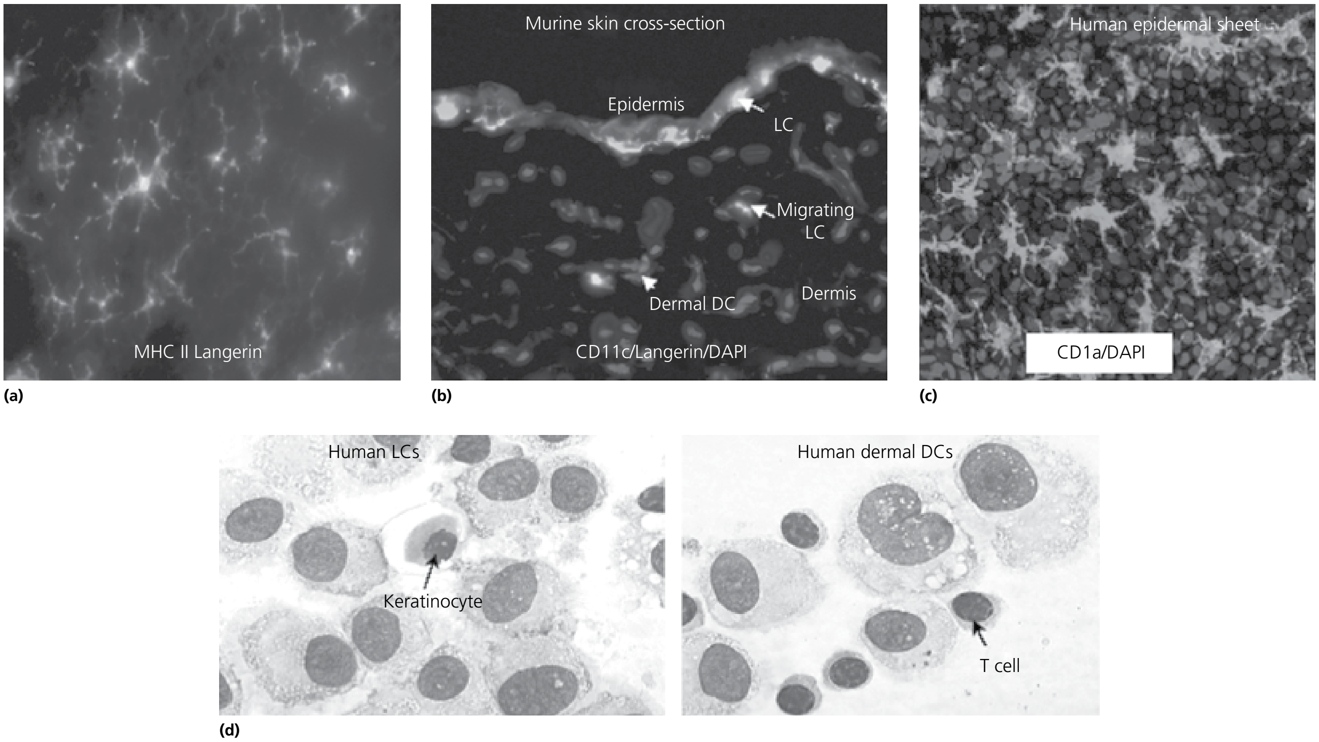
MHC II Langerin
(a)
Murine skin cross-section
Epidermis
LC
Migrating LC
Dermal DC
Dermis
CD11c/Langerin/DAPI
(b)
Human epidermal sheet
CD1a/DAPI
(c)
Human LCs
Keratinocyte
Human dermal DCs
T cell
(d)

Figure 17.2 Cutaneous dendritic cells (DCs) in mice and humans. (a) Epidermal sheet isolated from C57BL/6 mice and stained for major histocompatibility complex (MHC) class II molecules and langerin. Langerhans cells (LCs) coexpress MHC class II and langerin, and form a very tight network of cells that contrast with dermal DCs, which are more sparsely distributed. (b) Cross-section of murine skin stained with anti-CD11c, antilangerin, and DAPI (which stains nuclei) to reveal LCs and dermal DCs. LCs coexpress CD11c and langerin, whereas dermal DCs express CD11c but not langerin. LCs are present exclusively in the epidermis, but migrating LCs on their way to the LNs can also be found in the dermis. (c) Human epidermal sheet stained with anti-CD1a antibody. Human LCs express CD1a; they represent the major hematopoietic cell population in the epidermis, where they form a tight network of cells constituting the first immunologic barrier with the environment. (d) Epidermal and dermal sheets were isolated from human skin maintained in culture for 2 days to allow the LCs and dermal DCs to migrate into the medium. Migrating cells are stained with Giemsa. Arrows show a contaminating keratinocyte in the epidermis and a contaminating T cell in the dermis. Data source: M. Bogunovic and M. Merad (unpublished material) and M. Collin (unpublished material).

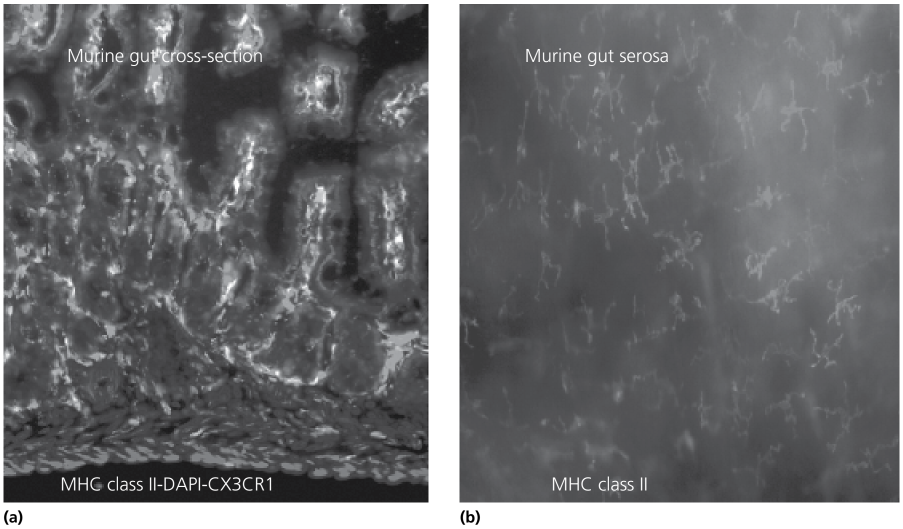
Murine gut cross-section
MHC class II-DAPI-CX3CR1
(a)
Murine gut serosa
MHC class II
(b)

Figure 17.3 Gut dendritic cells (DCs). (a) Cross-section of the colon isolated from transgenic CX3CR1^{-}GFP mice. CX3CR1 is a marker of DCs in the gut. The section is stained with anti-major histocompatibility complex (MHC) class II antibody and DAPI. Gut DCs are MHC class II^{+} CX3CR1/GFP^{+}. (b) Gut serosa whole mount stained with anti-MHC class II antibody. Serosal DCs form a network of cells that resembles the Langerhans network in the epidermis. Source: reproduced with permission of M. Bogunovic and M. Merad.

Although most tissue migratory DCs die in LNs, there is evidence that some DCs exit through efferent lymphatics to gain access to the blood and play a role in tissue immune response and tolerance (for a complete review, see [26,29]). Non-lymphoid tissue DCs constantly migrate through afferent lymphatics to the T-cell areas of LNs charged with antigens [26] in the steady state and this process increases manyfold in response to inflammatory signals (reviewed in [26,29,30]). DC migration to the draining LNs is controlled by CCR7 [30,31]. CCR7$^{-/-}$ mice that lack migratory DCs [31] have helped characterize tissue migratory DCs. In the steady state, migratory DCs are distinguished from resident DCs based on higher MHC II expression and low-intermediate expression of CD11c. However, these features become obsolete during the inflamed setting.

Plasmacytoid DCs (PDCs)

PDCs are extensively reviewed in [32]. Similarly to DCs, PDCs express MHC class II molecules constitutively and lack CD3, CD19, CD14, and CD56 lineage markers. Although human PDCs express very low-to-undetectable levels of CD11c, they express CD4 and CD45RA antigens, the c-type lectin receptor BDCA2, and BDCA4, a neuronal receptor often used to identify PDCs in vivo. Human PDCs also express high levels of the interleukin-3 (IL-3) receptor (CD123). Murine PDCs express low levels of the integrin CD11c, express low levels of the lineage markers CD45RA/B220 and ly6C/GR-1 molecules, lack CD11b, and express plasmacytoid DC antigen-1 (PDCA1) and siglec-H, a member of the sialic acid-binding immunoglobulin-like lectin (Siglec) family, recently identified as a specific surface marker of PDCs in mice. In the steady state, PDCs are generated in BM, circulate in the blood, enter LNs through high endothelial venules, and accumulate in the paracortical T-cell-rich areas of LNs. Hence the migration pattern of PDCs closely resembles that of lymphocytes but is clearly distinct from that of DCs. Migration of PDCs into LNs is greatly enhanced when the LNs drain a site of inflammation. PDCs are absent from non-lymphoid peripheral tissue such as the skin in the steady state, but they can be found in these tissues when they are inflamed.

Human DCs

Most of our understanding of human DC heterogeneity stems from studies of skin and blood DCs. However, increased refinement of flow cytometric approaches allowing cross-correlation of a large number of surface markers and the development of novel genomic profiling methods have recently fostered an improved understanding of human DC heterogeneity**.** Human DCs are defined as cells that lack lineage (Lin) markers (CD3, CD19, CD14, CD20, CD56, GPA) and constitutively express MHC II. Human PDCs were initially described as Lin$^-$MHC II$^+$CD11c$^-$CD4$^-$CD123 (IL-3 receptor)$^+$ and referred to as DC2 [33,34]. The term DC2 is not used any longer and PDCs are best characterized as Lin$^-$MHC II$^+$CD303$^+$(BDCA2) CD304$^+$(BDCA4$^+$), whereas human DCs are characterized as Lin$^-$ MHC II$^+$CD11c$^+$ [35–37], although in humans, CD11c is also expressed on the majority of human monocytes and macrophages. In contrast to mice, in which circulating pre-DCs differentiate into mature DC subsets once they reach peripheral tissues, in humans two DC subsets expressing the non-overlapping markers CD1c$^+$ (BDCA1$^+$) or CD141$^+$ (BDCA3$^+$) [38–40] are present in the blood circulation. CD1c$^+$ DCs represent by far the most numerous DC subset in human blood, whereas CD141$^+$ DCs form a minute blood population. Gene chip (meta)analysis of the transcriptome of multiple mouse and human DC subsets and functional studies of these cells revealed that human CD141$^+$ DCs are related to mouse CD8$^+$ DCs, whereas human CD1c$^+$ DCs are more related to mouse CD11b$^+$ DCs [41,42].

In vitro-derived DCs and PDCs

In humans, CD34$^+$ progenitor hematopoietic cells are commonly used to derive LC- and dermal DC-like cells [43]. Monocytes are also a common source of DC-like cells, referred to as monocyte-derived DCs. Granulocyte–macrophage colony-stimulating factor (GM-CSF) is the most common cytokine used to obtain DCs in vitro, although it is dispensable for DC development in mice in the steady state [44]. Transforming growth factor-β is required to obtain LC-like cells in vitro in humans and epidermal LCs in vivo in mice [45]. Murine BM cells can also be used to obtain DC-like cells in the presence of GM-CSF either alone or in combination with tumor necrosis factor [43]. PDCs can be generated from BM cells in cultures enriched with the cytokine fms-like tyrosine kinase ligand-3 [44]. Although in vitro-derived DCs differ from in vivo-generated DCs, these culture systems have been extremely valuable for analyzing the molecular basis of DC function.

DC functions

In contrast to most cell populations, DCs are defined by a set of properties that include both functional and phenotypic properties. The ability to take up, process, and present antigens, to migrate selectively through tissues, and to prime naïve T-cell responses are defining DC properties. The superior ability of DCs, particularly DCs, to stimulate T cells became clear in the late 1970s from studies that compared the ability of DCs, B cells, and macrophages to stimulate a primary mixed lymphocyte reaction, which could be used as a model for graft rejection if T cells were isolated from the donor graft and DCs from the recipient. Normally, mixed lymphocyte reactions are carried out with equal number of stimulator (APCs) and responder (T cells), but only one DC is necessary to activate 100–3000 T cells, consistent with the concept that DCs are specialized to initiate immunity [1]. Elegant studies have subsequently shown that mice deficient in DCs are defective in initiating adaptive T-cell responses [46], whereas mice that genetically lack macrophages (Csf-1$^{op/op}$) are not [47]. Now it is clear that DCs form a specialized system to prime T cells not only to mismatched MHC proteins, but also to minor histocompatibility antigens (mHAs) and foreign proteins.

More recently, several studies have demonstrated that, in addition to inducing immunity, DCs can play a critical role in the induction of central and peripheral tolerance, a function that might prove useful in the setting of allogeneic HCT. Although it seems counterintuitive that potent APCs induce tolerance, it is now clear that induction of tolerance in addition to immunity requires two critical functions that are performed uniquely by DCs [1]. First, the antigen sampling and migratory capacities of DCs effectively allow naïve T cells to come into contact with peripheral antigens that they would otherwise not encounter. Indeed, cell-derived antigens located in diverse anatomic sites in the body must be found and recognized by T cells that circulate in the bloodstream. This task is made more difficult by the low density of specific antigen–MHC complexes on the surface of most cells (<100 molecules per cell), which must be recognized by low-affinity T-cell receptors on T-cell clones with a frequency of 1/10^6 [1]. In addition, most somatic cells lack costimulatory molecules that drive the clonal expansion of T cells, production of cytokines, and development of cytotoxic T cells. DCs provide a means to overcome these challenges. Located in most tissues, DCs capture and process antigens and display large amounts of MHC–peptide complexes on their surface. DCs charged with antigens migrate to the LNs, where they locate in areas that are rich in T cells. The second major property of DCs is their capacity to translate environmental cues to T cells, in a manner that shapes the nature of the T-cell response to such antigens. The capacity of DCs to translate the environment is based on their capacity to respond to environmental stimuli through a maturation process [48].

One of the most complex and intriguing aspects of DCs is the process by which they mature, in situ, as first revealed in studies of LCs. Thus, freshly isolated LCs, which express low levels of MHC class II, are unable to present antigens to naïve T cells. However, upon culture in vitro, LCs increase their expression of MHC class II and T-cell costimulatory molecules, and are able to prime naïve T cells in vitro [1]. Subsequent studies showed that when LCs receive certain inflammatory signals, they can be induced to leave the epidermis and migrate to the draining LNs, where they localize in T-cell areas. These results establish the concept of DCs as immune sentinels, enabling naïve T cells that lack the ability to circulate through tissues to sample peripheral tissue antigens.

More recent studies have also suggested that not only are DCs able to present and initiate an immune response against tissue antigens, but they are also able to instruct T cells to migrate back to the tissue from which they originated, which could be particularly helpful if DCs acquired antigens at sites of infection [49]. Stimuli such as Toll-like receptor (TLR) signals, inflammatory cytokines, T-cell ligands such as CD40 ligands, and disruption of homotypic contacts between immature DCs have been shown to lead to a series of biochemical and phenotypic changes that result in DC maturation (reviewed in [50]). First, there is an increase in macropinocytic uptake, followed by downregulation of endocytic activity. Second, MHC molecules escape from the lysosomal compartment and are transported to the plasma membrane. Finally, the levels of MHC class II, costimulatory molecules, and MHC class I increase at the cell surface and extend into finger-like projections or dendrites, which dramatically enhance DC migration to the draining LNs and the ability of these cells to interact with other cells in their environment. Concomitant with these events, the cells also enhance their ability to form and accumulate peptide–MHC class II complexes, even using antigens internalized before exposure to the maturation stimulus. The end-point of the maturation process leads to functionally competent DCs able to engage naïve T cells.

Most studies of DC maturation have been performed in the context of injury signals, and therefore DC maturation has been wrongly thought to occur only in the context of inflammation. It now appears clear that mature DCs are present in LNs in the steady state, suggesting that maturation can occur independently of inflammatory signals [48]. The immunologic role of DCs in the steady state is becoming a focus of numerous laboratories. There is considerable evidence that DCs function not only to initiate immune responses, but also to maintain peripheral tolerance, by deleting self-reactive T-lymphocytes and/or expanding regulatory T cells amid a constant steady-state flow of mature tissue-derived DCs to the T-cell area of the draining LN [51].

Antigen presentation

Antigen presentation is extensively reviewed in [50]. All DC populations constitutively express high levels of MHC class II molecules dedicated to presenting peptides derived from exogenous antigens. Mechanisms for capture of these antigens can be distinguished on the basis of the size of the cargo internalized and/or the mechanisms of internalization, which include phagocytosis, macropinocytosis, clathrin-dependent receptor-mediated endocytosis, and caveolin-mediated endocytosis. Phagocytosis involves the ingestion of large particles or cells greater than 1μm in diameter. Macropinocytosis is constitutive in DCs and macrophages, and accounts for the uptake of large quantities of extracellular fluid and fluid-dissolved antigens. Receptors such as DEC-205 and FcRγ, upon ligation to their ligands, form complexes that are typically internalized by clathrin-coated vesicles. Proteins internalized by any of these mechanisms eventually reach the endosomal/lysosomal compartments, where they can be processed by resident proteases and loaded onto MHC class II molecules. Classically, the MHC class I pathway provides for the presentation of endogenous cellular antigens. For example, in virally infected cells, viral protein expressed in the cytosol is subject to proteosomal proteolysis, and the resulting peptides are translocated via transporters associated with antigen processing (TAP transporters) into the endoplasmic reticulum lumen and loaded onto MHC class I molecules. Although this process remains true for infected DCs, non-infected DCs can also take up, process, and present viral antigens to $CD8^+$ T cells through a process called cross-presentation. Soluble exogenous antigens can also be cross-presented to $CD8^+$ T cells. It is now clear that although cross-presentation occurs with greatest efficiency in DCs, other cells, such as macrophages and liver sinusoidal endothelial lining cells, also may have this potential. The mechanisms that regulate cross-presentation are currently an active area of research.

Stimulation of allogeneic T cells

Alloreactivity is the response of T cells to MHC complexes or mHAs not encountered during thymic development. The nature of the allogeneic T-cell response differs depending on whether the presenting MHC molecules of the recipient are matched or mismatched with those of the donor. The frequency of T cells specific for foreign antigens is thought to be approximately one in 10^6, whereas the frequency of T cells that recognize allogeneic MHC molecules is thought to be 1000-fold higher (one in 10^3), which results in a very strong primary alloresponse [52]. The molecular basis underlying the specificity of alloreactivity has been a matter of debate, and several studies have suggested that the response of T cells to allogeneic MHC–peptide complexes is less peptide specific than the T-cell response to conventional antigens, and that this “degenerate” response explains the high frequency of alloreactive T cells [52]. A study in mice elegantly demonstrated that the high precursor frequency of alloreactive T cells is due to polyspecificity and not to “degeneracy” as T cells have a germline-encoded capacity to recognize multiple distinct peptide-mismatch MHC molecules [53].

In current clinical transplantation settings, the MHC molecules of donors and recipients are usually matched. In these situations, donor T cells recognize MHC-bound mHA peptides derived from the protein products of polymorphic genes that are present in the recipient but not in the donor [54]. In contrast to solid tumor-associated antigens, all mHAs should theoretically be able to elicit T-cell responses since the donor immune system is not tolerant to these antigens. The clinical manifestations of immune responses against mHAs are likely to be determined by the specific tissue expression of the proteins encoding these antigens. mHAs constitutively expressed in many tissues are likely to be targets for a combined alloreactive immune response directed against the host tissue [graft-versus-host disease (GVHD)] and the tumor [graft-versus-tumor (GVT)], whereas T-cell responses directed against antigens that are restricted to the hematopoietic system, including the malignant hematopoietic cell clone, are likely to mediate GVT reactivity without severe GVHD. Numerous human mHAs have been described and include mHAs derived from autosomal genes such as HA-1, HA-2, HA-3, HA-8, HB-1, and BCL2A1, and those that derive from the H-Y gene [55]. Most mHA-specific T-cell responses identified to date have been directed against mHAs expressed on hematopoietic cells, which favors the possibility that direct presentation by host professional APCs may be a more efficient way to induce tumor-specific $CD8^+$ T cells than is cross-presentation by donor APCs, which requires uptake of exogenous antigens. Detailed genomic typing of the allelic variants of the targeted mHA in the patient and the donor, in addition to examining the expression and functional recognition of the target mHA on the malignant cells, has the potential to lead to novel immunotherapy strategies in clinical transplantation.

Functions of PDCs

One important function of human and mouse PDCs is to produce large amounts of interferon-α (IFN-α) in response to infectious pathogens. In addition to its antimicrobial properties, IFN-α can also promote T helper cell type 1 differentiation and has been shown to contribute to autoimmune disease [56]. The capacity of PDCs to capture and present antigens to T-lymphocytes is still being examined in several settings. Freshly isolated human and mouse PDCs are very poor inducers of T-cell proliferation; however, upon activation, PDCs can differentiate into mature DCs with high levels of MHC class II and costimulatory molecules and T-cell stimulatory activity [46]. More recent studies indicated that PDCs exposed to immune complexes can use their surface Fc receptors to take up the antigens present in these complexes, leading to their processing and presentation to both $CD4^+$ and $CD8^+$ T cells [57]. In humans, freshly isolated PDCs induce anergy in human $CD4^+$ T-cell clones with various antigenic specificities. IL-2 and IFN-γ production by T cells is impaired, whereas IL-10 production is increased. Anergy is reversed by the addition of exogenous IL-2. In the mouse, freshly isolated antigen-pulsed spleen PDCs induce minimal proliferation and no cytokine polarization in antigen-specific T cells. Thus, PDCs, particularly in their immature state, or in certain anatomic localizations, may have tolerogenic functions, although the exact nature and mechanisms of these functions remain to be explored [46]. In allogeneic HCT, donor PDCs are enriched in granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood mononuclear cells and are also present in donor leukocyte infusions (DLIs). Therefore, the immunomodulatory properties of PDCs should be examined in this context.

Non-professional APCs

Other cell types in addition to professional APCs can express MHC class II molecules, costimulatory molecules, and accessory factors including MHC invariant chains, HLA-DM and HLA-DO, under normal or pathologic conditions. These cells, sometimes referred to as non-professional APCs, include endothelial cells [58] and some epithelial cells [59]. These cells have a heterogeneous capacity for antigen processing and presentation of peptides that are often derived from endogenous proteins. Tissue inflammatory reactions such as those induced by GVHD may also increase the presentation capacity of non-professional APCs, enhancing local inflammation [60].

DC homeostasis in the steady state

Based on our current understanding of DC homeostasis, there is a continual recruitment of blood-borne DC precursors to peripheral tissue and lymphoid organs that gives rise to immature end-stage differentiated DCs. Peripheral tissue DCs migrate through the afferent lymphatics to draining LNs, where their life is limited to days [61]. Recent studies have led to a reappraisal of the importance of local renewal in DC homeostasis under quiescent conditions. Studies of parabiotic mice showed that, in contrast to other DC populations in the spleen and LNs, LCs were maintained exclusively by local hematopoietic precursors that had taken residence in the skin [62]. Local self-renewal of DCs has now been confirmed, although at much lower levels, in a number of tissues, including spleen, LNs, and dermis [63]. Short-pulse-labeling experiments label approximately 5% of tissue DCs without significant incorporation into monocytes or blood DCs, excluding a contribution from recent blood emigrants to labeling in the tissues [63]. In humans, 2–3% of LCs are in S/M_2 phase by cell cycle analysis, and a similar proportion label with Ki-67 antibodies (M. Bogunovic and M. Merad, unpublished data). Recipient LCs can be identified unequivocally 1 year after human BM transplantation [64], and donor LCs persist in transplanted human limbs [63].

Importantly, in contrast to the steady state, severe skin injuries lead to the elimination of self-renewing LC progenitors followed by the recruitment of blood-borne monocytes and their differentiation into LCs in a *csf-1*-dependent manner [63]. These data are consistent with earlier studies suggesting that, in experimental inflammatory conditions, DCs are clearly derived from classic monocytes [65,66], and establish the concept that DC homeostasis is critically dependent upon conditions of quiescence or inflammation.

Monocyte-derived DCs at sites of inflammation

Several studies have demonstrated that monocytes infiltrate inflamed tissues and differentiate into $CD11c^+$ DCs in vivo [67–69]. More recent experiments have shed light on the mechanisms responsible for DC formation in such sites. These studies have shown that T helper cells, which are often seen in association with monocytes/macrophages in inflamed tissues, can drive DC differentiation from monocytes [70]. For example, in the affected skin of patients with psoriasis, TH1 and TH17 cells interact with monocytes and instruct these cells to differentiate into TH1- and TH17-promoting DCs, respectively. Correspondingly, in acute atopic dermatitis, TH2 cells interact with monocytes and elicit the formation of TH2-promoting DCs. DC formation requires cell contact, whereas TH subset specific cytokines dictate DC function and the expression of DC subset specific surface molecules. Interestingly, the phenotypes of T-cell-induced DC subsets are maintained following subsequent stimulation with a panel of TLR agonists, suggesting that TH-derived signals may outweigh downstream TLR signals in their influence on DC function.

Although these results were obtained in studies of human cells, we recently demonstrated in mice that activated TH1, TH2, and TH17 subsets also promote the differentiation of monocytes into functionally specialized DCs (M. Davidson and E. Engleman, in preparation). Of interest, in the murine system whereas all T helper subsets induce the formation of DCs, TH17 cells uniquely promote the formation of IL-12/IL-23, producing DCs that can polarize both naïve and TH17 cells to a TH1 phenotype. This phenomenon was observed in the inflamed spinal cord of mice with experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis, and might be responsible for the well-documented conversion of previously committed TH17 cells into IFNγ-producing TH1 cells within the spinal cord of such mice [71].

These results suggest that TH cells regulate the secondary immune response by instructing monocytes to differentiate into DCs, which in turn trigger naïve CD4$^+$ T cells to acquire similar effector functions as the TH cell subset that initiated DC differentiation. Such an amplification loop would be beneficial during memory responses, but deleterious if uncontrolled during inflammatory diseases. The numerical predominance of TH cells in most chronically inflamed tissues suggests that these cells may be principally responsible for shaping the local DC repertoire. However, this does not exclude the possibility that monocytes also receive signals from other cell types. Thus, NK, CD8$^+$, and NK T cells can also initiate DC differentiation [72–74].

Response of DCs to conditioning therapy

Conditioning

A number of different modalities are used to condition the recipient for HCT. In humans, myeloablative radiotherapy and alkylator-based regimens are still practiced alongside a range of newer reduced-intensity and minimal or non-myeloablative protocols. Conditioning often takes several days, and maintenance of postgrafting immunosuppression is the rule. In murine experiments, radiation at myelosuppressive or lethal doses without postgrafting immunosuppression has been traditionally employed [75]. Reduced-intensity regimens have been developed [76,77], but the majority of murine studies continue to employ single-dose irradiation without immunosuppression.

Effect of conditioning on blood DCs

In common with other leukocytes, peripheral blood DCs are depleted by conditioning therapy. Clinical studies describe a rapid loss of both DCs and PDCs after myeloablative conditioning, which approximates the decline of neutrophils and monocytes [78]. Less is known about blood DC subsets following reduced-intensity conditioning, although the relative preservation of leukocyte counts in some patients suggests that DCs are also less depleted. One report suggests that PDCs are more labile than DCs after reduced-intensity regimens [79], and another study shows that monocyte-derived DCs are more easily isolated after reduced-intensity than high-dose regimens [80]. Alemtuzumab (monoclonal anti-CD52 antibody) is a component of some reduced-intensity regimens and effects a swift reduction in the number of monocytes and blood DCs, which express CD52, unlike their tissue counterparts [81–84]. Patients often have lower pre-transplant DC levels than normal controls, suggesting that BM hypoplasia associated with prior cytotoxic therapy or hematologic disease reduces peripheral DC counts [78].

Effect of conditioning on tissue DCs (Figure 17.4)

Surprisingly, in murine models, there are very few data on the immediate effects of radiation conditioning on DCs. We recently found that exposure to ionized radiation leads to increased complement production by recipient DCs yielding C3a and C5a proteins, which in turn controls donor T-cell survival and effector function [85]. Exposure to ionized radiation also leads to rapid loss of recipient DCs from the spleen within 3–5 days; the peripheral blood, LNs, and liver were also reported to be similarly depleted [86]. Most experimental work has utilized chimerism analysis of congenic transplants following stable engraftment to infer the radiosensitivity of various DC populations. In these studies, the spleen and liver DCs are almost completely replaced. In contrast, epidermal LCs [62,87], a component of the dermal DCs [88] and certain macrophage populations, including brain microglia [89] appear relatively radioresistant. LCs recover over several weeks almost exclusively from local host cells [62]; in the brain about 70% of cells are recipient long term [89] and in the dermis approximately 20% [88]. LNs contain some recipient cells depending on the tissue they drain; notably, skin-draining LNs may contain up to 30% recipient LC-derived DCs after transplantation [87]. Other organs such as the gut have not been evaluated in detail and may contain niche populations of resistant cells. The extent of depletion of murine DCs after reduced-intensity conditioning is currently unknown and may be quite different since previous studies on tissue macrophage populations have demonstrated a significant sparing effect simply due to fractionation of irradiation [90].

The analysis of conditioning effects on human tissue DCs has thus far been restricted to the skin. Several studies have documented depletion of epidermal LCs in the first week of transplantation [91–94], and recent data illustrate a greater effect of myeloablative conditioning [64]. An important variable in human transplantation may be the intensity and proximity of prior therapy, since patients often have lower numbers of leukocytes, including DCs, before transplantation than normal controls [64,95].

Mechanisms of conditioning-induced DC depletion

The mechanisms that govern DC depletion by conditioning therapy are poorly understood. Direct cytotoxicity may occur with some agents, although most observers suggest that the secondary release of cytokines, chemokines, and endotoxin is likely to be responsible for the mobilization of DCs from the tissues [96–98]. The role of

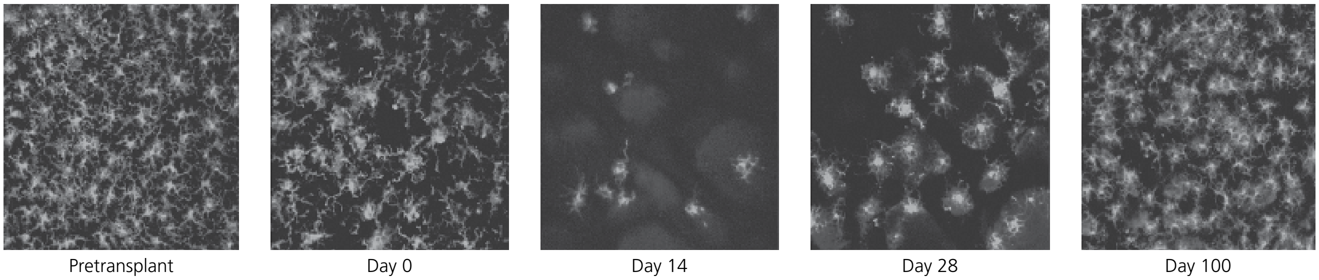
Pretransplant Day 0 Day 14 Day 28 Day 100

Figure 17.4 Cutaneous dendritic cell turnover in patients transplanted with allogeneic hematopoietic stem cells. Epidermal sheets isolated from patients at different times after transplant were stained with anti-CD1a. Langerhans cell numbers are markedly reduced around day 14 and return to normal around day 100 post-transplantation. Scale bar: 40 μm. Source: reproduced with permission of M. Collin.

locally derived tumor necrosis factor-α and IL-1β in mobilizing LCs from skin explants is well described [99]. It should not be overlooked that post-transplant aplasia alone, which accounts for severe leukopenia in the peripheral blood, could lead to a significant depletion of DC populations with rapid turnover. The relative contributions of precursor depletion and enhanced mobilization are difficult to resolve in humans, whereas in mice parabiosis and congenic transplantation have allowed a distinction between populations that are more or less dependent on blood-borne precursors [62,100].

Qualitative effects of conditioning on DCs

Conditioning exerts qualitative effects on DCs. Irradiation induces a transient upregulation of costimulatory molecules and IL-12 expression [86]. As with the stimulation of migration, secondary stimulation by cytokines and microbial products is likely to be important. Although this has not been directly shown in the context of transplantation, circumstantial evidence comes from the ability of cytokine and lipopolysaccharide (LPS) antagonists to diminish GVHD [97,101]. Some work also demonstrated DC activation by NK cells and other effectors of innate immunity (reviewed in [102]).

There are no data concerning the activation status of DCs as the intensity of conditioning is reduced, but the general assumption is that both the direct toxicity to organs and the immediate immunologic consequences are ameliorated. It is interesting that although more intensive conditioning should generate an increased inflammatory response, this may be offset to some extent by a greater depletion of recipient cells prior to transplant. Recipient DCs may "go quietly" after reduced-intensity conditioning, but there is also evidence of a high cumulative rate of late GVHD after some regimens, consistent with eventual recognition of a significant burden of recipient DCs [103,104].

Homeostasis after transplant: content of the graft

The recovery of DC populations after allogeneic HSC transplantation is influenced by complex and interacting variables. The donor hematopoietic cell inoculum contains DC precursors and mature DCs [105,106]. Although practically undetectable after infusion, the relative proportions of DC subsets in different grafts have been linked to transplant outcomes. In particular, PDCs are five times more abundant than DCs in G-CSF-mobilized peripheral blood progenitor cells (PBPCs), whereas DCs and PDCs are present in approximately equal numbers in BM [105,106]. In keeping with this, the proportion of T helper cells type 2 in G-CSF-mobilized PBPCs is much higher than in normal peripheral blood or BM [105,106]. These observations have been used to explain the equal incidence of acute GVHD following PBPC and BM transplant even though the total $CD3^+$ cell content of PBPCs is 10-fold higher than that of BM. Among PBPC recipients, a higher frequency of PDCs was associated with increased risk of death from relapse in one report [107], although in general total DC content is correlated with CD34 dose and a positive outcome [108]. The relatively low risk of GVHD after cord blood transplantation has also been linked to the apparently immature or tolerogenic status of cord blood DCs [109].

Numerical recovery of DCs

With the advent of accurate blood DC counting [78], many studies have analyzed the numerical recovery of blood DCs after allogeneic HCT [78,83,108,110–113]. In the first study, expression of DC activation antigens CD83 and CMRF-44 was used to confirm the identity of lineage-negative, MHC class II-positive cells as DCs. Subsequent work has defined DCs as $CD11c^+$ myeloid or $CD123^+$ PDCs of the lineage-negative, MHC class II-positive fraction. Overall, DCs recover faster than PDCs [79], and brisk reconstitution is associated with improved outcomes [111–113]. This is perhaps not surprising given that normal blood DC counts are promoted by good BM function and the absence of GVHD. DC recovery is apparently not related to the dose of CD34 cells in the graft itself [108].

From earlier work on the skin, it was apparent that LC numbers were reduced for several months post-transplant, although separation of conditioning from later effects was not clear [91–94]. Accurate blood DC counting has since revealed a profound depression of DC counts in association with GVHD [78,79,81,110,113]. In one study, temporal resolution was sufficient to reveal a transient increase in all blood DC subsets just at the onset of acute GVHD, and to demonstrate that treatment with corticosteroids was a major factor in DC depletion during GVHD [111]. A recent study has shown that activation antigens are expressed just prior to acute GVHD such that twice-weekly monitoring can be used to predict GVHD [114]. The mechanism of DC activation is not known, but activated DCs may be the source of IL-12, serum levels of which also have predictive value [115].

Blood DC chimerism

Several studies focusing on the chimerism of human blood DCs have shown rapid repopulation by donor cells, in parallel with blood myeloid engraftment. In all of these studies, a minority of patients had a small percentage of persistent recipient blood DCs [116–119]. Despite the large number of patients reported in the last two studies, no significant correlations have been observed between DC engraftment and conditioning, donor type, or GVHD. This is perhaps related to the robust conditioning regimens used by most investigators. Also, the potential of the studies to discriminate was limited by sampling different subgroups of patients relatively late, at 6 and 12 months post-transplantation. Taking all the data together, mixed DC chimerism appears slightly more frequent after reduced-intensity conditioning, although to date there have been no analyses of DC chimerism following true "non-myeloablative" or "minimal" conditioning [103,104]. Surprisingly, no clear relationship between GVHD and DC engraftment has been demonstrated, although prior studies on total myeloid engraftment predicted that acute GVHD would favor complete donor chimerism in all peripheral blood leukocytes [120,121]. One study of monocyte-derived DCs showed a positive relationship between donor engraftment, GVHD, and protection from relapse, but whether this simply reflects overall myeloid chimerism is unclear [80]. Although laborious, prospective studies of homogeneous patient groups with frequent and strict chronologic assessment will be required to clarify this in detail.

Peripheral tissue DC chimerism (Figure 17.5)

In animal models, there exists a wealth of data on peripheral DC chimerism of the tissues following BM transplantation. Two factors play a role in determining donor chimerism of DCs in the tissues: the radiosensitivity or extent of depletion by conditioning, and the alloreactivity of donor T cells in the graft. Thus, in congenic transplantation, a number of tissues, such as the epidermis, dermis, and cutaneously draining LNs, retain host DCs as described above

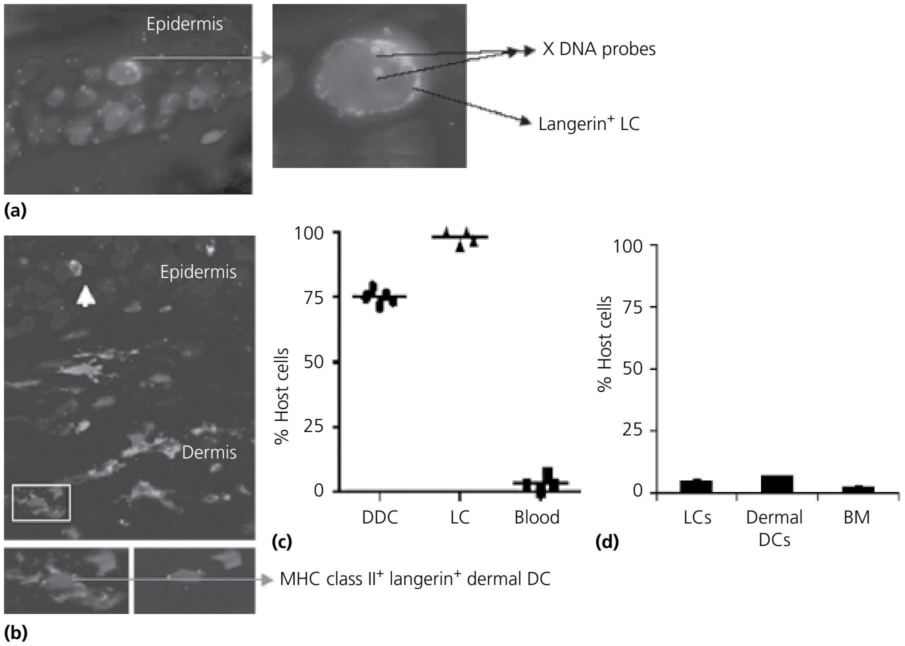

Figure 17.5 Fate of cutaneous dendritic cells (DCs) in patients after sex-mismatched allo-hematopoietic stem cell (HSC) transplant. (a–c) Persistence of human host cutaneous DCs despite complete donor-derived blood chimerism. Non-inflamed skin samples were obtained from patients who underwent reduced intensity for sex-mismatched allo-HSC transplant prior to the development of graft-versus-host disease (GVHD). Cross-sections of 2mm skin biopsies were subjected to fluorescence in situ hybridization of X (Cy3) and Y (Cy2) DNA probes followed by immunostaining of langerin (Cy3) and HLA-DR (Cy5). Nuclei were counterstained with DAPI. One representative image obtained from a female recipient transplanted with male donor hematopoietic cells. (a, b) Overlaid images of three of four color channels in indicated combinations show persistence of host female XX$^+$langerin$^+$ Langerhans cells (LCs) in the epidermis (a) and HLA-DR$^+$langerin$^-$ DCs in the dermis (b). Magnification: ×600. (c) Dot graphs show the percentage of remaining host langerin$^+$ LCs among total the LCs, and HLA-DR$^+$langerin$^-$ cells among the total dermal DCs (DDCs), and the percentage of remaining host leukocytes among the total bone marrow (BM) leukocytes, in each patient 30 days after transplantation. (d) Absence of residual host DCs in advanced cutaneous GVHD lesions. GVHD-affected skin samples were isolated from four patients who underwent myeloablative conditioning for allo-HSC transplant and developed GVHD. The dot graph shows the percentage of remaining host LCs, host dermal DCs, and host BM nucleated cells in five separate patients. Data source: M. Merad and M. Bogunovic (unpublished material).

[62,88]. In addition, several studies showed significant retention of host-derived macrophages in the brain, lungs, and liver [89,90]. However, the situation is radically different when host-reactive donor T cells are introduced in allogeneic transplantation. The effect of donor T cells is generally illustrated by experiments in which mixed myeloid chimeras are converted to complete donor chimerism by lymphocyte infusion [77]. The case for isolated persistent recipient DCs is most clearly demonstrated by the conversion of LCs to donor type by donor T cells [87]. Under these conditions, self-renewing local progenitors of LCs are depleted from the skin and replaced by inflammatory monocytes [122]. The fact that skin is a target organ of GVHD may be critical in this process, and it is perhaps less likely that donor T cells can also eliminate APCs in non-inflamed organs such as the brain.

DC chimerism in the tissues is more difficult to examine in humans, and for this reason all studies of human transplant recipients completed to date have been confined to the skin. An important early paper described the persistence of recipient LCs for up to 120 days after conventional transplantation, using quinacrine to identify the Y body of male CD1a$^+$ (OKT6$^+$) cells [123]. An interim report showed no clear engraftment of LCs with time, which may have reflected technical difficulties with combined immunofluorescence and fluorescence in situ hybridization prior to confocal microscopy [124]. More recent studies using emigrated LCs found that engraftment is largely complete by 100 days, although engraftment occurs more slowly after reduced-intensity conditioning [64,95]. The effect of prior GVHD in promoting LC engraftment is just discernible at 100 days, and patients without GVHD often harbor a small percentage of recipient LCs for at least 1 year after transplant [64]. Murine data also show persistence of recipient dermal DCs, especially after reduced conditioning [88]. It will be difficult to ascertain in humans whether other interstitial DCs, such as splenic or hepatic DCs, behave similarly to LCs or dermal DCs. Extrapolation from murine studies suggests that LCs have a unique mechanism of homeostasis and that other tissue DCs should turn over even more rapidly in parallel with the blood myeloid compartment. Given this assumption, the protracted survival of recipient tissue DCs appears unlikely in most human transplant recipients. Several factors may promote more rapid donor DC engraftment in humans than mice, including greater mHA disparity and higher T-cell content of the grafts. Prior cytotoxic therapy, which attenuates DC populations pre-transplantation, may also lead to a more rapid depletion of recipient cells. Data are also lacking on DC turnover after minimally intensive transplants, which show gradual engraftment [103] and significantly delayed GVHD compared with more intensive regimens [104]. A notable exception to the relatively rapid turnover of human tissue APCs is the long-term survival of recipient microglia. Their persistence may be of questionable relevance to transplantation immunology, but a post mortem study has shown that more than 98% of recipient microglia survive up to 6 years after transplantation [125].

Acute GVHD

Early work

Acute GVHD is primarily a cell-mediated disorder. The interaction between donor T cells of the inoculum and BM-derived elements of the host lymphoid system was first demonstrated by the elegant experiments of Sprent and colleagues (reviewed in [126]). In these studies, recipient mice were infused with T cells that were later collected from the efferent lymph by cannulation of the thoracic duct, a process known as blood–lymph filtering. Alloreactive T cells are initially trapped in the lymphoid organs for 24–48 hours and then emerge primed to induce GVHD in secondary recipients. Filtering governs the responses of both mHA-reactive T cells, thus demonstrated to be H-2 "restricted" [127], and T cells primed across H-2 differences [128]. By transplanting recipients with donor BM 6 months beforehand, so that recipient DCs are replaced by donor cells at the time of T-cell infusion, it is possible to show that CD4 and CD8 T-cell priming is attenuated, although not completely abolished, and is therefore largely dependent on recipient BM-derived APCs [129].

More recent studies

Blood–lymph filtering experiments heralded a series of tandem transplant studies that defined in detail the roles of recipient and donor DCs [130–132]. Using MHC class I-deficient BM grafts (from β_2-microglobulin knockout mice) to prepare recipient animals, the seminal study of Shlomchik et al. proved that recipient APCs are required for the efficient induction of CD8 T-cell-mediated GVHD across an mHA mismatch [130]. The interpretation of this result is that recipient APCs present their endogenous mHAs in association with class I MHC much more effectively than donor APCs, which must cross-present the same antigen acquired exogenously. Complementary studies in which modified donor cells lack functional antigen-presenting capacity showed that donor DCs do in fact amplify CD8 T-cell-dependent acute GVHD but cannot induce it [131]. It is important to note that $CD4^+$ T cells were absent in these experiments. Although this proves that $CD8^+$ T cells alone can mediate acute GVHD, donor APCs may be licensed much more efficiently to cross-present antigen in a full allogeneic response involving $CD4^+$ and $CD8^+$ T cells [133]. Another model has been used to demonstrate that donor DCs may also participate in the induction of acute GVHD indirectly by elaboration of IL-15 [134].

The contribution of recipient APCs to $CD4^+$ T-cell-mediated GVHD is less clear than in CD8 T-cell-mediated GVHD, partly because of the dual functions of $CD4^+$ T cells in GVHD: help for CD8-mediated cytotoxicity and direct cytokine-mediated organ damage. In turn, the removal of $CD8^+$ T and NK cells from an experimental system reduces IFN-γ-induced MHC class II expression by non-professional APCs and may artificially augment the role of professional APCs when $CD4^+$ T cells are put to the test [102]. The potential for direct CD4 T-cell-mediated pathology was clearly illustrated in one study in which a single MHC class II allele mutation (equivalent to a single mHA) expressed on recipient APCs alone was sufficient to induce lethal GVHD [132]. This finding, however, contrasts with the results of an earlier study in a multiple mHA-mismatched model, which demonstrated a strict requirement for alloantigen on target tissues, not only APCs [135]. Shlomchik and colleagues addressed the role of recipient and donor APCs also in a minor histocompatibility antigen-mismatched model, by using BM grafts or recipients deficient in costimulatory molecule expression [136]. In these experiments, both recipient and donor APCs were sufficient to initiate $CD4^+$ T-cell-mediated GVHD. Interestingly, there was a tissue-specific distribution of GVHD, with recipient DCs being more responsible for skin GVHD and donor DCs potentiating gut GVHD. The pattern of disease in these models was subacute with cutaneous and hepatic features of chronic GVHD.

Persistence of recipient LCs

An important modification of these results was introduced by the demonstration that some populations of APCs, notably LCs of the epidermis, are not necessarily replaced by donor-derived cells following BM transplantation [9]. Under conditions of T-cell depletion, nearly all recipient LCs survive MHC-mismatched transplantation and are able to mediate acute GVHD upon reinfusion of donor T cells and a second inflammatory insult such as irradiation [87,137]. Cutaneous GVHD, in turn, leads to the rapid turnover of recipient LCs and complete donor chimerism [62,87,137]. Persistent recipient APCs may account in part for the breakthrough of T-cell priming and GVHD at high T-cell doses when BM transplantation is used as an experimental means to replace or inactivate APCs [129,130].

The observation that activated T cells are able to clear residual recipient APCs is important since it makes the induction of acute GVHD a self-limiting process. Studies by Sykes and colleagues have explored the conversion of mixed chimeras by donor T cells in detail, demonstrating the essential role of inflammation in controlling access to the tissues [138] and the combined requirement for $CD4^+$ T cells and recipient APCs in mediating a robust graft-versus-leukemia (GVL) effect [139]. Under appropriate conditions, in which priming of alloreactive T cells is confined within the hematopoietic compartment, GVHD pathology can be prevented while allowing full expression of the GVL effect [77]. It remains to be determined whether a similar selective benefit can be obtained by targeted ablation of tissue-resident DCs, particularly those self-renewing populations such as LCs, while sparing visceral DCs in the spleen and liver.

APCs that induce GVHD

A limitation of using HCT to manipulate recipient APCs experimentally is that the identity of the APCs responsible for inducing acute GVHD remains obscure. While it is assumed that DCs play a significant role, PDCs, B cells, or macrophages may also contribute. Recipient "add-back" experiments have demonstrated that DCs are sufficient to induce GVHD in animal models [140,141], although removal of recipient macrophages with clondronate-loaded liposomes has also been shown to attenuate GVHD [142]. In animal models, recipient B cells have negligible or even negative effects on GVHD [140,143]. Alternative APCs are possibly more important in human transplantation, in which polyclonal memory T cells participate in the allogeneic response, than in model systems focusing on naïve T-cell priming to defined antigens. By developing new means to target host macrophages while sparing host DCs, we have recently revisited the role of host macrophages in GVHD [144]. Our data revealed that host DCs and macrophages have opposite contributions to GVHD outcome. In contrast to DCs, host remaining macrophages reduce the expansion of activated donor T cells through their ability to engulf allo-reactive T cells and modulate T-cell proliferation, and consequently limit the severity of GVHD [144]. The immune-modulatory role of macrophages has already been reported in several settings. In tumors, for example, macrophages modulate T-cell function through several mechanisms that include, but are not limited to, the production of inducible NO synthase (iNOS), arginase and indoleamine 2,3-dioxygenase (IDO) [145,146], all of which have all been shown to modulate GVHD after allogeneic HCT [147–151].

GVHD kinetics

From a clinical standpoint, it might be argued that the contribution of recipient APCs to GVHD is entirely predictable based on the simple fact that only recipient APCs are directly activated by conditioning and that classical acute GVHD occurs during or prior to engraftment when the presence of donor APCs is minimal. Although proof is likely to remain elusive in humans, a key role for recipient DCs was argued over 20 years ago in the pioneering studies of Perreault and co-workers [91,123]. Intriguingly, a more recent study showed that the pre-transplant CD14 count is linked to GVHD risk [152] and, as described above, there is clear evidence that recipient tissue DCs survive conditioning therapy [64,88].

Donor T cells and GVHD promote DC engraftment, but the effect of alloreactivity on DC turnover is often ignored in attempts to show that persistent recipient APCs constitute a “risk factor” for developing GVHD. Pre-transplant, every patient has 100% recipient DCs, and after transplant the majority of patients convert to 100% donor-derived DCs. The occurrence of GVHD is linked to a period of rapid conversion from recipient- to donor-derived DCs, which more than likely precedes the onset of clinical symptoms; thus, the faster recipient DCs are lost, the greater is the risk of clinical GVHD. It is therefore very difficult to demonstrate a causal link between persistent recipient DCs and GVHD. For this reason, although it is possible that persistence of recipient APCs correlates with de novo acute GVHD following the withdrawal of immune suppression, it is perhaps just as likely that primed donor T cells, which have already effected complete donor DC engraftment, are the real risk factor. There is currently no evidence for or against either of these mechanisms.

Perhaps a more promising approach to testing the role of persistent recipient DCs in humans is to examine correlations between DC chimerism and GVHD kinetics in cohorts of patients transplanted with regimens of varying intensity. There is no appreciable difference in LC chimerism at 100 days between high-dose and reduced-intensity fludarabine/melphalan transplants, but data are lacking on minimally intensive HCT that show significantly delayed GVHD kinetics [104]. It will be informative to determine whether DC engraftment is similarly retarded. Murine models, which incorporate the added refinement of elective donor T-cell infusion, broadly support this rationale. After a high-dose regimen, the transfer of donor T cells more than 1 month post-transplantation does not generate GVHD [141,153], but after reduced-intensity transplantation in which there is significant myeloid partial chimerism, GVHD can still be induced many months later. Even more directly relevant to these data is the clinical observation of progressive diminution of the toxicity and efficacy of donor lymphocyte infusions with time [121,154]. It has been suggested that the loss of recipient DCs is the cause as described in murine models [77,139], although this is not entirely consistent with human data. DLI is rarely given before 3 months, at which time all patients have at least 90% donor LCs [64] and probably complete engraftment of DCs in other organs. Unless a small percentage of recipient DCs are unusually potent or there is protracted survival of migratory cells in the LNs, other explanations for the reduction of the DLI effect, such as active regulation by donor cells, should be considered [155].

GVHD tropism

One of the major biologic questions concerning GVHD is its tropism for the skin, gut, and liver. It is likely that more than one mechanism explains this predilection. The skin and gut are both important epithelial barriers with commensal flora and dense APC networks. Simply from a numerical standpoint, these organs, together with the BM, spleen, and liver, contain many more F4/80+ resident APCs than the other viscera and skeletal tissues that are generally not affected by GVHD [156]. It is possible that DCs in these sites are also selectively primed by conditioning, either because the epithelia themselves are more readily damaged and release inflammatory mediators (hair loss and mucositis being prominent features of chemoradiotherapy) or because there is greater ingression of microbial products from commensal organisms. In support of the latter argument, there is good evidence for a role of LPS in gut GVHD [96,101,157]. T cells primed in the skin and gut also recirculate to these sites with high efficiency through the induction of specific homing receptors, creating feedback circuits of tissue damage [158]. Studies using bioluminescence dramatically indicate the early involvement of gut and spleen in T-cell priming [159], although in other models the Peyer’s patch itself appears dispensable [160].

It is conceivable that local DC renewal could play some part in the tropism of GVHD for certain organs. Even though splenic and hepatic DCs rapidly prime donor T cells [86], this does not exclude roles for self-renewing DCs in prolonging the initial sensitization phase of GVHD or influencing the spectrum of GVH responses. However, such effects will tend to be subtle and self-limiting since donor T cells, once primed, initiate inflammation, which promotes complete DC donor chimerism. In the skin, persistent recipient LCs are sufficient to generate cutaneous GVHD [87]. The radioresistant LC precursor may indeed be a dermal cell that gives rise to interstitial dermal DCs and LCs, both of which may persist after experimental transplantation [88,122]. Other target organs have not yet been closely scrutinized. The gut and associated Peyer’s patches may harbor radioresistant DC precursors, even though a rapid turnover of mesenteric LN DCs is apparent [62]. In the liver, the bulk of parenchymal DCs rapidly equilibrate with blood DCs [87], but this does not exclude a niche population of cycling DCs in focal GVHD targets such as the portal triad of the liver. The relative importance of persistent recipient DCs may be greater after reduced-intensity transplantation, and it is interesting that atypical patterns of single-organ GVHD are more frequently seen than in myeloablative transplant.

Chronic GVHD

The pathogenesis of chronic GVHD has been difficult to study, for several reasons. Clinical disease is very heterogeneous and animal models are less convincing than for acute GVHD. A number of fundamental features of human chronic GVHD must be explained by any successful model. First, chronic GVHD is delayed, classically to at least 100 days after transplant. Second, prior acute GVHD is a major risk factor, although chronic GVHD is less well controlled by T-cell depletion. Third, the tissue tropism is different from that of acute GVHD, although features of chronic GVHD may rapidly evolve and coexist with severe acute GVHD. Fourth, chronic GVHD has a very different response to therapy, being largely refractory to calcineurin inhibitors but responsive to corticosteroids and also rituximab, thalidomide, extracorporeal phototherapy, and other alternative agents. The features of chronic skin inflammation with sclerosis, mucosal ulceration, salivary and lacrimal gland dysfunction, and chronic hepatitis have parallels with human autoimmune disease. Whether this reflects the same induction process or simply a final common pathway of chronic inflammation is not clear.

From a simple perspective, the delayed onset of chronic GVHD suggests a greater dependence on donor-derived APCs. It has long been held by clinicians and immunologists that engraftment of donor DCs might underlie the progression of acute to chronic GVHD on the grounds that DCs of different origins favor alternative routes of antigen presentation (reviewed in [126]). In mice, acute CD8 T-cell-mediated GVHD dependent on recipient APCs contrasts with subacute forms of $CD4^+$ T-cell-mediated GVHD, which occurs with donor APCs alone [135,136,161]. In the MHC-matched setting, direct MHC class I presentation to CD8 cells is self-limiting and should decline as recipient APCs are removed. In contrast, MHC class II-restricted antigens continue to be presented to donor $CD4^+$ T cells indefinitely by donor APCs. This simple model accounts for the pathologic spectrum of acute and chronic disease by the selection of different minor antigens, potentially with tissue-specific distribution [162], and qualitative differences in $CD8^+$ and $CD4^+$ T-cell-mediated pathology [163,164]. It is easy to add that acute GVHD will accelerate the appearance of chronic GVHD through increased priming of donor $CD4^+$ T cells by activated donor DCs.

More complex models of chronic GVHD invoke thymic dysfunction, principally in terms of defective negative selection [165,166]. Impaired negative selection as a result of acute GVHD has been documented in animals [167]. The merit of this explanation depends on the extent of thymus-dependent immune reconstitution following a transplant. In adult humans, thymic recovery attenuates with age, and mature alloreactive T cells may survive to propagate chronic GVHD completely independently of the thymus [168]. In a number of animal models, mature T cells also mediate chronic GVHD syndromes that are thymus independent [164]. The mechanism of negative selection in the thymus involves BM-derived elements, and the switch from recipient to donor could compromise the availability of recipient mHAs for negative selection, although recipient epithelial elements would still be present. If donor APCs populating the thymus are completely unable to present antigen, as in the case of transplantation with MHC class $II^{-/-}$ BM, a convincing picture of thymic-dependent chronic GVHD is seen [165]. Thymectomy is protective, although this only proves that a dysfunctional thymus can be worse than no thymus at all. This mechanism is perhaps relevant to de novo human chronic GVHD in the younger patient and cautions against attempts to boost thymic function indiscriminately in older patients.

Studies of human DCs in chronic GVHD have revealed a relative depletion of tissue DCs [169] but a higher level of exclusively donor-derived blood DCs, especially PDCs [118]. There are no data on DC chimerism in the tissues, which makes it difficult to exclude a third hypothesis that chronic GVHD is the result of continuous immune activation by rarified long-term surviving recipient DCs. Although in humans all transplants attain at least 90% LC donor chimerism at day 100, just over one-third of patients continue to yield occasional recipient LCs for at least 1 year post-transplant [64]. Chronic allostimulation by these cells might explain the very frequent involvement of the skin and mucosa, often with a focal distribution. The main limitation of this idea is that it cannot account for the increased risk of chronic GVHD in patients with prior acute GVHD, which efficiently ablates recipient LCs. An interesting alternative suggested by results in a murine model is that persistent recipient B cells could contribute to chronic GVHD [170]. This must be tested in humans, and further experiments comparing lesional and non-lesional skin in cases of de novo chronic GVHD are required.

GVL effect

Immunologic protection from relapse, or the GVL response, is likely to be a manifestation of the GVH response in hematopoietic tissue and therefore dependent on the same cellular process of induction [54]. Transient pancytopenia often accompanies DLI responses [171] and is the cardinal feature of transfusion-associated GVHD [172], indicating that BM is indeed a target organ of the engrafting immune system.

Animal models confirm the importance of recipient APCs in the GVL response. Functional recipient, but not donor, APCs are required for a GVL effect mediated by $CD4^+$ and $CD8^+$ cells [131,173]. For a $CD8^+$ T-cell-mediated GVL effect, donor alloantigens must be present on both recipient APCs and tumor; tumor lines expressing costimulatory molecules are also unable to substitute for professional APCs [173]. Of interest, stably engrafted donor APCs, although not required for a GVL effect, are able to mediate some $CD8^+$ T-cell-dependent GVL activity at lower tumor burden while sparing the effect of GVHD [131,139,173].

The GVL effect appears to occur at a lower threshold than GVHD in studies of escalated DLI in mice and humans, suggesting that access to a narrow therapeutic window between GVL effect and GVHD is possible [174,175]. Changing the density or location of host DCs in the recipient may influence the therapeutic advantage in favor of a GVL effect. In murine models, recipient visceral DCs are sufficient to prime robust GVL responses and are readily accessible by donor T cells in the hematopoietic compartment [77,139,173]. In contrast, recipient peripheral tissues are not infiltrated by alloreactive donor T cells in the absence of inflammation [138]. The interaction of donor T cells with visceral, but not peripheral, DC populations may achieve a selective benefit in promoting a GVL effect without GVHD [77,138,139,173]. This argument underpins the logic of delayed T-cell add-back strategies [176] and preemptive DLI [121], which allow the inflammatory insult of conditioning to subside prior to the infusion of donor T cells. It is noteworthy that recent data indicate a more stringent requirement for priming the GVL effect in MHC-matched chimeras than in mismatched transplants. In the matched animals, additional lymphodepletion or Toll-like receptor signaling is required to initiate alloresponses, whereas GVL in mismatched chimeras requires T-cell infusion alone [177]. These results may put new constraints on the efficacy of DLI in humans, although in comparison with mice, humans are significantly lymphopenic post-transplant [169].

Finally, it has been proposed that, in certain malignancies, APCs may arise directly from the malignant clone, serving to prime donor T cells directly to tumor-associated antigens [120]. In chronic myelogenous leukemia, BCR-ABL DCs can be derived in vitro from monocytes [178], but the spontaneous generation of these cells in vivo has never been examined. A similar argument applies to B cells in follicular lymphoma [179]. It is likely that the susceptibility of these diseases to the GVL effect is due not only to efficient priming of donor T cells, but also to the slow kinetics of disease and relatively normal phenotype of the leukemic clone. In other diseases where the GVL response is less effective, relapse can easily occur during GVHD, suggesting that the target cells escape recognition or overwhelm the effectors rather than that there is a fault with T-cell priming.

DCs as potential therapeutic targets in transplant settings

DCs are potential therapeutic targets in the peri- and post-transplant settings. Conventional drugs such as calcineurin inhibitors, mycophenolate mofetil, and glucocorticoids are now known to mediate some of their actions through DC modulation [180–182] and, as described above, conditioning therapy and monoclonal antibodies have profound effects.

A number of novel approaches involving DCs have also attracted interest. The first, suggested 30 years ago, is that ablating recipient APCs might prevent GVHD [123]. The principal objection to this strategy, which parallels the problem of donor T-cell depletion, is that the GVL effect might also be lost. The absence of appropriate APCs indeed diminishes the GVL response in a number of experimental models and provides further evidence that GVHD and the GVL effect are facets of the same process [77,131,141,173]. There is also concern that regulatory T cells might not be formed, diminishing the potential therapeutic advantage [183]. A counter-argument is that different regional populations of DCs may have more or less propensity to induce GVHD, as previously outlined. Prevention of hepatic GVHD by removal of hepatosplenic APCs with liposomal clodronate lends credence to this concept [142]. It is likely that prior cytotoxic therapy of human subjects attenuates GVH reactions, given that a single non-myeloablative dose of cyclophosphamide pretreatment diminishes GVHD in mice [184]. This could explain the relatively high risk of GVHD seen historically in patients with chronic-phase chronic myeloid leukemia compared with patients given induction chemotherapy for acute leukemia, rather than biologic differences in the diseases themselves.

Murine experiments have raised the profile of ultraviolet (UV) irradiation of the skin as a means to accelerate the depletion of recipient LCs [87]. The regional effects of UV radiation may also be complemented by a general immunosuppressive effect [185,186]. However, reservations exist in view of the transient, cytokine-mediated nature of some responses [187] and subsequent recruitment of potent immunostimulatory APCs to the skin [188]. At least one early-phase clinical trial reported increased GVHD, although UV treatment was continued post-transplant, making it difficult to evaluate selective effects of UV radiation in the conditioning phase [189]. An alternative regional therapy is the preemptive use of topical corticosteroids [190]. In contrast, in a more recent study, 17 patients were treated with whole-body UVB irradiation after conditioning with a reduced intensity protocol. Strikingly, all nine patients with complete LC depletion (<1 LC per field) developed only grade I GVHD or no GVHD up to day 100 post-transplant, suggesting that prophylactic UVB irradiation post-transplant is safe and should be further explored as a clinical strategy to prevent acute (skin) GVHD [191].

Modulation of DC activation through the use of C3a receptor (C3aR) and/or C5a receptor (C5aR) blockade may also help reduce alloreactive T-cell immunity and improve GVHD outcome, as recently found in mice [85]. In addition to modulating host DC function, expansion of immunosuppressive macrophages may also provide a useful strategy to improve GVHD outcome. Adoptive transfer of tolerogenic DCs generated in the presence of GM-CSF, IL-10, transforming growth factor beta-1 (TGF-β1), and LPS [192,193], or histone deacetylase inhibitors such as suberoylanilide hydroxamic acid (SAHA) [194] or vasoactive intestinal peptide (VIP) [195], have been shown to reduce GVHD when injected during the peri-transplant setting in mice. We have recently found that pre-transplant administration of CSF-1, a cytokine required for macrophage development, survival and proliferation in vivo, expands the host macrophage pool and dramatically improves GVHD morbidity after allo-HCT [196].

Complementary to the removal of recipient DCs for attenuation of GVH responses is the possibility of stimulating donor immunity by infusing recipient DCs after transplant. This is supported by findings in experimental models [140,141] and could be an important adjunct to DLI, validating the concept of DC-based vaccination for malignancy. One encouraging trial of allogeneic HLA-matched DCs after transplant has already been reported [197]. Such a strategy might be particularly useful in cord blood transplantation, in which there is no access to the donor post-transplant, or when matched unrelated donors prove difficult to trace. Others have shown that regulatory or tolerogenic DCs from the recipient could also be given to treat GVHD in an MHC-mismatched model [193].

In general terms, therapeutic targeting of DC has the potential to control alloresponses beyond means currently available. Antibodies such as CMRF-44 recognize activated DCs in any site and are able to lyse blood DCs and LCs in the presence of complement [82]. Blockade of costimulatory receptors has been advocated for several years as a way of attenuating the initial encounter between DCs and T cells. Newer small molecules such as FTY720, a sphingosine receptor antagonist, have specific actions on DCs, preventing migration to the LNs, and a selective effect on GVHD has been reported with this drug [198]. Inhibitors of Flt3 may also target DCs since the Flt-3 receptor, which is related to c-kit and CSF-1, appears to be required for the differentiation and maintenance of DC populations [199]. Flt-3 inhibitors have shown some activity in ameliorating experimental autoimmune encephalomyelitis [200]. Another novel compound, NK026680, has been reported to interfere with DC activation [201].

Conclusion

The prominent role of recipient APCs in initiating GVH responses and the importance of the GVL effect in eradicating human malignancy have galvanized efforts to understand the homeostasis of peripheral DC populations. Many questions remain, such as the identity and location of the principal APCs in the GVH response, especially in fully complex immune systems. Elegant results have been achieved in animals by isolating different components of the GVH reaction, and new methods to manipulate APCs in vivo are evolving. However, proof that DC-targeted therapy can improve the therapeutic benefit of the GVL response in relation to GVHD has not yet been obtained.

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CHAPTER 18

The Experimental Basis for Hematopoietic Cell Transplantation for Autoimmune Diseases

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Introduction

Autoimmune diseases (ADs) are a heterogeneous group of disorders that affect an estimated 5–8% of the US population – 14–22 million persons [1,2]. These diseases occur when antigens within the body of an individual, called self-antigens, become targets of destruction by the immune system. Classically, both T- and B-cell responses are involved. Theoretically, all tissue types can be targets of autoreactivity and more than 80 clinically distinct ADs have identified. However, it is not known why certain organs are involved more commonly than others. Six of the most common ADs are listed in Table 18.1 and include rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), type I diabetes mellitus (T1DM), Graves disease, multiple sclerosis, and pernicious anemia. Collectively, these diseases represent ~50% of the ADs [3]. Autoimmune responses are generally sustained, persistent with manifestations of chronic tissue damage (presumably because self-antigens are continually produced on the targeted tissue), and, in severe cases, diminution of the response does not occur until the cells expressing the autoantigens are destroyed. Around four decades ago it was demonstrated that transfer of hematopoietic cells can alter the course of ADs in rodents. Bone marrow transplantation (BMT) experiments were shown to transfer disease from autoimmune prone rodents to unaffected ones [4–6] and, conversely, to prevent disease if the hematopoietic cells were transplanted from unaffected to susceptible rodents [5,7–9]. The goals of this chapter are to provide a basis for understanding how and why hematopoietic cell transplantation (HCT) may effectively treat severe ADs and to describe the preclinical studies that have contributed to this understanding. Clinical updates are provided in Chapter 67. This chapter begins with an overview of how normal antigen-specific immune responses develop, followed by a discussion of why autoimmunity occurs. Thereafter, studies on preclinical models using HCT for the treatment of autoimmune syndromes are presented.

The immune response

Induction and perpetuation of immune responses

Autoimmunity arises when there is dysregulation of the antigen-specific “adaptive” immune response. Adaptive immunity is induced when innate immune cells fail to eliminate a new infection. T- and B-lymphocytes constitute this adaptive arm, but act in concert with cells of the innate immune system to initiate and carry out their maximal effect. Innate immune cells, which include myeloid, natural killer (NK), and phagocytic cells, rely on a relatively limited number of invariant receptors to recognize microorganisms [10]. These receptors recognize simple molecules and regular patterns of molecular structure known as pathogen-associated molecular patterns (PAMPs) that are present on many microorganisms but not mammalian cells. The invariant receptors of innate immune cells include Toll-like receptors (TLRs) and other pathogen recognition receptors (PRRs). In contrast, adaptive immunity is based on clonal selection of lymphocytes that express a diverse array of highly antigen-specific receptors, and the adaptive arm develops immunological memory after an initial response to a pathogen, leading to an enhanced response to subsequent encounters with that same pathogen.

The adaptive response begins with the interaction between a professional antigen-presenting cell (APC) that presents its antigen to naïve $CD4^+$ T cells. Since activation of $CD4^+$ T cells can induce and perpetuate a cascade of downstream effector mechanisms with potentially disastrous consequences, stringent criteria are required for their activation [11–13]. B cells, macrophages, and dendritic cells (DCs) are the professional APCs that take up foreign protein antigens and process them via an intracellular pathway into peptide fragments. The fragments bind to class II major histocompatibility complex (MHC) molecules that make their way to the APC surface. In the presence of pathogens that signal the APC through their TLRs or PRRs, APC are induced to express costimulatory molecules [10]. APCs migrate to the draining lymphoid tissues where they encounter recirculating $CD4^+$ T cells. In order for a naïve $CD4^+$ T cell to be activated, two criteria must be met: (1) the T-cell clone must express the correct T-cell receptor (TCR) that recognizes the peptide bound to a self-MHC class II molecule; and (2) recognition and binding via the TCR must accompanied by simultaneous delivery of a costimulatory activating signal(s). In the absence of costimulation, TCR engagement can lead to T-cell anergy – a state of unresponsiveness to the antigen [14]. Only professional APCs that both express high levels of class II MHC molecules and express costimulatory molecules effect T-cell activation. Costimulatory molecules expressed by APCs include B7-1 (CD80) and B7-2 (CD86) – the ligands for CD28, CD40, inducible costimulator ligand (ICOS-L) and various adhesion molecules [12,13,15]. APCs, and in particular DCs, demonstrate phenotypic and functional plasticity, and the expression of these functional differences when encountering naïve T cells determines whether or not the encounter will result in the induction of immune reactivity versus tolerance [16]. The environmental milieu in which a T cell encounters

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Table 18.1 Associations of HLA type with susceptibility to autoimmune disease

Disease	Affected organ	HLA association	Relative risk[a]
Ankylosing spondylitis	Joints	B27	87.4
Graves' disease	Thyroid	DR3	3.7
Multiple sclerosis	Central nervous system	DR2	4.8
Type I diabetes mellitus	Pancreatic islets	DR3/DR4 heterozygote	~25
Rheumatoid arthritis	Systemic/joints	DR4	4.2
Systemic lupus erythematosus	Systemic	DR3	5.8

[a] The relative risk calculated by comparing observed number of patients with the HLA allele with the number that would be expected, given the prevalence of the HLA allele in the general population.

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an APC also influences the outcome. Specifically, the cytokine environment can skew the commitment to certain effector pathways, resulting in polarization of T-cell responses.

Following TCR binding plus costimulatory signaling, naïve CD4$^+$ T cells respond by rapidly proliferating and differentiating. As part of this process, they begin to express new receptor molecules and synthesize and secrete a number of chemokines and cytokines that constitute "signal 3" required for a continued response. Under the influence of signal 3, T-cell clones can expand and develop into distinct effector phenotypes. At least five CD4$^+$ effector phenotypes have been characterized (and the list continues to grow) based on their distinct cytokine-secretion patterns, which activate unique downstream cellular responses [17–19]. These subtypes are designated T-helper type 1 (Th1), T-helper type 2 (Th2), and T-helper type 17 (Th17), T follicular helper cells (Tfhs), and regulatory T cells (Tregs). Th1 development is induced when signal 3 is interferon (IFN)-γ and interleukin (IL)-12, and Th1 cells themselves produce large amounts of IFN-γ. Effector cells of Th1 immunity are macrophages, CD8 T cells, B cells, and IFN-γ-producing CD4 T cells. Th1 responses are particularly effective in protection against intracellular pathogens, including viruses and bacteria, and also tumor cells. Th2 cells are induced by IL-4 and secrete IL-4, IL-5, IL-10, and IL-13. Th2 cells recruit and activate eosinophils, mast cells, and basophils and promote class switching of B cells to produce immunoglobulin E (IgE). Their primary role is in the control of parasites. Th17 are triggered by IL-6 and transforming growth factor beta (TGF-β). They secrete IL-17, IL-17A, IL-17F, IL-21, and IL-22 and play an integral role in both tissue inflammation and activation of neutrophils to combat extracellular bacteria. The fourth subset of CD4 cells is now credited with being the primary population that provides B-cell help for high-affinity antibody production, not Th2 cells as was previously thought [18]. Tfhs mediate antigen-specific naïve or memory B-cell activation, and secrete cytokines characteristic of both Th1 and Th2 cells. They are identified by their location in the B-cell follicles of lymphoid organs and their constitutive expression of CXCR5.

Unlike the other four subtypes that respond to help clear pathogens, the fifth subtype, regulatory T cells (Tregs), suppress T-cell function, and have a primary role in limiting immunity and preventing autoimmune responses [20]. Tregs are a heterogeneous group of cells with different developmental origins [21]. Natural Tregs develop in the thymus and seem to be selected by high-affinity binding to MHC molecules containing self-peptides, whereas peripherally derived Tregs are found in both systemic and mucosal immune systems. Both CD4$^+$ Treg subsets express CD25 and the forked/winged helix transcription factor (FoxP3) [22], which interacts with other transcription factors that would otherwise facilitate activation of non-Treg cells [20]. Both types of Tregs can secrete IL-10 and TGF-β. IL-10 inhibits DCs from secreting IL-12, thus impairing their ability to promote T-cell activation and Th1 differentiation. Natural Tregs may also function by direct cell contact inhibition.

Following the activation of naïve CD4$^+$ T cells and their differentiation and proliferation, the downstream effector elements of the immune system are turned on. Regardless of whether or not the effector mechanisms involve predominantly a cellular or antibody-mediated response, during a normal immune response once the pathogen is destroyed the immune response ceases, leaving only an expanded cohort of memory lymphocytes. However, in autoimmunity, the self-antigen cannot be easily eliminated. Hence the mechanisms that normally function to limit the extent of an immune response do not apply to ADs. Instead, ADs tend to evolve into a chronic state.

Control of immune reactivity

To guard against the development of autoreactivity during the course of defending host tissues, several check points exist to prevent unwanted lymphocyte activation. These mechanisms are generally categorized as central versus peripheral tolerance

Central tolerance

During development, T and B lymphocytes undergo a rigorous selection process to delete potentially self-reactive cells [23,24]. Hematopoietic stem cells (HSCs) give rise to all lymphoid progenitor cells. For T cells, the progenitors migrate to the thymus and traverse this specialized microenvironment which permits T-cell maturation, clonal expansion, and clonal selection. In the thymic cortex, developing T cells (thymocytes) are positively selected in such a way that only those thymocytes whose TCRs can engage peptide–self-MHC complexes on cortical epithelial cells survive and mature, giving rise to thymocytes that are restricted to self-MHC. Negative selection primarily occurs in the thymic medulla. This process removes thymocytes whose TCR binds too strongly to self-peptides plus self-MHC, thereby eliminating potentially autoreactive cells. The thymic medullary cells are involved in negative selection of self-reactive thymocytes [25] and are known to express ectopically peripheral-tissue antigens (PTAs) [26]. It has become clear that many (but not all) tissue-specific antigens, such as insulin, are expressed in a subset of dendritic-like cells, and therefore self-tolerance against these antigens can be generated by central deletion. How these "peripheral" genes are turned on ectopically in the thymus is incompletely understood. However, it has been shown that a single transcription factor, AIRE (autoimmune regulator), is responsible for turning on many peripheral genes in the thymus [27]. Studies in mice revealed that defects in the AIRE gene could explain a rare inherited form of autoimmunity – APECED (autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy) which is manifested by destruction of multiple endocrine tissues including insulin-producing pancreatic islets. Highly relevant to the utilization of HCT for the treatment of autoimmunity is the long-standing observation that bone marrow (BM)-derived APCs present in the thymus are at the top of hierarchy of cells that mediate negative selection of developing thymocytes.

Similarly to T cells, immature B cells that express immunoglobulin receptors that bind too strongly to components of self either die within the BM or become impaired in their ability to respond to antigen (anergic) [24,28]. Deletion occurs at the immature B-cell stage, following expression of IgM on the cell surface (sIgM). sIgM has a pentameric structure and deletion of immature B cells of a particular antigen specificity seems to predominate when the interacting self-antigen is multivalent, indicating that strong cross-linking of an autoreactive receptor causes developmental arrest. Immediate elimination of the B clone is not the only outcome of such strong binding. Autoreactive B cells can be rescued by further gene rearrangements that replace the autoreceptor with one that is not self-reactive. This mechanism is termed receptor editing [28].

Peripheral tolerance

Central deletion is imperfect and self-reactive clones can escape elimination and enter the circulating pool. Moreover, by virtue of the T-cell selection process itself, only strongly autoreactive clones are eliminated and those that recognize self-MHC molecules plus foreign peptides are positively selected. Thus, many lymphocytes have some degree of low affinity for self-antigens. Outside the thymus in the "periphery" several mechanisms to avoid lymphocyte autoreactivity are utilized.

Immunologic ignorance and anergy

Most self-proteins are expressed at levels that are too low to serve as targets for lymphocyte recognition and thus cannot serve as autoantigens. It is likely that only very few self-proteins contain peptides that are presented by a given MHC molecule at a level that is sufficient for effector T-cell recognition but too low to induce tolerance. Lymphocytes able to recognize these rare antigens will be present in the individual but will not normally be activated; they are said to be in a state of immunological ignorance [29,30]. Most autoimmunity likely reflects the activation of such immunologically ignorant cells that occurs when the threshold of reactivity is altered by antigen presentation and milieu in which the events take place.

Stimulation of an antigen specific receptor which in the case of T-cells occurs in the absence of costimulation and for immature B-cells when their sIgM encounters a weakly cross linking antigen of low valence such as a small soluble protein, enter a state of long-lived functional unresponsiveness known as anergy [13–15,31]. Anergy in T-cells involves an acquired blockage of the TCR signaling pathways and anergized T-cell clones produce negligible amounts of IL-2, which is crucial for clonal expansion following T-cell activation. Anergy can be overcome by elevated levels of exogenous IL-2 during re-stimulation [14]. Anergic B cells cannot be activated again by their specific antigen, even with help from antigen-specific T-cells.

Regulation or suppression

Unwanted immune responses can also be controlled by populations that suppress lymphocyte activity. $CD4^+CD25^+FoxP3^+$ Tregs [20,22,32,33] have been shown to inhibit a variety of immune responses both in vitro and in vivo. Treg suppression differs from other forms of self-tolerance because Tregs can suppress lymphocytes that recognize antigens different from those recognized by the Tregs themselves, provided that the antigens are from the same tissue or are presented by the same APC. Two types of Tregs have been experimentally defined. "Natural" Tregs are moderately autoreactive $CD4^+CD25^+FoxP3^+$ that escape thymic deletion and respond to self-antigen by inhibiting other cells. "Peripherally derived" Tregs develop in tissues when antigens are recognized on "immature" DCs that produced TGF-β in the absence of pro-inflammatory cytokines. The importance of Tregs in the maintenance of immune tolerance is evident from the fact that humans and mice that carry the mutation for the master regulator transcription-factor in Treg development, FoxP3, develop severe systemic autoimmunity known as the IPEX syndrome (immunodysregulation polyendocrinopathy enteropathy X-linked syndrome).

A regulatory role for B cells in autoimmune diseases was first reported by Janeway and colleagues in a murine model of multiple sclerosis [34]. Like Tregs, Bregs are a source of inhibitory cytokines such as IL-10 and TGF-β [35]. The issue of how to identify these cells by surface markers or transcription factors, and whether or not Bregs exist naturally in humans, is yet to be resolved [35].

Natural killer T cells (NK-T cells) that express both NK receptors and TCRs qualify as belonging to a heterogeneous population termed NK-T cells with regulatory functions [36]. The most prominently studied subclass are termed invariant natural killer T cells (iNK-T cells) since their TCR diversity is very limited owing to their expression of a single α-chain (Vα 14–Jα 18 in mice, Vα 24–Jα 18 in humans) coupled with a β-chain rearranged with limited Vβ gene segments. NK-T cells recognize glycolipid antigens presented by a monomorphic glycoprotein CD1d. Upon activation, iNKT cells produce large amounts of IL-4 and IFN-γ, and may be an initial source of cytokines that polarize T-cell responses, particularly in the direction of Th2 cells. iNK-T cells have been shown to suppress autoimmunity in animal models, and also graft-versus-host disease (GVHD) responses [37–39].

Autoimmune pathology

ADs arise when immune effector cells cause damage to tissues. The response may be organ specific or systemic, and more than one autoimmune syndrome can exist in an individual Classically, loss of tolerance to self-antigen(s), such as insulin in the case of T1DM or DNA in the case of SLE, are invoked as the triggering events. However, for the inflammatory bowel diseases (Crohn disease, ulcerative colitis), the dysregulated immune response is against commensal microbiota resident in the intestines, not against intestinal tissue itself [40]. Histologic studies have shown variability in the apparent causes of tissue destruction since predominance of antibodies, activated by T or B lymphocytes, or non-specific inflammatory cells, can be found in the tissue lesions. On the basis of such studies, it was concluded that different diseases are predominantly mediated by either humoral (antibody) versus cellular driven immune responses. A traditional categorization of immunologic diseases divides this syndrome into four types, designated type I–IV hypersensitivity responses. Type I responses are caused by antibodies of the immunoglobulin E (IgE) isotype and are considered to be allergic responses, not classical autoimmune responses. Types II–IV involve tissue damage. Type II responses are mediated by antibodies directed against the targeted tissue, type III responses by antibody–antigen complex deposition, and type IV by cellular processes. It should be emphasized, however, that these classifications do not illuminate the more fundamental and important issue of what triggers autoimmune responses since, by the time an autoimmune process becomes clinically evident and classifiable by this scheme, the initiating events are obscured by the downstream effector mechanism causing the actual tissue damage. It is therefore more accurate to consider that autoimmunity involves the integrated activity of T cells, B cells, and innate immune cells. For example, $CD4^+$ T cells are almost always required for the production of pathogenic autoantibodies. Conversely, B cells often have a central role in maximal activation of T cells that effect tissue damage.

Genes and environment

Both genetic and environmental factors appear to be required in the development of ADs. Family studies, animal models, and human epidemiologic studies all support the role of these factors in AD susceptibility. The importance of genetic predisposition was first identified by analyses of disease incidence in monozygotic twins. The concordance rates in twins ranges from ~15% for RA [3,41] to a robust ~57% for SLE [3,42]. Comparisons of these rates with disease incidence in the general population predict that genetic predisposition is a dominant factor. For example, the lifetime risk of developing T1DM in the general population in the United States is 0.4%, whereas for monozygotic twins the concordance rate is in the range 30–50% [43,44]. For siblings, the rate is still significantly increased above the general population at ~6% but is lower than for twins. This decrease in sibling concordance rates compared with monozygotic twins suggests that multiple genes contribute to genetic predisposition. Thus, while genetic susceptibility is a dominant factor, the pattern of inheritance of most ADs is complex [1,3]. With the exception of a few known monogenic causes of autoimmune syndromes, the overwhelming majority of autoimmune diseases are inherited as polygenic traits, meaning that they arise from several independently segregating genes that interact in a cumulative way. The oldest and most consistent genetic marker for susceptibility to specific ADs are certain alleles of the genes located within the MHC (Table 18.1). In recent years, genome-wide association studies (GWAS) have provided a long list of other candidate susceptibility genes, albeit the effect of these alleles confers far less risk [45].

Inbred rodent strains exist that reliably develop spontaneous ADs (Table 18.2) with features similar to those of human disease. These animals are highly inbred, and therefore genetically identical. Like human twins, many, but not all, animals in these inbred colonies develop disease. This lack of 100% concordance in genetically identical humans and rodents provides evidence for the essential role of environmental interactions in AD development. Observations made in rodents where environmental elements can be controlled revealed that some of the factors affecting disease incidence include exposure to infectious pathogens and diet [46–50]. For example, a germ-free environment has been shown to suppress or enhance autoreactivity in mice with spontaneously arising forms of multiple sclerosis [51] and diabetes [46], respectively. Furthermore, it is well known amongst investigators who raise non-obese diabetic (NOD) mice, a model for T1DM, that certain common mouse pathogens, such as pinworms, lead to a dramatically reduced incidence of diabetes. Oral ingestion of protein antigens has been shown to lead to marked suppression of systemic humoral and cell-mediated immune responses when animals are later immunized with the same antigen. This phenomenon is called oral tolerance [52]. A high-fat, high-protein diet has been shown to increase the rate and severity of diabetes in NOD mice [53].

Another factor contributing to autoreactivity is sexual dimorphism. Sexual dimorphism refers to a pattern of skewing of disease incidence and/or severity towards one's sex. It has been observed in many human ADs, such as SLE and autoimmune thyroid disease, that human females demonstrate a disproportionately higher incidence than males [54]. Rodents with ADs, such as NOD mice, show a similar pattern of sexual dimorphism. In NOD mice, castration studies [55] and administration of exogenous male hormones [56,57] have shown that sex-related hormones contribute significantly to the dimorphism. Data from human epidemiologic studies confirm the contributions of genetic and environmental factors in AD incidence. Such studies show clear associations with race, geography, and susceptibility to disease. Again using T1DM as an example, the incidence of disease is ~40 times higher in Finland than in Japan [44].

Table 18.2 Animal models of autoimmune disease

Strain or designation	Disease model	Induction/manipulation
NOD mice	Type I diabetes mellitus	Spontaneous
BB rats	Type I diabetes mellitus	Spontaneous
(NZB/NZW)F1 mice	Systemic lupus erythematosus	Spontaneous
MRL-lpr/lpr mice	Systemic lupus erythematosus	Spontaneous
BXSB mice	Systemic lupus erythematosus	Spontaneous
NZB/KN mice	Polyarthritis	Spontaneous
C57BL mice	Multiple sclerosis [Experimental autoimmune Encephalomyelitis (EAE) in rodents]	Induced – Immunize with peptide of myelin oligodendrocyte glycoprotein (MOG)
SJL mice	Multiple sclerosis (EAE)	Induced – immunize with mouse spinal cord homogenate (MSCH)
Buffalo rats	Multiple sclerosis (EAE)	Induced – immunize with rat spinal cord homogenate (RSCH)
Mice – strains variable	Multiple sclerosis (EAE)	Induced – transfer of lymphocytes from myelin antigen- immunized syngeneic donors
DBA1 mice	Rheumatoid arthritis	Induced – immunize with type II collagen
Buffalo rats	Rheumatoid arthritis	Induced – administer Freund's adjuvant

MHC genes and susceptibility to ADs

The most established genetic association for predisposition to ADs is the genotype of the MHC [58]. This association was noted in the mid-1970s. Initially, correlations were made with the class I MHC type and the spondyloarthropathies. Ankylosing spondylitis, an inflammatory and presumably AD of vertebral joints, was found to be strongly associated with the class I human leukocyte antigen (HLA)-B27 allele, conferring a ~90–100 times greater chance of developing this disease. Later, the emphasis shifted to associations with class II rather than class I MHC molecules, since frequent associations were found in subsequent studies with class II gene products and other ADs, such as Graves disease and T1DM (Table 18.1]. In the last 30 years, the technology of HLA typing has advanced from serologic assays to the more sensitive molecular-based assays that detect variations of the nucleotide level (see Chapter 10). As this technology for HLA genotyping has become more precise, allowing examination of specific regions of the MHC, the associations have become stronger. For example, it has been observed that up to 95% of white subjects developing T1DM express the HLA alleles DR3 or DR4 compared with about 40% of normal individuals, and that individuals heterozygous for both DR3 and DR4 have the highest risk of T1DM development [59]. Subsequent to these observations, it was learned that the association between T1DM and the DR3 and DR4 alleles is due to the tight linkage between DR with DQ alleles, and that the DQβ alleles actually confer susceptibility to the disease [60]. A similar evolution in the association of HLA-type and disease susceptibility has occurred for Graves disease. Graves disease was amongst the first autoimmune disorders noted to have an association with HLA haplotype and the initial association with the MHC class I genotype HLA-B8. Later, however, it became evident that stronger association was with HLA-DR3, which is tightly linked to HLA-B8 [61].

Function of MHC in AD pathogenesis

Although genetic associations are firmly established between ADs and defined MHC haplotypes, the way in which these molecules contribute to autoimmune pathogenesis remains hypothetical. MHC molecules play central roles in both T-cell selection and during T-cell ontogeny and in the presentation of antigen to T cells. It has been hypothesized that certain AD-associated MHC haplotypes permit faulty selection of T cells during development and/or allow aberrant presentation of self-peptides to T cells that results in inappropriate T-cell activation [62–64]. The hypothesis that faulty T-cell selection permits autoreactive T cells to escape central tolerance is supported by the observation that the I-A^{g7}, the disease-associated MHC class II allele in NOD mice, binds peptides poorly and may therefore be less effective at driving intra-thymic negative selection. Alternatively, given that binding affinities differ between MHC alleles and peptides, which can affect the conformation of the MHC–peptide complexes seen by T cells, and that polymorphic residues of the MHC molecules can make direct contact with TCRs and thus affect antigen recognition, it is possible that the disease-associated MHC haplotypes make certain self-antigens appear foreign and/or the haplotypes generate a strong enough immune response to self-antigen to induce T-cell activation. The above hypothesis presumes that the association of ADs with MHC haplotype derives directly from the function of the MHC gene products. Although this hypothesis has been amply supported by data from both humans and rodents, it is not conclusively proven. Disease association clearly maps to the MHC region; however, contained within this region are a number of other genes. Alternative hypotheses include that the MHC haplotype serves only as a marker and that the true (and as yet undetermined) disease-associated genes are closely linked to the MHC alleles.

Non-MHC genes and susceptibility to ADs

The importance of genetic predisposition in AD susceptibility and the conclusion that several genes contribute to an AD phenotype have motivated the search for predominant non-MHC susceptibility genes. To achieve this goal, international coalitions have been formed aimed at collecting large cohorts of families afflicted with specific ADs and employing state-of-the-art technologies to perform genome-wide analyses to locate and identify susceptibility genes. Analyses of such GWAS data have confirmed the complex nature of genetic associations with autoimmunity and the importance of MHC-linkage. In addition, hundreds of non-MHC loci have been identified but have only modest or fairly weak effects [65]. Nevertheless, some loci that show associations with multiple diseases reveal categories of associated genes that, in turn, implicate involvement of particular functional pathways (Table 18.3). These pathways include the intracellular signaling that drives the activation of T and B cells, signaling by cytokines and cytokine receptors, and pathways that mediate immunity and microbial responses [45,65].

Two widely cited examples from GWAS of non-MHC genes with polymorphisms associated with risk of developing multiple autoimmune diseases are protein phosphatase non-receptor type 22 (*PTPN22*) and cytotoxic lymphocyte-associated protein (*CTLA-4*) [45,65]. *PTPN22* encodes an intracellular tyrosine phosphatase that modulates T-cell receptor signaling and is crucial in setting the threshold for thymic negative selection. Knockout of the murine homolog of *PTPN22* results in hyperactive T cells. Single nucleotide polymorphisms (SNPs) of *PTPN22* are associated with human T1DM, SLE, RA, and Graves disease. This clustering of genetic risk factors for many autoimmune diseases suggests that these diseases might share, at least partly, similar underlying causal mechanisms. CTLA-4 is a surface molecule produced by activated T cells that acts as a negative regulator through its binding to the inhibitory costimulation molecules, and has a central role in the maintenance of peripheral tolerance [66]. *CTLA4* polymorphisms have been associated with T1DM, RA, celiac disease, and alopecia areata, again supporting the idea that dysregulation of a central gene pathway involved in lymphocyte signaling thresholds can be a common link to autoimmune pathogenesis (Table 18.3) [45].

Leveraging the information obtained via GWAS is in its early phase, and many concerns have been voiced regarding the utility of such studies. One focus of concern is that most of the loci identified

Table 18.3 Selected non-MHC major associations with autoimmune diseases

Candidate gene	Possible function and mechanism of action	Chromosome location	Autoimmune diseases
Lymphocyte activation and intracellular signaling			
Protein tyrosine phosphatase non-receptor type 22(PTPN22)	Modulation of lymphocyte receptor activation	1p13	T1DM, RA, SLE, Graves disease, Crohn disease[a]
Cytotoxic lymphocyte-associated protein (CTLA4)	Transmission of inhibitory signals in T cells	2q33	T1DM, RA, celiac disease, alopecia areata
Cytokines and cytokine receptors			
Interleukin 2/21 gene region	T-cell trophic growth factors; multiple associations flanking both cytokines	4q26	T1DM, RA, celiac disease, Crohn disease, ulcerative colitis
Interleukin-7 receptor (IL7R)	Differentiation/activation of T cells affected by IL-7 signaling	5p13	MS, primary biliary cirrhosis, alopecia areata
Innate immunity and microbial recognition			
Nucleotide oligomerization domain 2 (NOD2)	Sensing of bacterial peptidoglycan in nuclear factor κB activation	16q12	Crohn disease
Interferon regulatory factor 5	Inducement of interferons, regulation of activation of pattern recognition receptor	7q32	SLE, RA, systemic sclerosis, primary biliary cirrhosis
Transcription factors			
Signal transducer and activator of transcription4 (STAT4)	Mediation of multiple cytokine signals, including interleukin-12	2q32	SLE, RA[b], primary biliary cirrhosis[b], systemic sclerosis

[a] A potentially distinct association within this implicated genetic region has been shown for this disease.

[b] Genetic associations with this disease have been observed in patients of Asian or European ancestry.

Source: Cho and Gregersen 2011 [45]. Reproduced with permission of the Massachusetts Medical Society.

are non-coding, and many are far from discovered genes; therefore, they are not immediately informative for experimental work. However, a recent report by Lee et al. [67] provides an example of the integration of genetic, clinical, and functional data in mice and humans to shed light on the pathway influenced by a non-coding SNP. By using a resource of local genotyped controls, they showed that one specific allele of a non-coding SNP correlated with the activity of FOXO3, a transcription factor found in APCs and T cells. FOXO3 downregulates TNF and other pro-inflammatory cytokines in response to lipopolysaccharide (LPS) and other TLR-4 ligands, and conversely, upregulates IL-10, an anti-inflammatory cytokine in response to TLR stimulation. These results were consistent with the more indolent clinical course observed in patients with Crohn disease who carry this SNP haplotype. Hence in the future we can look forward to extending the application of GWAS to encompass genetic influences on disease outcome, independent of the usual focus on disease susceptibility.

Monogenic ADs

Most ADs occur due to the combined effect of multiple genes. However, there are a few known monogenic ADs. These diseases are usually recessive or X-linked. Although the human syndromes have been recognized for decades, discovery of the controlling genes has occurred within the last 15 years and was made possible by studies in mice wherein gene manipulation was used interrogate the gene effect. These syndromes are described in detail later in the section Genetic engineering of ADs.

What triggers autoreactivity?

Several hypotheses exist to explain what triggers and perpetuates AD pathology. Although lymphocytes undergo a rigorous selection process during development to eliminate high-affinity self-reactive clones, most circulating lymphocytes have low affinity for self-antigens but make no response to them. These latent, potentially self-reactive, cells can be recruited into autoimmune responses if their threshold for activation is lowered by coactivating factors. Inappropriate presentation of antigens to T cells via activated APCs can occur in many settings, including infection or non-specific tissue trauma. It is generally thought that few self-peptides can trigger a cascade of cellular events that results in targeted tissue damage [63,68,69]. Experimental data show that immune response against a self- or foreign protein is usually focused on one or two peptide sequences (called epitopes) contained within that protein, which are termed *dominant epitopes*. Once a response is triggered against the dominant epitope, other peptide epitopes from the same protein become targets, thus expanding and perpetuating the immune response (Figure 18.1). This hierarchical extension of an immune response from a dominant to a subdominant epitope is termed epitope spreading [69]. Most self-peptides also cannot serve as autoantigens simply because they are present at levels that are too low to be detectable by naïve T cells. However, a few self-peptides that failed to induce tolerance may be present at high enough levels to be recognized by T cells. These peptides are likely the breakdown products of tissue-specific proteins, and it is thought that only certain proteins can act as autoantigens since there are relatively few distinct autoimmune syndromes, and individuals with a particular AD seem to recognize the same antigenic targets. Thus, autoimmunity can occur if an APC presents a dominant epitope of a self-protein on conjunction with activating costimulatory molecules resulting in activation of CD4$^+$ T cells. For example, autoreactive B cells appear to capable of driving this process in SLE by internalizing antigens bound through their antigen receptor and presenting the derived peptides to T cells, elaborate pro-inflammatory cytokines, and organize ectopic lymphoid tissue [70,71]. In a "normal" immune response elicited by exogenous pathogens, elimination of the pathogen would result in cessation of the response. However, in autoimmunity, self-antigens generally cannot easily be eliminated because they exist in vast excess or are ubiquitous, as is the case of the SLE autoantigen chromatin. Hence ADs tend to evolve into a chronic state.

Two predominant hypotheses on how spontaneous autoreactive cells can be induced to respond are by *molecular mimicry* or *tissue injury*. The hypothesis of *molecular mimicry* (shown in Figure 18.1) suggests that immune responses directed against infectious agents can cross-react with self-antigens, causing autoimmune destruction. Thus, the inciting antigen could be a bacterium- or virus-derived protein that shares an amino acid sequence with a prevalent tissue-specific protein. Antibodies or cytotoxic T cells directed against the pathogen will also selectively destroy the normal tissue that expresses the cross-reactive protein. There are clear examples of rodent neurotropic viruses, such as Theiler's murine encephalomyelitis virus (TMEV) and mouse hepatitis virus (MHV), which infect neurons and other cells within the central nervous system (CNS) that result is dysregulation of antiviral immune mechanisms and culminate in the development of autoimmunity [72]. In certain mouse strains, intracranial injection of TMEV results in a self-limited infection, whereas in other strains, TMEV infection results in a chronic demyelinating disease resembling multiple sclerosis. In this TMEV-induced demyelinating disease, autoimmunity can be worsened by engineering the virus to express a known immunodominant peptide of a spinal protein, myelin proteolipid protein. In human multiple sclerosis, Epstein–Barr virus (EBV) and measles virus (MV) have been implicated in the pathophysiology; however, a precise causal relationship between these viruses and multiple sclerosis has yet to be elucidated [72].

The *tissue injury* hypothesis attributes activation of localized inflammatory mechanisms in response to organ injury as the inciting event. During inflammation, the release of chemoattractants and cytokines recruits APCs, lymphocytes, and other effector cells. The result may be release of tissue-specific antigens and uptake by APCs that can result in presentation of self-antigen at high enough levels to act as an immunogen. The two hypotheses are not mutually exclusive as virus infection and direct damage to target tissues such as the CNS or pancreatic β cells could lead to localized inflammation and death of normal parenchymal cells. In this setting, presentation of cellular antigens that are not normally presented in the context of costimulatory signals and cytokines could incite activation of a few bystander autoreactive clones.

Animal models of ADs

Rodent models of ADs have contributed significantly to the understanding of disease pathogenesis. Three major types of animal ADs serve as models for study: (i) ADs that arise spontaneously; (ii) ADs that are induced by antigen immunization or by adoptive transfer of autoreactive mature immune cells; and (iii) ADs that are created by genetic engineering technology. Many of the concepts regarding the causes of autoimmunity have either originated from or been confirmed by observations made in these animal models. Examples of these models and their homologous human diseases are shown in Table 18.2. The models rely on genetic homogeneity so that recipients and donors are from highly inbred strains.

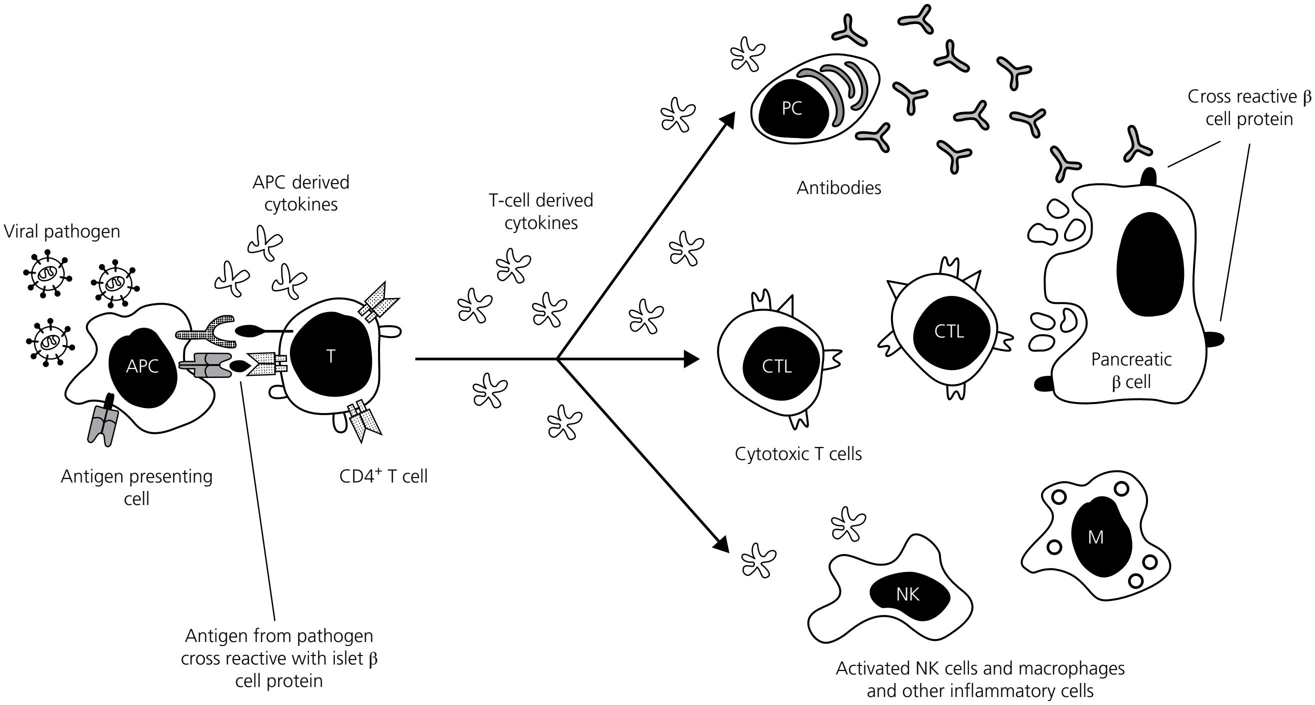

Viral pathogen
APC derived cytokines
T-cell derived cytokines
APC
T
Antigen presenting cell
CD4⁺ T cell
Antigen from pathogen cross reactive with islet β cell protein
PC
Antibodies
Cross reactive β cell protein
CTL
CTL
Cytotoxic T cells
Pancreatic β cell
NK
M
Activated NK cells and macrophages and other inflammatory cells

Figure 18.1 Induction of autoimmunity. Schematic illustration of events thought to cause an autoimmune response according to the hypothesis of molecular mimicry. An activated antigen-presenting cell (APC) internalizes a pathogen and presents a peptide derived from the pathogen to a naïve CD4$^+$ T cell by a disease-associated class II major histocompatibility molecule (MHC) on its surface. The peptide sequence cross-reacts with a component on pancreatic islet β cells. The CD4$^+$ T cell is activated upon receipt of this first signal by its cognate antigen and costimulatory signals from the APC. Following activation, CD4$^+$ T cells then activate downstream effector mechanisms through direct cell–cell contact and the secretion of cytokines. The pancreatic β cells are destroyed since they express a protein that contains the same peptide sequence as the inciting antigen. CTL, cytotoxic lymphocyte; M, macrophage; NK, natural killer; PC, plasma cell.

Spontaneously arising ADs

Animals that spontaneously develop autoimmune syndromes have been instrumental in revealing the complex nature of genetic susceptibility to ADs and in understanding the cellular events that lead to tissue destruction. The observations that even amongst inbred animals there is reliable but not 100% development of an autoimmune syndrome underscores the importance of the interaction of genetic plus environmental factors in autoimmune pathogenesis [3,73,74]. The essential role of certain MHC alleles as the primary susceptibility genes, the interactions between other minor susceptibility genes, and the role of T-lymphocytes in driving autoimmune pathogenesis have all been confirmed by studies in these animals. The most extensively studied models of spontaneously arising ADs are mice that develop SLE-like syndromes and NOD mice that develop a disease resembling T1DM.

The lupus-prone mice include the F_1 hybrid of New Zealand black (NZB) and New Zealand white (NZW), designated (NZB×NZW)F_1, MRL-*lpr/lpr* mice, and BXSB mice [73,75]. Like human SLE, these animals develop autoantibodies to nuclear antigens and progressive severe glomerulonephritis. Extra-renal disease manifestations occur variably in the individual models and include lymphoproliferation with both splenomegaly and lymphadenopathy, hemolytic anemia, autoimmune thrombocytopenia, vasculitis, thrombosis, and arthritis. All of these lupus-prone strains exhibit premature thymic atrophy, the significance of which is unknown. In the (NZB×NXW)F_1 model, heterozygosity at the MHC (MHC designation H-2$^{d/z}$) has shown to directly impact on disease severity. Data on the non-MHC genes linked with murine lupus come primarily from the New Zealand hybrid model for which genetic crosses have demonstrated confirmed linkage with ~12 loci from the NZB or NZW strain [3,73]. In MRL-strain mice, homozygosity for the *lpr* or *gld* mutations results in acceleration of lupus autoimmunity [76]. *Lpr* is a spontaneous mutation of Fas (CD95) and *gld* is a mutation of Fas ligand. Binding of Fas ligand to Fas signals Fas-bearing cells to undergo apoptosis. MRL-strain mice have a massive accumulation of lymphocytes and autoantibody production. Although typical SLE patients do not have Fas defects, in the mid-1990s it was determined that a rare human syndrome called autoimmune lymphoproliferative syndrome (ALPS), which resembles MRL/*lpr* mice, was in fact due to Fas mutations [77]. BXSB mice defects carry the Yaa (Y chromosome-linked autoimmune acceleration) gene [73,78], which results in more rapid and severe lupus-like disease in male than female BXSB mice. The skewing towards higher disease severity in male contrasts the more common pattern of severity seen in female (NZB×NZW)F_1 mice, NOD mice, and many human ADs where disease severity is often skewed towards females.

NOD mice develop a syndrome resembling human T1DM and are the most exhaustively studied model of an animal AD [74,79]. The disease pathogenesis begins with the infiltration of mononuclear cells into the insulin-producing islets of Langerhans at ~3–4 weeks of age. Infiltration of the islets progresses slowly over the course of several months until the islets are destroyed and the mice manifest symptoms of hyperglycemia at ~6–9 months of age. NOD mice express only one MHC class II gene product. The sequence of this class II molecule, designated I-A^{g7}, is unique to the NOD strain and it binds peptides poorly – a characteristic which has been suggested to explain the association of this genotype with autoreactivity [62,80]. In addition, gene mapping studies have revealed that >20 non-MHC loci contribute to the disease process [81]. NOD mice are reported to have other immune defects [82,83], including differentiation and functional defects of macrophages, and also defects in Tregs or alternatively the ability of effector T cells to be regulated by Tregs [84]. Progression to overt diabetes can be blocked in these mice by prolonged administration of antibodies directed against $CD4^+$ T cells in the prediabetic phase, demonstrating that NOD disease is $CD4^+$ cell mediated [85,86]. Pathogenic T cells capable of transferring the disease have been cloned from these mice and TCRs from such clones have been expressed in non-NOD background transgenic mice causing diabetes [87–89]. Once hyperglycemia develops, NOD mice are difficult to cure since, like humans, physiologic normalization of hyperglycemia can only be corrected by islet or pancreas transplantation, and such transplantations are vulnerable to immune destruction by both anti-islet specific immunity, and also rejection on the basis of allogeneic differences.

Immunization and cell transfer models of ADs

Conventional strain rodents can also be induced to develop autoimmune syndromes by immunization with proteins or peptides derived from defined tissues, or by transfer of pathogenic lymphocytes (Table 18.2). Genetic susceptibility appears to play a major role since certain strains are more prone to mount pathogenic responses than others. One of the best studied examples of an antigen-induced AD is the rodent disease experimental autoimmune encephalomyelitis (EAE) [90,91]. EAE-affected mice or rats develop symptoms analogous to the human neurologic disease multiple sclerosis. The animals show symptoms of paralysis that, as in multiple sclerosis, can be either progressive or fluctuating in severity. EAE is induced by immunization with components derived from the spinal cord (Figure 18.2). These components range from the emulsified spinal cord itself, proteins derived from the spinal cord, or defined peptides from spinal cord proteins. The proteins that are known to induce EAE in mice include myelin-basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG). Defined peptides derived from these proteins can also induce EAE, and the exact sequence of such peptides depends upon the MHC haplotype of the mouse strain immunized. For any given mouse strain, only certain peptide sequences are pathogenic and are termed immunodominant. It was the study of the immunodominant peptide in EAE that led to the hypothesis of epitope spreading [68,69] as a mechanism by which immune responses can be perpetuated and expand to other antigenic specificities. In order to induce pathogenic autoimmune responses by immunization with peptides or proteins, it is critical that these antigens be administered as part of a mixture of adjuvants, such as Freund's adjuvant and/or pertussis toxin. Complete Freund's adjuvant is an oil-in-water emulsion containing dead mycobacteria that is thought to enhance immunogenicity in two ways. First, the bacterial products in adjuvant signal the APCs via the Toll pathway resulting in the expression of costimulatory molecules and cytokines, and converting resting APCs to activated and migrating cells [92]; second, emulsification of antigen in adjuvant serves to convert soluble protein antigens into particulate forms, which are more readily ingested by APCs.

Another way to induce ADs reproducibly is by transfer of lymphoid cells from affected to unaffected immunocompetent recipients. Populations capable of transferring disease include cells taken from peripheral lymphoid organs, such as the spleen or lymph nodes [93,94], or cells extracted from autoimmune target tissues, such as infiltrative pancreatic islets in NOD mice. It is also possible to clone pathogenic T cells of a single antigen specificity that can transfer disease [87,95,96]. Cloned T cells are particularly valuable for tracking immune responses in vivo since they express a monotonous TCR that can be identified by labeled monoclonal antibodies (mAbs) that bind the variable region of the TCR β chain (Vβ) or MHC–peptide tetramer complexes that can bind the antigen recognition portion of the TCR. Receptors from such clones can also be used to generate transgenic animals. Interestingly, adult T cells from normal mice that are depleted of $CD4^+CD25^+$ T cells can transfer an autoimmune syndrome with a wide spectrum of organ-specific manifestations including gastritis, oophoritis, orchitis, and thyroiditis [32]. Indeed, these transfer studies were one important means by which the existence of regulatory $CD4^+CD25^+FoxP3^+$ T cells was demonstrated [32,97,98].

Appropriate recipients for adoptive transfer studies are irradiated animals, or genetically immunodeficient strains that cannot produce T- and/or B-lymphocytes [99]. Two prominent examples of naturally occurring mutations that prevent normal lymphocyte development are a defect in DNA repair resulting in mice with severe combined immunodeficiency syndrome (SCID), and a defect in the Wnt signaling pathway, which results in mice that are both hairless and lack a thymus (*nude* mice). Mice with the *nude* defect cannot produce T cells. However, transfer of their hematopoietic progenitor cells to recipients with normal thymuses results in normal T-cell development. Hematopoietic progenitors from SCID mice cannot generate functional T or B cells even in a normal recipient. Genetically engineered knockouts of the recombination activating genes (*RAG-1* or *RAG-2*) required for T- and B-cell receptor rearrangement result in defects phenotypically similar to the SCID mutation in that *RAG-1* or *RAG-2* knockout mice cannot generate functional T or B cells.

Genetic engineering of ADs

Genetically engineered mice that either express molecules at supraphysiologic levels or that have been knocked down for the expression of specific molecules have been generated in order to model and study defined aspects of AD pathogenesis. Numerous varieties of such genetically manipulated mouse strains now exist that have resulted in the generation of animals with altered expression at key regulatory molecules of the immune system [100]. These gene targets include but are not limited to the MHC, lymphocyte receptors, transcription factors, and soluble molecules such as cytokines and chemokines. A category of genetic aberrations that have been particularly informative are those that give rise to autoimmune syndromes as monogenic traits. Included in this category are knockout of the *AIRE* (autoimmune regulator) gene, which results in a decreased expression of self-antigens in the thymus. AIRE knockout mice develop a syndrome of autoimmunity that resembles human APECED [27]. Mutation of the FoxP3 transcription factor, a key differentiation factor for some Tregs, results in the mouse version of the human IPEX syndrome, which is an X-linked trait that presents as immune dysregulation, polyendocrinopathy, and enteropathy [101]. In IPEX, the numbers of $CD4^+CD25^+$ Tregs are comparable to those in healthy individuals. However, the suppressive function of these cells is impaired. Knockout of CTLA-4, the high-affinity inhibitory receptor on T cells for the B7 molecules, results in a fatal syndrome of multi-organ lymphocytic infiltrates and severe enlargement of lymphoid organs consistent with a

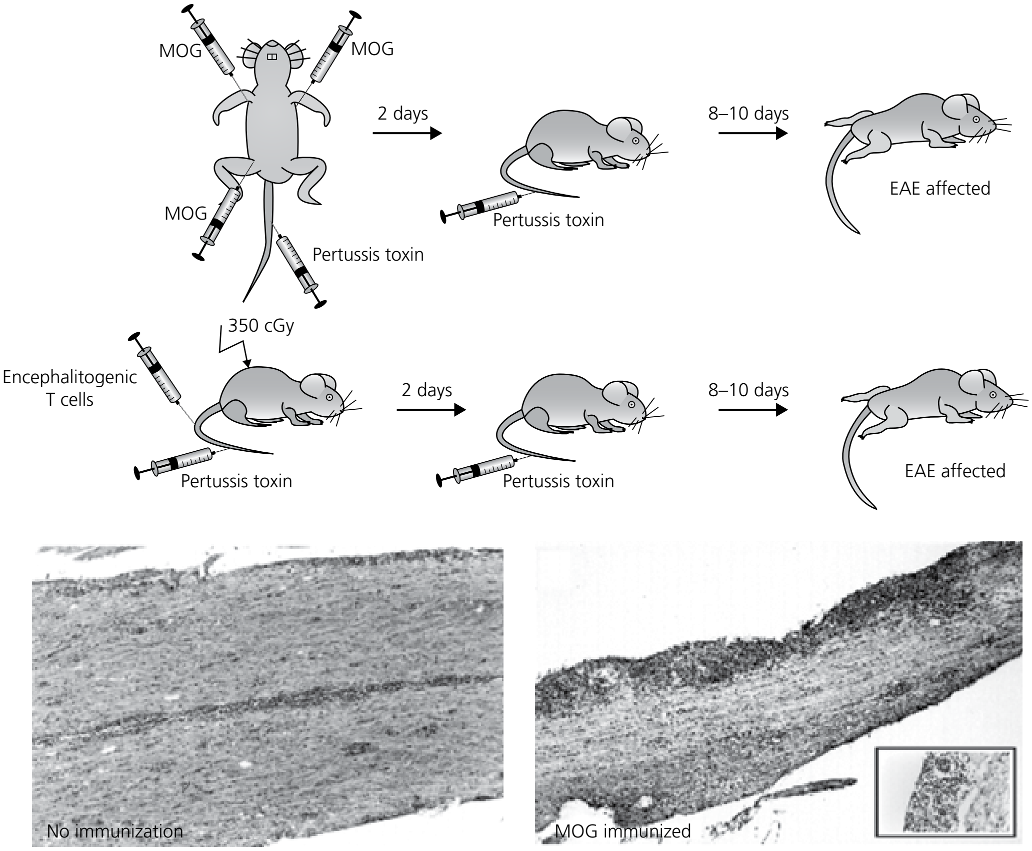


Figure 18.2 Experimental approach to causing an autoimmune disease (AD). Experimental autoimmune encephalomyelitis (EAE), a rodent disease resembling multiple sclerosis, can be induced by immunization of normal mice with components derived from mouse spinal cord. The top panel shows a schematic for EAE induction using a peptide derived from myelin oligodendrocyte glycoprotein (MOG). The peptide is emulsified in Freund's adjuvant. On day 0, the mixture is injected subcutaneously into the inguinal and bilateral axillary regions. Immunized mice receive an additional adjuvant injection (pertussis toxin) by intravenous route on days 0 and +2. Alternatively, encephalitogenic T-cell lines, T-cell clones, or lymphocytes from syngeneic mice immunized with myelin components can be transferred into mice prepared with low-dose radiation plus pertussis toxin on days 0 and +2. EAE is graded on a scale of 0–5 as follows: 0, no clinical signs; 1, loss of tail tonicity; 2, flaccid tail and hind limb weakness; 3, hind limb paralysis; 4, complete hind limb paralysis; 5, moribund or death. The bottom panel shows hematoxylin and eosin-stained spinal cords from (left) a non-immunized mouse as compared with (right) a MOG-immunized mouse. Note that the intense mononuclear cell infiltrate is most prominent in the meningeal areas of the spinal cord of the immunized animal.

systemic autoimmune reaction presumably directed against multiple, as yet unknown, self-antigens. This profound phenotype in mice led to a search for associations of CTLA-4 polymorphisms with human ADs, and the discovery that several such diseases, including Graves disease, T1DM, and other endocrinopathies, show a striking association with a *CTLA4* polymorphism (Table 18.3) [102]. Although animals with these types of monogenic aberrations have been instrumental in revealing the basic biology of autoimmunity, they have not been used for purposes of clinical translation of HCT to the treatment of ADs, hence their use in HCT transplantation will not discussed in this chapter.

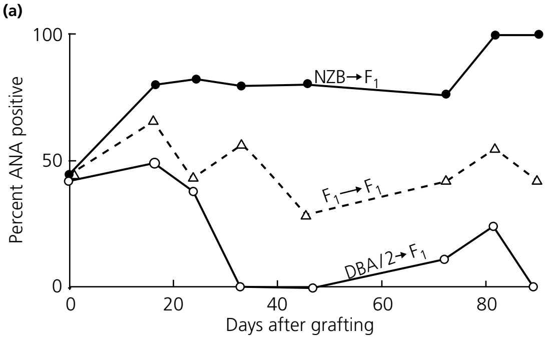
HCT and the treatment of ADs

Historical perspective

Preclinical studies and case reports in the human transplantation literature support the use of HCT for the treatment of severe ADs [103,104]. The clinical literature on this topic (reviewed in Chapter 67) contains several case reports demonstrating that patients undergoing allogeneic HCT for conventional indications (e.g. hematologic malignancy) with a coincidental AD experience long-term improved or even full remission of both disorders. Conversely, there have been case reports of transfer of ADs from AD-affected allogeneic HCT donors into previously unaffected recipients. In evaluating the preclinical literature for its relevance to the treatment of human ADs, it is important to bear in mind that heterogeneity exists among the experimental systems, and that often the studies were designed to answer basic questions about AD pathogenesis rather than to form a basis for direct translation to human therapy. A goal of the early investigators was to establish what cellular elements transfer or prevent disease. Thus, initial studies were directed towards the creation of allogeneic radiation BM chimeras to determine if transplantation of hematolymphoid elements could alter susceptibility to AD in rodents. As early as the 1960s, it was known that certain mouse strains, such as the NZB, develop a syndrome resembling human SLE. In 1969, Denman et al. [6] demonstrated that transfer of BM or spleen cells from NZB mice to MHC-matched non-autoimmune prone BALB/c mice (both H-2^d) resulted in disease in the recipients. Later, in 1974, Morton and Siegel [4] demonstrated that BM transferred into irradiated recipients could, on the one hand, transfer disease from NZB donors into MHC-matched BALB/c or DBA/2 recipients and, on the other, BM from BALB/c or DBA/2 donors could result in transient normalization of antinuclear antibody (ANA) titers in NZB recipients (Figure 18.3) [5]. Donor chimerism was not measured in these studies, and one explanation proposed by the authors for the transient rather than persistent nature of ANA depression was that perhaps only short-term chimerism was achieved in the NZB recipients. Later studies supported this latter idea since permanent allogeneic chimerism appears to be necessary to achieve long-term disease control [105,106]. Following the landmark studies of Morton and Siegel, other investigators confirmed in different SLE models that BM contains the cellular components capable of transferring disease [7,8].

ADs as "stem-cell disorders"

These seminal experiments led to the concept that the etiology of autoimmunity is determined by innate properties of the HSCs and its differentiated lymphocytic progeny, and independent of host environment. Indeed, it was later observed by other groups, and in different animal systems, that the genotypic origins of the BM (i.e. from susceptible or non-susceptible strains) determined whether or not the animal developed or was protected from disease. However, not all studies have been consistent with this concept. For example, NOD disease (diabetes) has been transferred by parental NOD BM into F_1 offspring of NOD mice crossed with different strains [93,107]. However, when NOD BM was transferred into genetically disparate recipients, the radiation chimeras engrafted with NOD BM and developed lymphocytic infiltrates of their islets, but most did not progress to overt diabetes [108]. These data show that while anti-islet reactivity can be transferred by BM, the host environment provides additional elements that permit the perpetuation of an immune response, which ultimately results in tissue destruction. (See also the later section Genotype and susceptibility.)



(b)

Strain or chimera[a]	ANA incidence[b]
Intact DBA/2	0/20 (0%)
DBA/2 ⟶ DBA/2	0/14 (0%)
DBA/2 ⟶ NZB	9/45 (20%)
NZB ⟶ NZB	25/26 (86%)
Intact NZB	59/61 (97%)

[a] Chimeras were established by exposing recipients to 800 to 1000 R when 3 months old, followed by grafting of 3×10^6 bone marrow cells from 3-month-old donors. Mice were assayed 3 months after transplantation or, in the case of intact DBA/2 and NZB animals, when 5 to 6 months old.

[b] Individual plasma samples were diluted 1:20 and assayed for ANA by an indirect immunofluorescence method using chicken erythrocyte nuclei as antigen. Values represent number of mice per total assayed (percent) showing ANA positivity.

Figure 18.3 Seminal study showing that allogeneic bone marrow transplantation reverses autoimmunity in autoimmune prone mice. (a) Breeding of autoimmune prone New Zealand black (NZB) mice with non-autoimmune histocompatible DBA/2 mice yielded (NZB × DBA/2)F_1 progeny with intermediate antinuclear antibody (ANA) production. Lethal radiation of (NZB × DBA/2)F_1 recipients and transplantation with NZB, DBA/2, or syngeneic F_1 bone marrow (BM) showed that mice transplanted with non-autoimmune DBA/2 BM had suppression of ANA reactivity. Neither syngeneic F_1 nor NZB transplants suppressed ANA reactivity. In fact, responses of the different recipient groups displayed remarkable fidelity to donor-type ANA post-transplantation. (b) NZB or DBA/2 mice underwent lethal radiation and transplantation with syngeneic or allogeneic BM. Plasma ANA followed at 3 months post-transplant. Intact control mice were unmanipulated. Only NZB recipients of allogeneic DBA/2 BM showed significant reversal of ANA production. Source: Morton and Siegel 1979 [5]. Reproduced with permission of Lippincott Williams & Wilkins.

The inconsistencies that exist in this literature are not surprising given that autoreactivity arises from a combination of interacting genetic and stochastic factors that affect hematolymphoid cells, and also other tissues. Furthermore, BM grafts are complex mixtures of cells with different functions. Among the populations transferred and generated de novo in a recipient by BM are those that control antigen-specific immune responses – APCs and lymphocytes. APCs express the MHC-restricting elements that are instrumental in shaping the T-lymphocyte repertoire. Hence it is logical that the BM genotype contributes significantly to autoimmune susceptibility. However, the concept that ADs are solely disorders of HSCs is overly simplistic. The other factors that modulate immune reactivity should be considered when interpreting the studies discussed in the following sections.

Rationale for HCT to treat ADs

Both autologous and allogeneic HCT have been studied in preclinical models of ADs. The rationale for efficacy differs between these two procedures. The use of *autologous* HCT is based on the idea that near complete ablation of autoreactive cells, primarily T cells, can be achieved by high-dose therapy followed by rescue with a hematopoietic graft that contains few or no pathogenic cells. Such an approach is analogous to the treatment of cancer wherein the conditioning regimen results in cytoreduction of malignant cells and the patient is "rescued" with a hematopoietic graft that contains none or very few passenger cancer cells. It is thought that the aberrant events that induce autoimmunity (such as infection with virus that cross-reacts with normal tissues) occur only rarely, and the manifestations of disease in AD patients reflect the perpetuation of effector responses which continue even though the inciting event has passed. Therefore, lymphoablation and reconstitution with autologous grafts that lack mature lymphocytes are thought to "reset" the immune system, and the disease will not recur, assuming that the likelihood a second pathogenic event will trigger autoreactivity is extremely low.

The rationale for *allogeneic* HCT is similarly based on the assumption that replacement of a defective immune system with a normal one will eliminate the autoreactive cells. Genetically distinct hematopoietic cells likely express genes that can modify recipient immune responses favoring tolerogenic rather than immunogenetic ones. In addition, mature donor cells (primarily T cells) confer an effect that has been termed "graft-versus-autoimmunity" (GVA), meaning that graft alloreactive cells eliminate host autoreactive cells. There is no expectation that GVA is specific for autoreactive cells, since it is a graft-versus-host effect that can result in broad host T-cell depletion. The majority of preclinical studies favor allogeneic over autologous HCT as the more efficacious approach to blocking autoreactivity. However, the recent multicenter clinical trials of human disease have been exclusively directed to the use of autologous HCT (see Chapter 67) [103,109–111]. The reluctance to perform allogeneic rather than autologous HCT for ADs is based on concerns about unacceptable procedure-related morbidity and mortality in the former as compared with the latter.

Definition of terms and experimental approaches

Autologous, syngeneic, congenic

Genuine autologous HCT has only rarely been performed in rodents because of the pragmatic limitations of harvesting autologous hematopoietic cells from small donors. Instead, syngeneic donors from the same inbred strain as the recipient, or congenic donors that differ from recipients by one or a limited number of non-histocompatibility genes, are used. The advantage of using congenic donors is that the gene difference(s) permits the determination of the origin of hematopoietic cells (residual host as opposed to graft derived) in the transplanted recipient. For example, mice that are identical except for a congenic difference at the CD45 allele are common laboratory tools. CD45 (previously designated Ly5) is expressed on all lineages of hematopoietic cells, and in mice there are two alleles, CD45.1 and CD45.2 [112,113]. mAbs exist that can distinguish between these two alleles. Thus, staining assays employing labeled mAbs, such as fluorescence-activated cell sorter (FACS) analysis or immunohistochemistry, permit the detection of donor-versus-host hematopoietic cells in the transplant recipients. Male into female transplantations or vice versa can also be used, in which case the chimerism analysis involves in situ hybridization looking for the presence or absence of the Y chromosome. Another type of donor has been termed *pseudoautologous*, which means that in models wherein the disease and recipients are induced by antigen immunization [114], the congenic or syngeneic donors undergo similar immunization. Grafts from such donors may contain contaminating mature autoreactive cells with the potential to cause disease, therefore the use of such donors more accurately reproduces clinical autologous HCT. Grafts from congenic or syngeneic donor strains that develop spontaneous disease are not called pseudoautologous, although a similar potential exists to transfer autoreactive cells in unmanipulated hematopoietic grafts. In this chapter, the HCT studies are described as they were originally performed using congenic, syngeneic, or pseudoautologous donors with the understanding that all of these graft types serve as models for autologous HCT.

Genetic differences and measurement of chimerism in allogeneic HCT

By definition, allogeneic HCT uses donors that are genetically disparate from the recipients. Genetic disparity means that the donor and recipients are mismatched at multiple gene regions and are distinct from congenic pairs wherein the genetic differences are limited. Many of the studies were performed between donor and recipient pairs that were derived from distinct ancestral strains, and therefore differed at both MHC and multiple other minor histocompatibility antigen genes. Determination of donor chimerism during the early years was possible using antisera or mAbs in cytotoxicity or staining assays. For transplantations involving MHC differences (MHC-mismatched and haplo-identical), reagents that recognize MHC determinants have been used. Detection of chimerism between MHC-identical strains has been more difficult because the reagents are more limited. In fact, studies that predate the late 1970s when allele-specific mAbs were developed did not evaluate chimerism. The most widely used approach to measure chimerism is antibody-based assays. These assays require that allelic differences exist between strains for gene products expressed on the surface of hematopoietic lineage cells, and that antibody reagents exist that are capable of distinguishing these informative allele markers. Examples of antibody reagents used for this purpose are those that recognize Lgp100 [115], a glycoprotein expressed on lymphocytes of certain strains, or CD45 [112,113], an allelic marker expressed on all hematopoietic cells. An alternative non-surface-staining approach is to utilize polymorphisms identified within the mouse genome [116] and perform polymerase chain reaction (PCR) assays to differentiate hematopoietic cells between MHC-matched strains.

Preparative regimens

In most rodent studies, recipients are prepared for transplantation with lethal radiation. Myeloablative radiation doses are strain specific and require titration studies to determine the dose(s) at which death occurs because of hematopoietic failure, and not other organ toxicities [7,117,118]. Non-uniformity of radiation dose exists in the literature. Because such dose variations affect the level of lymphoablation and the degree of resistance to engraftment of allogeneic hematopoietic cells, radiation dose variability must be taken into consideration when comparing outcomes between different experiments.

The use of non-myeloablative radiation-based approaches in rodent models has lagged behind the studies in humans and large animals. Compared with the explosive development of reduced-intensity and non-myeloablative conditioning protocols in humans, relatively few rodent studies have been performed. However, given the acknowledged concern about the morbidity of conventional allogeneic HCT for the treatment of patients with ADs, the more recent rodent literature tests the idea that with reduced-intensity conditioning the establishment of mixed chimerism may be sufficient to cure ADs.

Chemotherapy that includes reagents used in human patients such as cyclophosphamide (CY) and busulfan (BU) have been employed in studies wherein the goals were to model explicitly clinical transplantation [119,120]. CY is the best studied of the chemotherapeutic drugs in rodents and reports from the early literature show that this agent alone without hematopoietic cell rescue is highly effective at ameliorating manifestations of ADs such as EAE in rats [121] and antibody production and immune abnormalities in SLE-prone mice [122,123]. Dimethyl myleran is an alkylating agent related to BU with profound marrow suppressive activity and little immunosuppression that has been used for rodent HCT preparation [124,125]. Fludarabine, an agent that is widely used in human reduced-intensity regimens, has also been tested in animals. Unfortunately, mice are highly resistant to the lymphoablative effects of fludarabine and its congeners, making it difficult to model homologous reduced-intensity regimens in mice utilizing this drug.

An attractive advantage of working with rodent model systems is the availability of numerous antibody reagents that specifically target immune cell subsets and hematopoietic stem/progenitors. Although there has been no study demonstrating that antibodies alone will effectively allow engraftment of allogeneic HSCs in wild-type animals, there are reports demonstrating that antibody treatment alone can permit engraftment in severely lymphoid-deficient mice [126], and in NOD mice if high doses of $CD8^+$ cells accompanied the BM graft [127]. Cell targeting with mAbs has proven ability to augment engraftment when used in combination with non-myeloablative radiation in NOD mice [128–130] and non-autoimmune strains.

Hematopoietic graft types

Unfractionated or T-cell-depleted (TCD) BM from wild-type donors or BM from mice with the *nude* (*nu/nu*) gene defect have been the graft source in most studies. *Nude* mice are useful as donor animals because they lack *a* thymus [99]. Hence, BM grafts from *nude* mice are not contaminated with T cells, but when engrafted into recipients with functional thymuses, their hematopoietic cells give rise to mature T-lymphocytes. One group of investigators used positive selection by binding BM cells to the plant lectin wheat germ agglutinin (WGA) [131]. The rationale for this approach was that WGA-positive BM cells were enriched for stem and progenitor cells, as well as an immunoregulatory "natural suppressor" population [132]. Highly purified HSCs isolated using a combination of magnetic bead and fluorescence-activated cell sorting for the composite phenotype of $cKit^{+}Thy\text{-}1.1^{lo}Lin^{-/lo}Sca\text{-}1^{+}$ (KTLS) [133,134] (see also Chapter 4) have been transplanted into autoimmune-prone mice [129,135,136]. The T-cell content of KTLS HSC grafts is reduced by >5 logs compared with BM. Of note, the use of KTLS HSCs or other manipulated BM populations in allogeneic transplantations results in marked differences in the level of resistance to engraftment by the host compared with unmanipulated BM. Furthermore, KTLS HSCs lack mature immune cells and therefore do not confer GVA activity. Consistent with this lack of GVA activity, significant amounts of host T cells remain following KTLS HSC transplantations [129,135–138]. Hence graft content has the potential to affect directly the outcome of autologous and allogeneic HCT in the treatment of ADs.

Timing of the HCT procedure

Since the progression of ADs in most rodent models is well characterized, it is possible to choose the time of HCT relative to the expected disease course. For example, in antigen-induced models, immunized animals have been transplanted either before or following the onset of overt manifestations. In spontaneously arising ADs, the transplantations can be performed during the phase when mice have documented abnormalities in immune function but few clinical signs of impairment, or at later disease stages. The studies show that animals are more consistently cured of the ADs if they undergo the HCT procedure at a very early stage of disease, even when there is measurable pathology such as lymphocytic infiltration of islets in NOD mice or evidence of glomerulonephritis in SLE-prone animals, but not at the point when they have suffered end-stage organ damage. If a single organ has been destroyed which is itself replaceable by transplantation, then it is possible to perform simultaneous organ plus HCT transplantation (see Chapter 12). The best example of this approach in AD-affected animals has been in older NOD mice that have undergone simultaneous allogeneic HCT plus donor-matched pancreatic islets in order to cure them of overt diabetes [128,129,139].

Autologous HCT

Proof of concept

The preclinical literature contains conflicting reports regarding the efficacy of syngeneic HCT in curing autoimmune syndromes [7]. Until the late 1980s, it was generally believed that syngeneic HCT would have no effect on AD pathogenesis. In fact, mice transplanted from syngeneic donors served as negative controls in allogeneic HCT studies to differentiate the effects of the preparative regimen alone from the effects of the allograft – a logical conclusion since syngeneic grafts were thought merely to perpetuate ongoing tissue destruction unless complete elimination of pathogenic cells was achieved. Indeed, in many studies [4,129,135,140–142], the animals in the syngeneic control group showed no amelioration of disease whereas allogeneic HCTs were curative. However, a series of reports emerged beginning in 1989 from van Bekkum and colleagues demonstrating that significant remissions could be achieved with syngeneic transplantation in rats affected with antigen-induced ADs [7,143]. In the original studies of arthritis caused by Freund's adjuvant, the syngeneic BMT "control" group was unexpectedly noted to show equal resolution of disease as the allograft recipients (Figure 18.4) [143]. Interestingly, rats that had undergone syngeneic

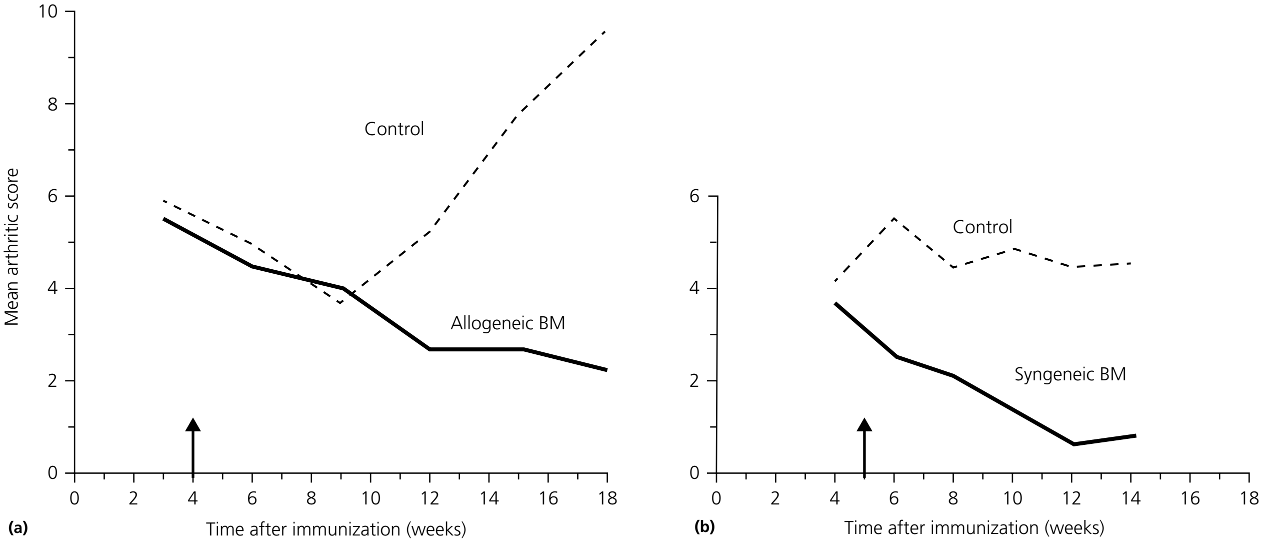


Figure 18.4 Seminal study showing that syngeneic and allogeneic bone marrow transplantation reverses autoimmunity in rats with adjuvant-induced rats arthritis. (a and b) Chronic adjuvant arthritis was induced in male Buffalo rats by inoculation with *M. tuberculosis*. Rats were divided into two equal groups: (i) animals with no further treatment, controls; (ii) animals irradiated (8.5 Gy TBI) and transplanted with bone marrow (BM) cells [(a) syngeneic; (b) allogeneic (MHC-mismatched). Arrows indicate the time of transplantation. Results are expressed as the arithmetic mean of the arthritic score. Source: van Bekkum et al. 1989 [143]. Reproduced with permission of the National Academy of Sciences of the United States of America.

BMT prior to arthritis induction with adjuvant developed disease that was equivalent in frequency and severity as in naïve rats, whereas reimmunization of rats that were disease affected at the time of syngeneic BMT did not reinduce disease. Later studies reproduced this observation that syngeneic BMT is effective only after antigenic challenge and immunity has occurred and not before immunization [144]. These data suggest that one element in the effectiveness of HCT is due to disruption of an ongoing immune response, and that antigen exposure at the time of the procedure (and not before) is required for shifting the response from immunogenic to tolerogenic.

The positive results from syngeneic BMT were repeated in a genuine autologous transplantation study wherein BM was harvested from arthritic rats by surgical removal of a femur, followed by preparation with myeloablative radiation and intravenous return of their own BM cells [145]. Thereafter, in order to reduce the suffering of the affected animals, transplantations were performed using pseudoautologous donors with the same stage of disease severity as the recipients at the time of transplantation.

The curative effect of syngeneic and pseudoautologous BM transplantation was confirmed by van Bekkum and colleagues in a different AD rat model – EAE [120,146,147]. Myeloablative radiation (850–1000cGy) administered shortly after the appearance of clinical symptoms induced a transient exacerbation of disease but most rats achieved remission from EAE following transplantation of syngeneic BM [147]. That said, a certain percentage of these animals spontaneously relapsed. Less intensive conditioning with a non-ablative dose of 750cGy total body irradiation (TBI) plus CY also resulted in complete remission in many rats, but the rate of spontaneous relapse was much higher than that observed with the myeloablative treatment. Hence EAE appeared to be more resistant to the curative effects of syngeneic BMT compared with adjuvant induced arthritis.

Success using syngeneic BMT in treating a different rodent genus, mice, with EAE or spontaneously arising SLE (MRL-*lpr/lpr*) was also demonstrated by Karussis and colleagues [94,148,149]. Their EAE studies [94,150] tested recipient conditioning in early-stage disease with two doses of myeloablative radiation (900 vs. 1100 cGy) or single-dose CY (300 mg/kg). Recipient preparation with CY yielded slightly better results than 1100 cGy of radiation, and both of these treatments proved significantly better than with 900 cGy. The inclusion of CY as a conditioning agent by this group and others was highly clinically relevant given the widespread use of CY in the treatment of human ADs, and its use was based on several earlier reports demonstrating the efficacy of CY alone in the treatment of rodent ADs [121–123]. Mice treated with either TBI or CY plus syngeneic BMT were resistant to disease relapse when rechallenged with the encephalitogenic inoculum, leading to the conclusion that immunoablation followed by reconstitution induces immune tolerance to the autoantigen. The effect of high-dose CY without syngeneic BM rescue [150] was also tested. Although disease symptoms were ameliorated, survival was inferior compared with the BMT groups, suggesting that early rescue of hematopoietic function optimized the therapeutic outcome. Of note, mice that had paralysis for 14 days or greater experienced only a mild therapeutic effect. These investigators extended their studies on syngeneic BMT to the SLE-prone MRL-*lpr/lpr* mice (a spontaneously arising disease) [149]. Preparation with either lethal TBI or high-dose CY and rescue with TCD or unmanipulated syngeneic BM resulted in improved survival and amelioration of serologic and pathologic evidence of disease in all treatment groups, unlike untreated controls. However, long-term follow-up at >20 weeks post-transplantation revealed a significant incidence of relapse. Under both preparative regimens, recipients of TCD BM grafts produced superior results to unmanipulated BM.

Autologous HCT in different disease phases

The early studies suggested that the timing of HCT relative to disease phase is critical to the success of the procedure. Since it is expected that only patients with severe and long-standing ADs will be candidates for HCT, the relevance of timing to clinical translation prompted studies comparing HCT at early and late time points relative to disease induction. Most reports have focused on EAE. Burt et al. [151] showed in a mouse model of EAE of adoptive lymphocyte transfer that clinical improvement and prevention of glial scarring were achieved by syngeneic BMT if mice were treated in the acute but not the chronic phase (day +14 versus day +75 post-lymphocyte transfer, respectively). Herrmann et al. [144] observed that disease symptoms in rats affected with MOG-induced EAE could be attenuated by transplantations from syngeneic, pseudoautologous, and allogeneic donors if rats were treated at the peak of acute disease after immunization (day +17) but not when the disease was in a chronic phase (day +140). Cassiani-Ingoni et al. [152] tracked green fluorescence protein (GFP)-marked congenic BM cells in the CNS of EAE-affected mice that were irradiated and infused with these cells early in the disease or during the chronic phase. In agreement with other reports, transplantation was more effective when performed in the early stage of disease. However, changes in the peripheral immune system, that is, reconstitution by donor elements, did not influence host glial cell inflammatory responses to injury in the CNS which appeared to perpetuate the disease process. Their results suggest that the pathophysiology of an AD occurring within a target tissue may evolve over time such that even aggressive modulation of lymphoid responses will be insufficient to interrupt the process of tissue injury.

Failures of autologous HCT: does T-cell depletion improve the outcome?

Why some animal ADs respond to syngeneic HCT whereas others do not is a subject of debate. One hypothesis for the discrepancies is that antigen-induced diseases are more amenable to treatment with autologous HCT than the spontaneously arising diseases. Indeed, all of the reports showing successful long-term “cures” with syngeneic HCT have been in animals with antigen-induced diseases. van Bekkum [7] suggested that antigen-induced ADs are the more realistic models of human disease, since their etiology seems to resemble more closely events proposed to induce disease in human counterparts, such as exposure to antigens cross-reactive to normal tissues. Although this point is not proved, the successes described above have paved the way to the establishment of large clinical trials testing the use of autologous HCT for the treatment of severe and refractory human ADs [103,109–111] (see Chapter 67).

One possible reason why spontaneously arising diseases did not respond to syngeneic BMT is because preformed autoreactive cells were not completely removed from the grafts. Indeed, syngeneic BMTs in the spontaneously arising lupus and MRL-*lpr/lpr* model showed better outcomes in recipients of TCD versus non-TCD BM [149]. Results were also different in an antigen-induced rat EAE model wherein comparisons were made between pseudoautologous, syngeneic, and TCD BM. Significantly higher incidences of spontaneous relapse were observed in rats that received BM plus peripheral lymphoid cells from pseudoautologous donors [146]. Given the newer technologies that permit the isolation of highly purified KTLS HSCs devoid of T-cell contamination, and the capability to deplete endogenous host T cells more rigorously with mAb targeting, studies have been carried out to re-evaluate whether with such maneuvers syngeneic HCT might be more successful in treating spontaneously autoimmune mice. In experiments by Beilhack et al. [129], prediabetic NOD mice prepared for transplant with myeloablative radiation and anti-T-cell antibodies were rescued with grafts composed of purified KTLS cells plus marked T cells from standard NOD mice and Thy-1.1 congenic NOD mice, respectively. Despite this treatment, most mice developed hyperglycemia within 6 months post-transplant. In the same study, KTLS allogeneic HSCs blocked diabetes development in 100% of mice (Figure 18.3). Similarly, syngeneic KTLS HSC transplantations failed to alter disease manifestations in lupus-prone (NZB×NXW) F_1 mice conditioned with high-dose (1450 cGy) radiation [135], whereas haplo-identical HSC grafts resulted in significant resolution of lupus parameters and prolonged survival. Thus, aggressive myelo- and lymphoablation and rescue with genetically identical grafts devoid of mature lymphoid cells were insufficient to block autoimmunity in at least two of the models of spontaneous AD. Although these data seem to support the idea that autoimmunity arises as a result of "defective stem cells," as discussed earlier in this chapter, autoimmunity in animals such as NOD and (NZB×NXW) F_1 mice is based upon multiple genetic defects that reside both within the hematopoietic lineage and outside.

Autologous transplantation augmented by gene therapy

The intent of autologous HCT to treat ADs is to eliminate disease-causing cells and establish a new immune repertoire devoid of activated pathogenic cells. This "conventional" approach involves the regeneration of a hematolymphoid system that follows a genetically scripted program driven by stochastic events encountered in the environment. Thus, the desired outcome of an immunologic "reset" to alleviate or cure disease is partly left to chance. Because experimental biologists can routinely produce genetically altered cell lines, the idea has emerged that BM can be engineered to express molecules capable of tipping the balance towards immune tolerance following transplantation. Several laboratories have reported success in blocking autoimmune pathology by the expression of selected "protective" molecules in hematolymphoid cells and transplantation into autoimmune-susceptible or -affected hosts [153,154]. Enforced expression of auto- or neoantigens by APCs has been pursued based on the known importance of APCs in inducing and maintaining central and peripheral tolerance. In that regard, expression targeted via the MHC class II promoter has allowed molecules known to be major targets in specific ADs to be expressed on APCs. Murphy et al. [155] used a double-transgenic model of autoimmune gastritis to demonstrate that transfer of BM from disease-resistant transgenic mice that express a gastric antigen (H/K/ATPase) driven by an MHC class II promoter into mice that develop gastritis due to expression of the pro-inflammatory granulocyte–macrophage colony-stimulating factor (GM-CSF) established tolerance to the antigen. Steptoe et al. [156] tested a similar strategy in NOD mice. Proinsulin, the prohormonal precursor of insulin, is implicated as a key autoantigen that drives β-cell destruction in humans and mice. NOD transgenic mice were generated that expressed proinsulin under the control of an MHC class II promoter. Transplantation of these transgenic hematopoietic cells significantly inhibited the development of diabetes in prediabetic NOD mice [156].

Proof of concept that this type of gene therapy can be performed in humans was demonstrated by transplantation of syngeneic cells that were retrovirally transduced ex vivo with autoantigens rather than harvested from transgenic donors. In one study, proinsulin-transduced BM inhibited insulitis in NOD recipients [157], and in another MOG-transduced cells both protected mice against EAE development and interrupted disease progression [158]. Although promising, this approach is applicable only to ADs in which a major offending antigen is known (so that tolerance develops specifically to the autoantigen), which for most human ADs is generally not the case; and the same constraints regarding the timing of transplant relative to end organ damage apply to such gene-manipulated cells. Furthermore, consistent with the idea that intensive immune ablation is required for optimal therapy from autologous HCT, transplants of autoantigen-expressing cells into non-myeloablated recipients have been less efficacious that myeloablative approaches.

Given the known role of disease susceptibility MHC class II alleles, the class II molecule itself has been a target for genetic manipulation (Figure 18.5). NOD mice express a single MHC class II molecule that is strongly associated with disease susceptibility (I-A^{g7}). Tian et al. [159] performed retroviral transduction of NOD BM cells with non-susceptible I-$A\beta^{d}$–GFP or I-$A\beta^{k}$–GFP constructs. Expression of either of these retrovirally encoded MHC class II genes in NOD BM prevented islet inflammation and hyperglycemia when transplanted into young irradiated NOD mice. They also showed that the MHC class II–GFP-transduced BM cells could protect against recurrent autoimmunity against islets, since overtly hyperglycemic NOD mice could be cured of their disease if transplanted with transduced BM plus NOD–SCID islets, whereas controls that received GFP-transduced BM plus islets quickly destroyed their islets [160]. It is worth remembering, however, that MHC class II gene products are histocompatibility antigens. Therefore, for the purposes of gene therapy and transplantation of the modified cells, expression of these molecules can result in untoward effects such as increased engraftment resistance and even potentially chronic GVHD [30,161].

Another related area of exploration is adoptive immunotherapy, which targets gene expression to cells of specific hematolymphoid lineages. Primary T cells, Tregs, T-cell hybridomas, B cells, and DCs have been tested [162]. When transduced with regulatory proteins such as IL-4, IL-10, anti-TNF, and others, these populations have been shown to home preferentially to sites of inflammation and ameliorate disease in autoimmune animal models. Although this form of cellular therapy has proven efficacy in vivo, its effectiveness would likely be potentiated in the context of autologous HCT. Autologous HCT results in significant cytoreduction of pathogenic cells and provides a host environment that could promote greater expansion of gene-modified populations (e.g. via mechanisms of homeostatic proliferation) compared with unconditioned recipients.

Allogeneic HCT

In contrast to the conflicting preclinical literature supporting the use of autologous HCT for ADs, several studies have demonstrated that both spontaneous and induced forms of rodent ADs can be successfully treated by allogeneic HCT [7–9]. Indeed, the seminal studies by Morton and Siegel (Figure 18.3) showing success with allogeneic but not syngeneic BMT [5] motivated this area of research. Variability exists among the experiments, including the

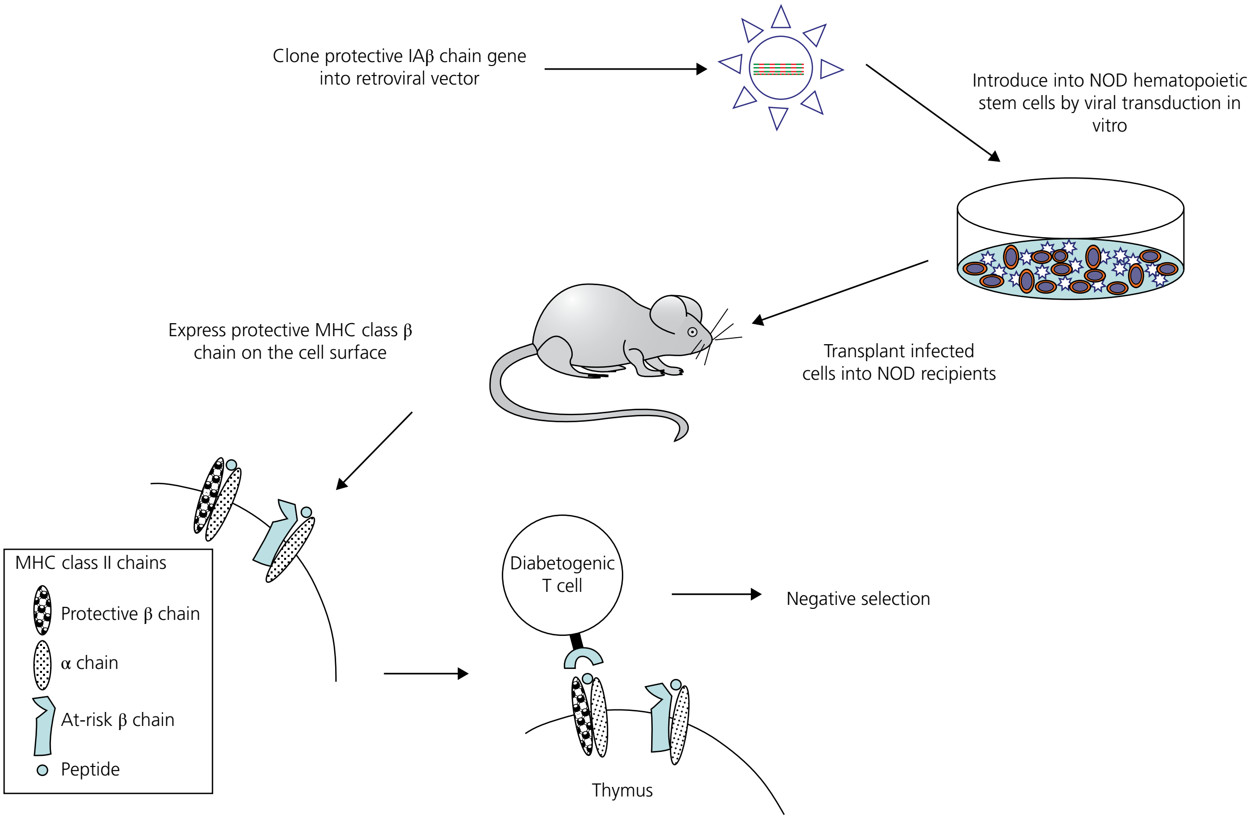

Clone protective IAβ chain gene into retroviral vector
Introduce into NOD hematopoietic stem cells by viral transduction in vitro
Transplant infected cells into NOD recipients
Express protective MHC class β chain on the cell surface
MHC class II chains
Protective β chain
α chain
At-risk β chain
Peptide
Diabetogenic T cell
Negative selection
Thymus

Figure 18.5 Strategy to overcome T1DM in NOD mice by genetic engineering of hematopoietic stem cells (HSCs). Retroviruses carrying MHC class II β chain genes from protective haplotypes are cloned into retroviral vectors. The produced recombinant viruses are then used to deliver the genes to NOD mouse HSCs which are then transplanted into conditioned NOD mice. The product of the protective β chain gene competes for the endogenous α chain to reach the cell surface of MHC class II positive cells, restoring expression of non-autoimmune, wild-type MHC class II molecules on the cell surface. Expression of these restored molecules prevents development of T cells that cause diabetes. Source: Tian et al. 2009 (http://www.discoverymedicine.com/Chaorui-Tian/2009/07/24/preventing-autoimmune-diabetes-through-gene-therapy/). Copyright 2014 Discovery Medicine. All rights reserved.

models tested, degree of donor/host genetic disparity, measurements of donor chimerism outcome, the hematopoietic graft type, the preparative regimens, and timing of the HCT procedure relative to the onset of disease manifestations. Given these heterogeneities, it is therefore striking that rodents are consistently cured by an allogeneic HCT approach.

Treatment of spontaneous ADs with allogeneic HCT

In 1985, Ikehara and Good began a large series of studies demonstrating that allogeneic BMT could successfully treat spontaneously arising ADs in affected mice with overt clinical symptoms [8,9]. Similar to earlier reports, mice with spontaneously arising SLE-like syndromes were transplanted, including MRL-*lpr/lpr* (NZB×NBW) F_1 and BXSB strain mice [163,164]. Resolution of symptoms was achieved even though treatment was initiated at relatively advanced stages of disease. They went on to show protection from progressive insulitis and development of diabetes in young NOD mice [165]. Mice in their studies were prepared for transplantation with lethal (myeloablative) radiation and in the initial reports recipients were rescued with either TCD BM from wild-type mice or BM from mice with the *nu/nu* defect. Thus, mature T cells were not transferred, but grafts gave rise to functional T cells. Such grafts were incapable of causing GVHD, but were also limited in their ability to eliminate host T cells. Most of the experiments were performed between MHC-mismatched donor–recipient pairs. Donor chimerism was evaluated by H-2-specific antisera plus complement assays, and uniformly revealed that >90% of spleen cells were of donor type. With the exception of the MRL-*lpr/lpr* SLE-affected mice [106] and NZB/KN mice that develop a spontaneous inflammatory polyarthritis [105], such allogeneic HCT was uniformly successful not only at blocking disease progression, but also in resolving already established inflammatory lesions [163].

Competitive advantage of autoimmune HSC?

The reasons why allogeneic BMT was unsuccessful in the MRL-*lpr/lpr* and NZB/KN mice were further studied by Ikehara and colleagues [105,106]. In these strains, HCT resulted in initial reversal of clinical manifestations and restoration of other immune aberrations; however, the effects of the transplants were transient and mice regularly relapsed [163]. H-2 typing of the relapsed mice revealed correlation with the return of symptoms and loss of donor chimerism. These graft failures were attributed to abnormal radioresistant HSCs in the autoimmune host strains. An additional competitive advantage of HSCs from MRL-*lpr/lpr* mice was linked to enhanced adhesion to stromal cells. MRL-*lpr/lpr* mice express high levels of the neural cell adhesion molecule (NCAM) [166], which supports hematopoiesis on stromal cell lines [167]. Successful engraftment of MHC-mismatched BM and long-term disease-free survival were achieved in both MRL-*lpr/lpr* and arthritic NZB/KN models [105] by intensifying the conditioning regimens and placement of donor bone grafts [106]. The rationale for the added bone grafting was based upon the investigator's earlier studies, which showed better colonization and proliferation of donor BM on H-2-matched bone grafts [8], consistent with the idea that H-2-compatible BM stroma facilitates enhanced hematopoietic cell engraftment.

Studies characterizing hematopoietic transplantations in spontaneously diabetic NOD mice similarly suggested that HSC/progenitors from this disease-prone strain exhibit enhanced engraftment potential compared with those derived from disease-resistant strains [168]. NOD progenitors exhibited a competitive advantage when co-administered with host-type HSCs in irradiated recipients. They also produced more spleen colony-forming units (CFU-Ss) in vivo in allogeneic recipients and more granulocyte–macrophage colony-forming units (CFU-GMs) in vitro compared with at least four disease-resistant control strains. Enhanced chemotaxis to stromal cell-derived factor 1 (SDF-1) and adherence to BM stroma were proposed as potential mechanisms that permitted a superior NOD HSC engraftment.

The observations that HSCs from certain autoimmune-prone strains dominate hematopoiesis are relevant to the translation of the allogeneic HCT approach to the treatment of human ADs. If HSCs in AD-affected individuals do indeed have a competitive advantage, then designing the appropriate conditioning regimens and graft content that will permit permanent donor cell chimerism will be all the more challenging. However, it is yet to be definitely proven whether or not HSC engraftment potential and autoimmunity are linked. It is known that among non-AD-prone strains, differences exist in the cell autonomous behavior of HSC [169–171]. Hence it is possible that the observations made in the MRL-*lpr*/*lpr*, NZB/KN, and NOD mice are reflective of HSC characteristics that are separate from autoimmunity.

Transplantation of purified HSCs

Reports in the NOD diabetes [129,136] and lupus-prone (NZB×NXW)F_1 models [135] (described above) demonstrated that AD in genetically prone mice was not altered by myelo- and lymphoablation and rescue with purified syngeneic or congenic HSCs. However, in the same studies, engraftment of allogeneic KTLS HSCs successfully blocked autoimmune pathogenesis. Beilhack and co-workers [129,136] showed in prediabetic NOD mice that engraftment of either purified MHC-mismatched HSCs or BM conferred equal protection and resolution of islet inflammation. Of note, the pattern of blood chimerism differed significantly between these groups. NOD mice transplanted with purified HSCs remained mixed T-cell chimeras for an extended period of time post-transplantation, whereas BM-transplanted animals converted promptly to complete donor T-cell type. In both groups, the other blood lineages (B cells, macrophages, granulocytes) were 100% donor derived. The significance of persistent host T cells in animals with spontaneously arising ADs is that these host cells have the potential for autoreactivity (discussed in detail in the later section Mechanisms by which allogeneic HCT abrogates autoreactivity). Smith-Berdan et al. [135] made similar observations when transplanting haplo-identical KTLS HSCs in (NZB×NXW)F_1 lupus-prone mice. Stabilization or reversal of lupus signs was achieved and recipients were mixed T-cell chimeras, whereas the B-cell and myeloid compartments were donor derived. Together, these data provide evidence that allogeneic HSCs alone give rise to elements capable of modifying the activity of radiation resistant autoreactive cells and that complete replacement of donor T cells is not a requirement to obtain curative benefit from allogeneic HSC transplantation.

Allogeneic HCT and antigen-induced ADs

Genotype and susceptibility

The important distinction between induced ADs and spontaneous ADs is that the latter generally arise in the context of a genetic background with multiple immune defects, whereas the former are induced in wild-type animals and require purposeful immunization to break T-cell tolerance. Nonetheless, susceptibility to develop an induced AD also appears to be strain specific since it is observed that for any given antigen immunization protocol, certain rodent strains are more likely to develop autoimmunity whereas others are resistant. Similarly to the spontaneously arising ADs, much of the early literature on BM transfer experiments in autoantigen-immunized rodents sought to identify the cellular elements controlling autoreactivity. Hence the studies were not designed to examine the curative potential of BMT that would involve BMT after disease induction – rather, they focused on the induction of disease in already established radiation BM chimeras. In agreement with the studies in spontaneously arising ADs, the BM genotype seemed to dictate disease susceptibility or resistance [7]. Furthermore, there were at least two independent reports in 1981 [172,173] in which the authors surmised that the mechanism by which the BM genotype exerts its autoreactive or protective effects is at the level of antigen presentation to T cells – a hypothesis that remains compelling, although yet to be proved.

A notable exception to the results demonstrating that BM genotype is the sole determinant of induced AD development was published by Korngold et al. [142] in a model of EAE. In that study, reciprocal transplantations were performed between MHC-matched strain mice that were either highly responsive to mouse spinal cord homogenate (MSCH) (SJL) or low responders (B10.S). Challenge of chimeras with MSCH derived from SJL spinal cord led to a high incidence of disease in the B10.S into SJL chimeras, but not in the SJL into B10.S mice, demonstrating that the BM from the low responders did not protect the recipients. The outcome differed if chimeric mice were immunized with MSCH derived from B10.S mice in that both B10.S into SJL and SJL into B10.S chimeras developed severe disease. These data suggested that non-hematopoietic factors, such as elements within the CNS, control the EAE development. The same group published a separate study in a relapsing EAE model [141]. These experiments showed that immunization with MSCH derived from third-party MHC-disparate BALB/c or B10.S mice resulted in disease in B10.S into SJL chimeras but not SJL into B10.S chimeras, indicating once again that restriction in the development of EAE involves elements outside the hematopoietic system.

Treatment of induced ADs with allogeneic HCT

Success in curing advanced-stage induced ADs with allogeneic HCT was reported beginning in the late 1980s. van Bekkum and co-workers established the adjuvant-induced arthritis and EAE models in rats and tested the efficacy of MHC-mismatched transplantation in conjunction with the autologous HCT studies described above [7]. It was in the adjuvant arthritis model that equivalence in the effect of syngeneic as compared with allogeneic BMT was first described (Figure 18.5) [143]. Susceptible rats were lethally irradiated and received either BM from non-susceptible MHC-mismatched rats or syngeneic BM at weeks or many months following immunization with the adjuvant (*Mycobacterium tuberculosis*). The most effective results were obtained when treatment was initiated shortly after evidence of clinical manifestations at 4–7 weeks post-immunization. Rats treated at the later stage had limited recovery with stabilization of disease, but not complete regression.

Equivalent responses were seen in disease-affected recipients of allogeneic or syngeneic BM.

Comparative studies of syngeneic versus MHC-mismatched allogeneic BMT have been performed in the rat EAE model [120,144]. In 1995, van Gelder and van Bekkum performed transplantations in the rat spinal cord homogenate (RSCH) plus adjuvant model comparing syngeneic grafts with TCD BM or BM from TCD *nude* rats [120]. Syngeneic transplantation was initially effective in inducing remissions in all recipients. However, as discussed earlier, these results differed from those in the adjuvant-induced arthritis studies, since syngeneic and pseudoautologous recipients demonstrated significantly increased incidences of relapse that occurred spontaneously or following rechallenge with the spinal cord antigen. In contrast, all allografted rats had complete remissions with markedly reduced spontaneous and induced relapses. The superior outcome of allogeneic HCT was attributed to a subclinical graft-versus-host reaction (which would later be termed GVA). The rat EAE studies were extended to the use of MHC-matched donors [174]. Again, recipients of allogeneic BM were observed to induce complete remissions with low relapse rates compared with pseudoautologous recipients. Mixed chimeric recipients were created by using grafts of TCD syngeneic plus TCD allogeneic BM. These mixed chimeras relapsed more frequently than the complete chimeras, leading the investigators to conclude that clinical protocols for the treatment of multiple sclerosis should be designed to achieve full donor chimerism. The issue of whether or not a major goal of clinical allogeneic HCT for ADs should be mixed or full donor chimerism is still an open question. Subsequent animal studies that tested reduced-intensity preparative regimens (see the following section) have resulted in numerous reports demonstrating that mixed hematopoietic cell chimerism results in successful amelioration of disease.

Herrmann et al. [144] reproduced and extended the observations made in the rat EAE model. MHC-matched and also syngeneic BM transplantations conferred significant clinical improvement in MOG-immunized EAE-affected rats. In agreement with other reports, HCT was effective in the acute but not the later phase of disease, and in the later phase allogeneic transplantation was no better than syngeneic. Rats transplanted during the peak of EAE disease and that improved were protected from relapse when challenged again with the original MOG immunogen, but not when a different epitope of MOG was used for the rechallenge, a finding that supports the idea that HCT induces antigen-specific tolerance. Furthermore, disease protection correlated with the presence of increased numbers of $CD4^{+}CD25^{+}FoxP3^{+}$ cells. Van Wijmeersch et al. [175] also studied amelioration of EAE in affected mice that underwent allogeneic HCT from susceptible versus non-susceptible donors. Their data indicated that high levels of donor chimerism from a non-susceptible donor were required to achieve the best therapeutic effect. Furthermore, alloreactivity induced by donor lymphocyte infusions (DLIs) enhanced the protective effect, thus supporting the idea that strong GVA is important for effectively treating ADs.

MHC-mismatched versus matched HCT

Most preclinical studies that have tested the efficacy of allogeneic HCT on AD pathogenesis utilized grafts mismatched at both MHC and minor histocompatibility loci. Surprisingly few have used transplantations of MHC-matched hematopoietic cells [4,131,136,174]. The importance of MHC identity or non-identity relates both to the direct translation of the animal data to clinical protocols as ideally HLA-matched donors will be used, and to the mechanism by which allogeneic HCT mediates its AD protective effect. Given the well-known association of MHC-genotype with autoimmunity (Table 18.1) and the importance of MHC molecules in shaping and maintaining the T-cell repertoire, the effect of MHC-matched transplantation on AD recipients should be a central issue. As a generalizable principle, it might be expected that donor grafts mismatched at the MHC would be more effective in blocking autoimmune pathogenesis than grafts that are MHC-matched.

In 2005 Beilhack et al. [136] and in 2011 Racine et al. [176] examined the effect of MHC-matched HCT in rodent disease with the strongest MHC association, namely diabetes in NOD mice. Disease development in NOD mice has a well-known linkage to the expression of a single unique class II MHC molecule, designated I-A^{g7}. In fact, NOD disease susceptibility appears to depend upon the presence of the I-A^{g7} molecule. Transgene expression of non-I-A^{g7} in the germline of NOD mice and also transplantation of transgenic BM expressing non-susceptibility class II alleles into young NOD mice result in protection against the development of hyperglycemia [159,177]. B6.H-2^{g7} mice that were C57BL/6 strain but congenic for the entire NOD MHC region and disparate at minor histocompatibility loci were used as donors of BM or purified KTLS HSC [136]. Transplantation of either graft type into myeloablated prediabetic NOD recipients resulted in 100% protection against diabetes. Because of the rigorous T-cell depletion, KTLS HSC recipients were partial T-cell chimeras with significant residual NOD host T cells remaining. These data give confidence that HLA-matched donor HCTs can be effective in the treatment of human ADs, since non-MHC "background" genes conferred protection even in the presence of a strong disease-linked MHC allele. Of course, a next important step is to identify and characterize the protective non-MHC genes.

Reduced-intensity allogeneic HCT and establishment of mixed chimerism

The major limiting factor in treating human ADs with allogeneic HCT is concern about the morbidity and mortality of the transplant procedure. Two ways in which the dangers of the procedure can be significantly decreased are by reduced-intensity conditioning (RIC) and reduction of donor T cells to remove the risk of GVHD. By performing these kinds of maneuvers, the expectations are that recipients will develop mixed donor–host chimerism rather than convert to complete donor type. The establishment of mixed donor chimerism has not been an end-goal for clinical transplantations, since the majority of HCTs are done for hematolymphoid malignancies and graft-versus-tumor (GVT) responses with conversion to donor type are thought to be an essential element in the therapy. However, for ADs where GVT is not needed, the question remains, will the establishment of mixed chimerism be sufficient to block autoimmunity? The prevalence of RIC protocols in the human HCT literature is in no way matched by animal studies. There are relatively few preclinical reports on the topic of RIC transplantation for the treatment of ADs.

Li et al. [178] demonstrated that disease in NOD mice could be successfully blocked with reduced doses of radiation when delivered in conjunction with infusion of high amounts of MHC-mismatched unmanipulated BM, and that despite the lower dose radiation, the high BM dose resulted in high levels of donor chimerism (>95%). Mixed chimeric NOD recipients and disease protection were also shown by treatment with lethal radiation and rescue with grafts that contained both NOD plus donor cells

[179,180]. Beilhack et al., showed that stable multilineage chimerism and prevention of diabetes in NOD mice could be more readily achieved using MHC-matched (B6.H-2^{g7}) purified HSC grafts [136]. These latter studies addressed the clinical question raised above, as it was shown that reduced-intensity treatment and the establishment of "true" mixed chimerism using grafts that were rigorously depleted of T cells and from MHC-matched donors were curative of this AD (Figure 18.6). Liang et al. [127] generated MHC-mismatched mixed chimeras in NOD mice using a novel non-radiation-based regimen of anti-CD3 antibody and grafts composed of high-dose MHC-mismatched BM plus CD8$^+$ T cells. Islet destruction was blocked [127] and even reversed in newly diabetic NOD mice [181], suggesting that effective disruption of the autoimmune process by allogeneic HCT in new-onset diabetics in whom some islet mass is preserved (a period in humans with T1DM termed the "honeymoon" phase) may prevent or delay the need for exogenous insulin. However, in contrast to the earlier studies by Beilhack et al. [136] when this same regimen was used to generate MHC-matched partial chimeras, recipients were not protected from diabetes development. These conflicting studies using MHC-matched grafts suggest that the mere establishment of mixed allogeneic chimerism may not be sufficient for protection from autoreactivity, but rather in addition, the method of conditioning as well as graft content will impact the outcome.

Proof that both non-myeloablative regimens and allogeneic HCT can induce tolerance to autoantigens and alloantigens has been shown by studies that combine both HCT and islet transplantation to cure overtly diabetic NOD. Seung et al. [128] used a preparative regimen of sublethal radiation plus anti-CD40 ligand (CD40L) mAb that resulted in high levels of MHC-mismatched donor chimerism (>99%) in most mice. Diabetic chimeric NOD mice were transplanted with donor-matched islets and achieved long-term normoglycemia. Sykes and colleagues [130] employed a conditioning regimen that combined anti-CD4, anti-CD8, anti-Thy-1.2, and anti-CD40L mAbs plus low-dose radiation [400cGy) to achieve engraftment of MHC-mismatched BM in

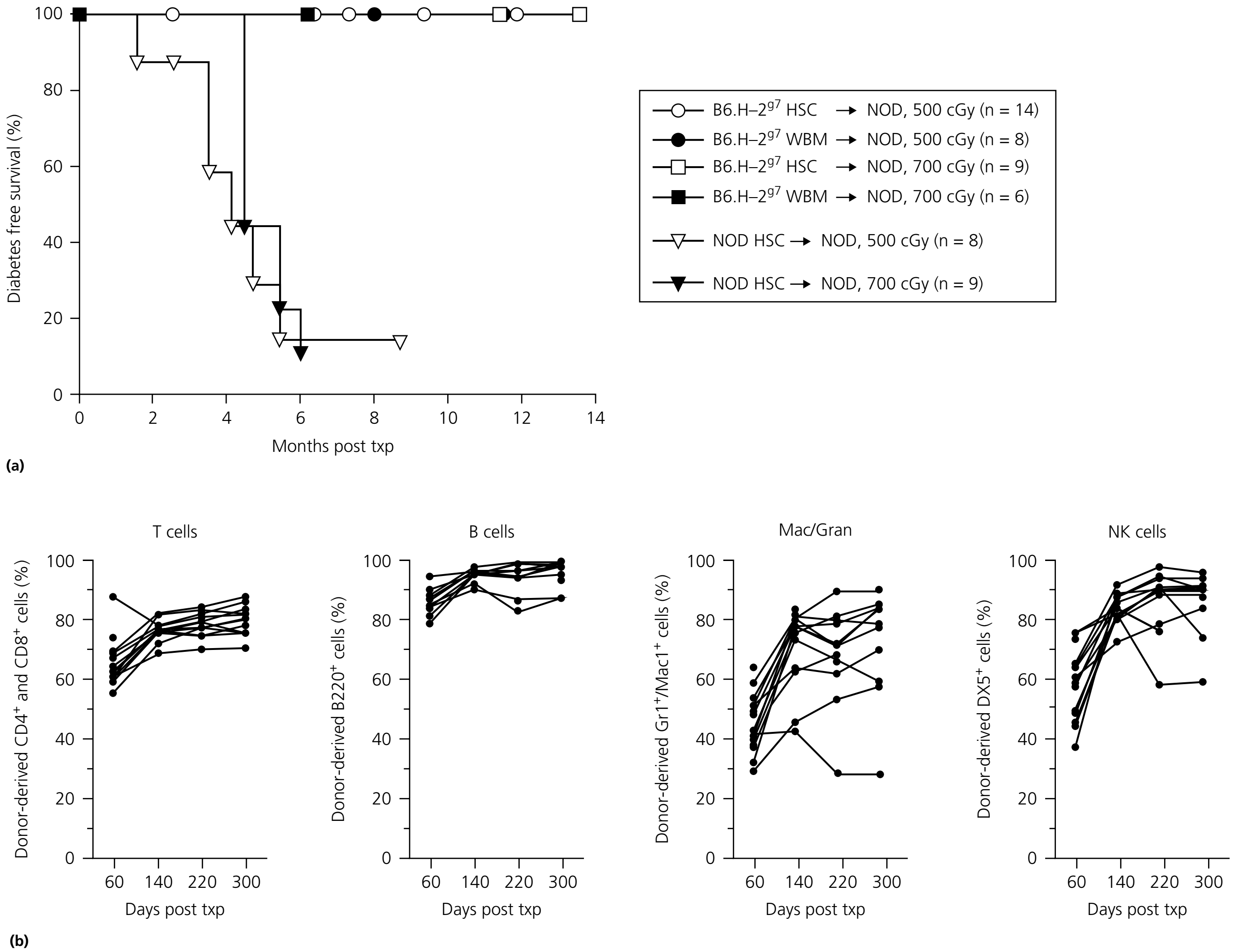


Figure 18.6 Non-myeloablative transplantation of MHC-matched hematopoietic stem cells (HSCs) results in multilineage chimerism and blocks autoimmunity. (a) Prediabetic NOD mice were prepared for transplantation with sublethal radiation (500 and 700 cGy) which was sufficient to permit engraftment of MHC-matched purified HSCs from B6.H-2^{g7} donors. Recipients of B6.H-2^{g7} HSCs or whole bone marrow (WBM) were protected from diabetes development. Recipients similarly prepared and transplanted with syngeneic HSCs progressed to hyperglycemia. (b) Testing of blood revealed the non-myeloablated NOD recipients to be stable mixed chimeras. Shown are the chimerism studies for the different blood cell lineages from NOD mice that received 500 cGy and B6.H-2^{g7} HSCs and that were followed up to 1 year post-transplantation. The mixed granulocyte–monocyte lineage demonstrates that these recipients had continued hematopoiesis from host HSC.

overtly diabetic NOD mice. Multilineage chimerism was established with amelioration of autoimmunity and the induction of tolerance to donor-matched islets.

In other non-diabetic autoimmunity models, reduced-intensity conditioning with allogeneic HCT has been shown to halt disease pathogenesis. Flierman et al. [182] showed efficacy in the collagen-induced arthritis model using sublethal radiation conditioning plus anti-CD40L and MHC-mismatched donors. Interestingly, both syngeneic and allogeneic BMT led to clinical suppression of arthritis, although allografted mice demonstrated superior reduction of pathogenic antibodies. Smith-Berdan et al. [135] achieved durable multilineage mixed chimerism following transplantation of haplo-identical KTLS HSCs in radiation-resistant SLE-prone (NZB×NZW)F_1. These stable chimeras had superior survival and exhibited stabilization or reversal of their lupus symptoms, which included proteinuria, circulating immune complexes or autoantibodies, compared with untreated controls and syngeneic recipients.

In summary, many animal studies using reduced-intensity conditioning have been consistently successful in blocking autoimmune pathology, even when significant levels of potentially autoreactive host cells persist. That said, the majority of the proof that mixed chimerism is sufficient to block autoimmunity comes from transplantations of MHC-mismatched grafts. When the anti-host immune effect of the allograft is weaker, as in the case of MHC-matched transplants, we expect that the type of conditioning and graft type will play a more prominent role in whether or not the procedure is a success.

Mechanisms by which allogeneic HCT abrogates autoreactivity

The way in which allogeneic HCT interrupts AD pathogenesis is by a combination of mechanisms that extend beyond the standard activities required for treatment of malignancies. Similar to transplants for cancer, conditioning regimens provide cytoreduction of pathogenic host immune cells, and donor allografts that contain T cells can also reduce host T cells and other immune cell populations, an effect referred to as graft-versus-autoimmunity (GVA). In addition, within the autoimmune-prone host, the allograft establishes its own immune system capable of generating cells that can control immunity and that follow the genetic programming of the donor. The superior outcomes in allogeneic animal models that result in mixed chimeras compared with syngeneic models underscores this latter point. Autologous HCT succeeds because of the stochastic interactions that occur between a regenerating immune system and non-hematopoietic factors that determine restoration of self-tolerance. It is thought that allogeneic HCT demonstrates higher success rates because in the process of regenerating immunity certain susceptibility genes expressed on host hematopoietic cells are either replaced or differentially expressed by the donor. These expressed gene products impact on the pathways of central and/or peripheral tolerance favoring a protective outcome.

T-cell depletion

Depletion of host T cells is the most extensively studied mechanism by which allogeneic HCT can alter immune function. There is ongoing discussion amongst clinical transplant professionals regarding the need to achieve full donor T-cell chimerism in order to obtain cures of human ADs. Separate from the effects of conditioning, unmanipulated allogeneic hematopoietic grafts replete with mature immune elements have the capacity to eliminate or reduce residual host hematolymphoid elements. GVA is the term that describes this pan-alloreactivity and conversion to complete donor type serves as a surrogate marker for GVA activity. Although elimination of host T cells by this mechanism certainly increases the likelihood that autoreactivity will be reduced, GVA will be accompanied by significantly increased risk of GVHD. Of note, GVHD is in and of itself immune suppressive because lymphoid organs are major targets of alloreactive T cells. Even low levels of mature donor T cells can result in a syndrome of subclinical GVHD wherein immune responses are impaired [183]. Therefore, attention should be paid as to whether or not autoreactivity is blocked because of globally impaired function versus more specific alterations in immunity.

Many of the animal studies on the topic of HCT for the treatment of ADs predate the technologies that allowed the measurement of donor-versus-host hematopoiesis. Hence T-cell chimerism was not assessed and it was assumed that animals surviving lethal radiation and BMT converted to donor type. Indeed, later studies by Ikehara et al., in which chimerism of the blood or spleen was assessed, showed chimerism levels of >90% [163] although lineage subset analyses were not performed. From more recent reports using purified HSCs [129,135,136,138], clinical studies examining chimerism in patients of TCD grafts [184], and analyses of chimerism following RIC transplantation [185], it is evident that the T-cell lineage is the most resistant to conversion to donor type. HSCs and TCD BM lack or have reduced amounts of alloreactive GVA populations that mediate elimination of residual host immune cells [137,138]. Transplantation of allogeneic unfractionated BM into lethally irradiated mice results in near complete conversion to donor type, whereas HSC transplantations result in persistent partial T-cell chimerism [138,186]. Patients who have received TCD BM also demonstrate long-term mixed chimerism [184,187,188]. It is therefore reasonable to conclude that in the many preclinical experiments wherein T-cell chimerism was not assessed, but in which TCD or BM from *nude* mice was used as the graft source, those autoimmune affected recipients remained as partial T-cell chimeras.

Central and peripheral T-cell deletion

Beyond T-cell depletion by conditioning or overt alloreactivity, potentially autoreactive T cells can be eliminated by negative selection. Negative selection, a fundamental mechanism by which the immune system maintains T-cell tolerance, occurs by both central thymic deletion, and peripheral deletion of post-thymic cells [189]. BM-derived APCs are the most efficient mediators of negative selection. In a non-transgenic animal, it is virtually impossible to demonstrate directly negative selection of T cells for any particular self-antigen because, generally, antigen-specific T cells are too few in number to detect. One way to follow the fate of an antigen-specific T cell in experimental models is to utilize TCR transgenic mice that have high levels of a single clone which can be identified by reagents such as a labeled MHC tetramer plus peptide known to bind the transgenic T cells. Racine et al. [176] used this approach to show that hematopoietic allografts could mediate thymic deletion of autoreactive T cells in TCR-transgenic NOD mice that have high levels of an anti-islet T-cell clone. In this study, MHC-mismatched but not MHC-matched hematopoietic grafts were capable of deleting the anti-islet clone.

Another method to track T-cell deletion is via methods that follow responses to a class of non-conventional antigens called superantigens [190]. Superantigens are viral or bacterial proteins that interact with T cells via the class II MHC and Vβ segment of the TCR. T cells bearing the Vβ segment specific for the antigen die by apoptosis, resulting in nearly complete elimination of all cells of the responding Vβ subclass. Some superantigens exist as endogenous genes in mice of certain strains, and during T-cell development the cells with receptors that have the Vβ capable of reacting to these “self antigens” are deleted by negative selection. Hence tracking T cells based upon Vβ staining in experimental systems in which exposure to superantigens can be manipulated (such as BM chimeras) serves as a surrogate assay for assessing negative selection. Using the superantigen system, Beilhack et al. [129] demonstrated the ability of purified HSC allografts to mediate negative selection of T cells reactive to endogenous superantigens by measuring Vβ subsets in chimeric NOD mice transplanted HSCs from donors that differed in superantigen expression. Such mixed chimeric NOD mice had significant residual T cells, yet both donor and host reactive Vβ subsets were deleted. These data not only showed that the HSC graft mediated negative selection of developing T cells, but additionally that the allografts eliminated potentially self-reactive post-thymic T cells of NOD origin in the periphery.

Replacement of susceptibility genes of the host

The cumulative experience on genetic predisposition to the predominant forms of autoimmunity, reinforced by the GWAS data, shows that activities of multiple, interactive susceptibility genes drive these diseases. Since many of these genes are expressed on hematolymphoid cells, another way in which allogeneic HCT can modify pathogenic immune responses is by “replacement” of these key regulatory genes in the hematopoietic compartment. MHC genes are the prototypic, and indeed the only, gene family identified to date that fit this description, and there are numerous examples that demonstrate that adding or replacing the MHC susceptibility allele by HCT, formal genetic crosses or transgenic technology provide disease protection [159,177]. That gene products outside the MHC can also halt autoimmune pathology has been demonstrated in preclinical models of MHC-matched HCT transplantations [4,131,136,191]. However, given that most preclinical studies demonstrating successful treatment of advanced ADs were performed with MHC-mismatched BM, there is some doubt whether similar consistent positive outcomes will be observed when AD-affected humans are transplanted with HLA-compatible grafts.

The GWAS analyses highlight the spectrum of non-MHC genes expressed in hematopoietic cells that can potentially alter the course of autoreactivity (selected genes shown in Table 18.3). These genes include those that affect overall immunoreactivity such as cytokines, cell proliferation, lymphocyte or APC cell activation, and apoptosis. For example, cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), a hematopoietically expressed molecule and critical regulator of T-cell activation, has been identified as a major non-MHC susceptibility gene associated with ADs in humans and mice, including NOD diabetes [102]. The studies imply that common allelic variation in the expression of levels of alternative splice forms of CTLA-4 is a primary determinant of AD susceptibility.

An approach that we have taken in NOD mice to identify the non-MHC genes expressed on hematopoietic cells that confer protection in the setting of HCT is to use as donors congenic mice generated by recombinant inbreeding. Extensive efforts by several groups led to the generation of NOD mice that contain congenic intervals in their genome, termed *Idd*, derived from the C57BL strain [74,81]. Depending on the introduced chromosomal interval, these congenic NOD mice demonstrate different degrees of diabetes protection. Among the known *Idd* loci, the most protective non-MHC loci introduced map to chromosome 3 (*Idd3*), chromosome 1 (*Idd5*), and chromosome 4 (*Idd9*). Our earlier studies showed that HSC transplantation from MHC-matched B6.H-2^{g7} donors that express the full compliment of C57BL background provided 100% disease protection [136]. Therefore, to determine whether one or more of these congenic intervals contained a transferable protective gene(s), BM from NOD *Idd3*, *Idd5*, and/or *Idd9* congenic donors were transplanted into lethally irradiated prediabetic wild-type NODs. Mice that received grafts carrying one or two protective loci developed diabetes. These results were disappointing, particularly because the mouse CTLA-4 gene maps within the *Idd5* region [192]. However, significant diabetes protection was conferred when NOD mice that express multiple (*Idd3*, *Idd9*, *Idd10*, *Idd17*, and *Idd18*) protective alleles were used as donors. Hence our NOD congenic studies show that in this model of autoimmunity, protection by HCT depends upon multiple disease-resistant loci outside the MHC. Progress in the genetic basis of T1DM supports this finding, as several studies described interactions of CTLA-4 with other susceptibility loci that regulate expression and therefore contribute to the pathogenesis [192,193].

Regulatory cells

Despite the prominence of Tregs in the basic and clinical immunology literature, few reports exist on the role of regulatory T cells as a mechanism by which allogeneic HCT blocks autoimmunity; and studies to date do not show a definite role for regulatory cells as a major mechanism by which allografts control autoimmunity. We studied the potential contribution of Tregs to disease amelioration in NOD chimeras in two different types of experiments [136]: (1) by measuring the percentage and absolute numbers of Tregs ($CD4^+CD25^+$ cells) in the blood of control and transplanted mice, we observed that unmanipulated NOD mice had higher levels than MHC-matched disease-resistant B6.H-2^{g7} donors, and diabetes protected chimeras had levels equivalent to wild-type NODs, (2) B6.H-2^{g7} SCID mice lack both $CD4^+CD25^+FoxP3^+$ cells and another subset of regulatory T cells, NKT cells, were used as hematopoietic cell donors. Despite the inability of the donated cells to generate these T-cell subsets, NOD recipients enjoyed nearly 100% disease protection. In contrast to our negative results, Herrmann et al. [144] reported in a MOG-induced EAE rat model that increased levels of $CD4^+CD25^{bright}$ cells in lymph nodes and increased expression of Foxp3 in the spleens were observed in EAE-protected transplanted rats compared with untransplanted controls. However, similar to our studies, they found that the EAE-susceptible rat strain had higher levels of $CD4^+CD25^{bright}$ cells in their lymph nodes than disease-resistant rats. Given the known importance of Tregs in the control of immunity, these negative results should not deter testing of Tregs as adjunctive cellular therapy in HCT for autoimmunity, since it is clear that shifting the balance in this way towards tolerogenic outcomes is in line with “standard operating procedure” of the immune system.

Conclusion

The knowledge that autologous and allogeneic HCT cause profound alterations in immune reactivity resulting in cures of ADs has been present in the scientific and clinical literature for decades. The animal studies provide a fundamental basis for understanding both the potential benefits and limitations of HCT to treat these complex diseases effectively. The experimental details discussed in this chapter are meant to provide a foundation for the thoughtful translation of these studies into clinical reality.

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CHAPTER 19

Pharmacologic Basis for High-dose Chemotherapy

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Introduction

High-dose chemotherapy utilized to "condition" patients prior to a transplant was based on the idea of eradicating the tumor and "creating space," then engraftment would occur. This was accomplished by applying an empiric combination of total body irradiation (TBI) and cyclophosphamide [1]. Later, combination chemotherapy without TBI gained popularity [2]. The most commonly used regimen in allogeneic stem-cell transplantation (allo-SCT) was for many years the (oral) busulfan–cyclophosphamide combination introduced by Santos et al. [3,4], and subsequently revised by Tutschka et al. [5]. Cyclophosphamide (CY) was used mostly for its immunosuppressive properties. However, agents such as melphalan (MEL) used by itself in multiple myeloma, and in various combinations, have also been successfully employed. A listing of the most commonly used cytotoxic agents currently used in (high-dose) conditioning chemotherapy preceding allo- and auto-SCT is presented in Table 19.1.

The concept of "creating space" has gradually been replaced by a more nuanced view of the mechanisms involved in engraftment of allogeneic cells and a complex picture is gradually emerging. Similarly, the idea of eradicating tumor cells with high-dose chemotherapy while simply replacing the patient's immune system has been substituted by a model where engraftment along with tumor killing is facilitated by the conditioning regimen. Once engrafted, the new immune system will provide an immunologic graft-versus-tumor effect against residual tumor cells to accomplish long-term freedom from progression. Conditioning regimens were previously designed by using cytotoxic agents in empiric combinations near their maximum doses in single-agent therapy. More recently, data from preclinical studies of combinations of drugs which optimize synergistic antitumor interactions while minimizing the risk for normal tissue damage are increasingly used to guide the design of new conditioning programs. Such novel regimes allow us to improve both the antileukemic effects and the immunosuppressive capability of the preparative regimen. Incorporation of pharmacogenetic information about metabolic drug handling permits the use of agents that target different metabolic pathways, thereby increasing patient safety. Much developmental work in the last decade has contributed to introducing innovative combinations of nucleoside analogs or other DNA damage-modifying agents with external-beam radiotherapy (XRT) and/or alkylating agents [6–10]. These regimens appear safer and possibly more efficacious than the older empiric programs. Further, treatment intensity can be modulated in proportion to the effectiveness of immune-mediated tumor killing by the graft. These approaches aim at optimizing patient safety and minimizing serious treatment-related adverse events while maximizing antitumor effects.

The more recent introduction of nucleoside analog (NA) combinations in pretransplant conditioning provides both the intense immunosuppression necessary to facilitate engraftment and an improved antitumor effect by inducing DNA damage and inhibiting DNA repair. Further, the different metabolic pathways for DNA-alkylating agents and NAs contribute to retaining or improving patient safety; each drug can be used at virtually full dose compared with its use as a single agent. Thus, the high incidence of overwhelming toxicity which directed the development of reduced-intensity conditioning is now being challenged with a graded approach that includes "myeloablative, reduced-toxicity" therapy. This methodology has been facilitated by an increasing level of mechanistic understanding of interactions between different cytotoxic agents. It is also clear that studies of genetic variability in the enzyme systems involved in the metabolic pathways that determine both activation and deactivation of the cytotoxic agents will assist in designing more efficacious and less toxic regimens. In this chapter, we review the cytotoxic agents that are most commonly used in pretransplant conditioning and some of the emerging molecular, pharmacokinetic, pharmacodynamic, and pharmacogenetic data that are of importance for the development of even more effective high-dose therapy. These data will not, however, suffice to optimize the drug administration in individual patients, where we look forward to an increasing role for therapeutic dose monitoring based on pharmacokinetics. We also examine some data for clinical dose-limiting toxicities and their pharmacologic basis. We limited our review to regimens commonly used in the period 2009–2011.

Pharmacology of drugs used in high-dose chemotherapy

Alkylating agents

Busulfan

Busulfan (BU) belongs to the general class of bifunctional methanesulfonic acid esters that have varying length of the interspacing methylene groups. There are several members of this class that have cytotoxic activity; busulfan (1,4-butanediol dimethylsulfonate,

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Figure 19.1) is the clinically most used. Its cytotoxic action is generally believed to be through alkylating guanine residues in DNA-forming adducts [11]. These adducts can be monoadducts, or DNA intra- and inter-strand cross-links and also DNA–protein cross-links. BU is chemically unstable in an aqueous environment and does not need cellular activation. The most important detoxification step is glutathione *S*-transferase (GST) A-mediated BU conjugation with glutathione (GSH) to form a tetrahydrothiophenium ion (Figure 19.2) [12,13]. The GSTA1 isoenzyme [14] constitutes about 65-80% of hepatic GST activity [15]. Depletion of GSH increases the target tissue sensitivity to BU. Experimental overexpression of GSTA1 induces relative resistance, but cellular BU resistance is multifactorial, and GSH depletion in resistant tumor cells may not restore sensitivity to this drug [16]. In the clinical setting, it is uncertain how much a varied cellular concentration of GSTA1 or any of the other GST isoenzymes contributes to BU resistance, since systematic studies are needed to confirm the clinical relevance of the intriguing experimental data.

Table 19.1 Conditioning regimen drugs given to HSCT recipients registered with the CIBMTR between 2009 and 2011[a]

Allogeneic ($n = 26538$)			Autologous ($n = 27005$)		
Drug	***N***	**%**	**Drug**	***N***	**%**
Fludarabine	14438	54	MEL	21488	80
Cyclophosphamide	13765	52	VP-16	11162	41
Busulfan	11673	44	BCNU	8486	31
TBI	9764	37	Ara-C	6694	25
ATG	8301	31	Cyclophosphamide	4297	16
MEL	4147	16	Busulfan	1943	7
MAb	2065	8	Carboplatin	1468	5
VP-16	1430	5	MAb	1214	4
ThioTEPA	1193	4	ThioTEPA	987	4
Ara-C	679	3	TBI	640	2
BCNU	479	2	Gemcitabine	214	1
Clofarabine	315	1	Radiolabeled MAb	205	1
Treosulfan	311	1	Bortezomib	195	1
TLI/TNI/TAI	257	1	CCNU	151	1
Pentostatin	154	1			

[a] Excludes drugs used for less than 1% of patients.

Data kindly provided by the Center for International Blood and Marrow Transplant Research, (CIBMTR) (2012).

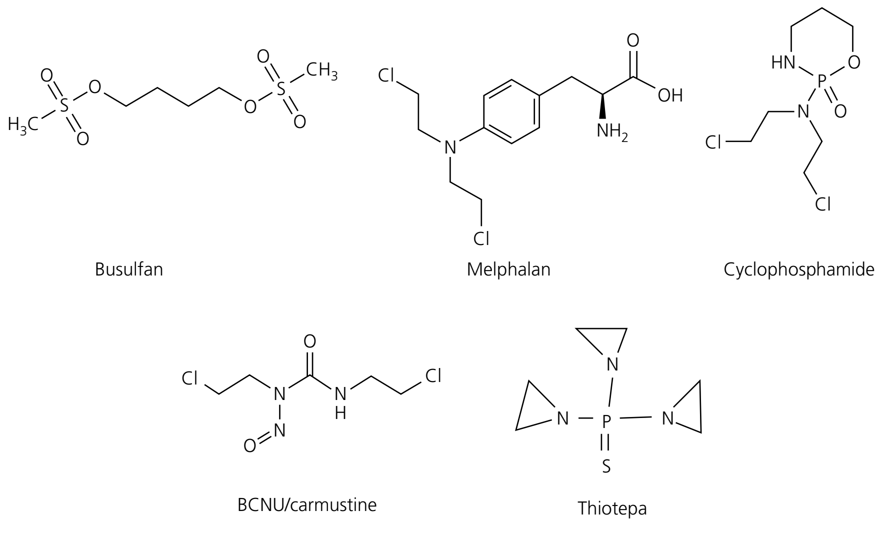
Metabolism and mechanism of action

BU is very lipophilic and is thought to diffuse freely into the target cells/tissues [17]. Although BU does not seem to be a substrate for any of the hepatic cytochrome P450 isoenzymes, its GSH-conjugated sulfonium ion is a substrate for hepatic P450 metabolism. Its further metabolism is partly determined by tissue alkylation and partly by hepatic and urinary excretion through the formation of sulfolane, 3-hydroxysulfolane, and tetrahydrothiophene 1-oxide (Figure 19.2). These metabolites constitute approximately 70% of a given dose and are excreted in urine within the first 72 hours. Only about 1–2% of a given dose is excreted unchanged in urine, and a similar amount of intact drug is excreted through the bile [12].

The relative importance of the various GST isoenzymes in BU metabolism and clearance and their importance for clinical adverse events after high-dose therapy and treatment outcome have been studied by several groups; both GSTA1 and GST-M have been correlated with altered drug clearance and cellular drug resistance [14,18]. The combined effects of genotypic isoenzyme variants have been implicated in altered drug clearance to a magnitude that may influence treatment outcome. In addition, there is still somewhat limited knowledge about intracellular BU disposition beyond GSH conjugation. Finally, BU efflux from cells has been inadequately studied. BU systemic exposure is a (good) surrogate marker for anticipated drug/regimen-related toxicity, but many clinical reports include patients conditioned with (oral) BU–CY, and both the CY component and its timing and sequencing relative to BU contribute to the risk for hepatotoxicity [19–21]. The effects of prior chemotherapy and concomitant medications given shortly before and during the preparative regimen have also been inadequately studied [22]. The metabolism and systemic clearance of BU, and of most other alkylating agents, are complex and at best incompletely understood [23].

Pharmacokinetics

BU is available both as a tablet and as a parenteral solution. Oral drug bioavailability is highly variable, ranging from less than 10% to virtually 100%; on average it is approximately 70–80% [24,25]. BU pharmacokinetics are linear and follow first-order kinetics in a wide dosing interval, ranging from the conventional 2–6 mg once daily up to 3–4 mg/kg given once daily (or about 130–160 mg/m^2) in



Busulfan

Melphalan

Cyclophosphamide

BCNU/carmustine

Thiotepa

Figure 19.1 Structures of DNA alkylating agents used in HSCT.

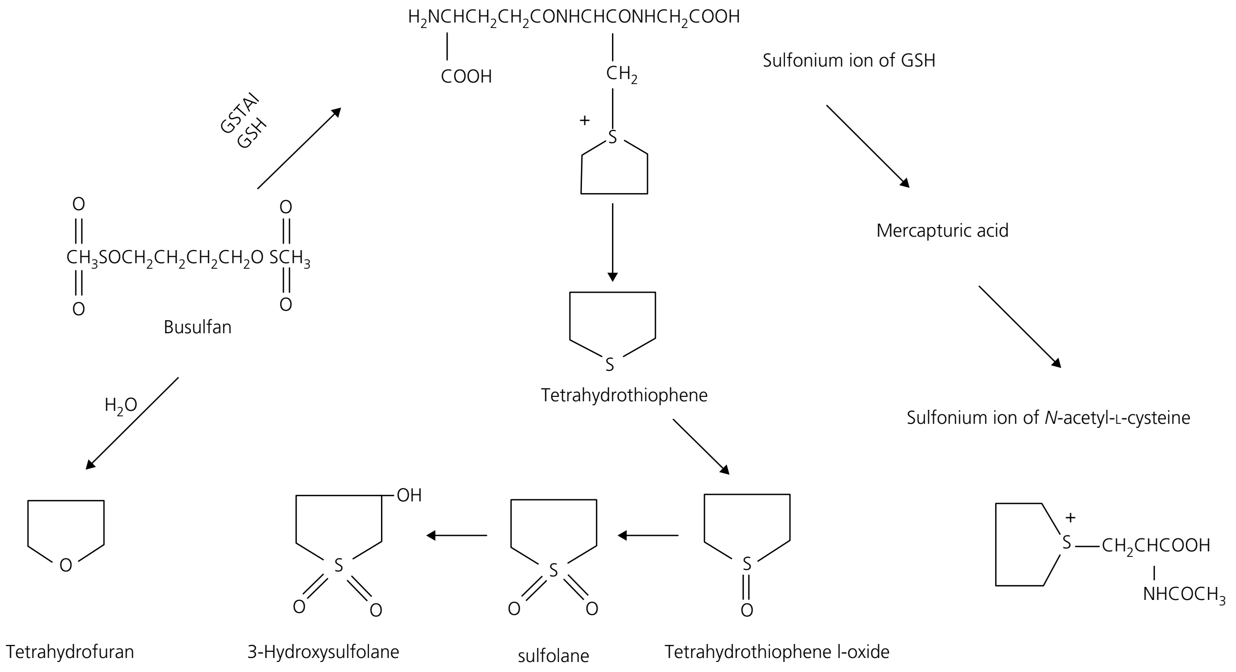


Figure 19.2 The metabolic pathway of busulfan. Source: Hassan and Andersson 2013 [13]. Reproduced with permission of Future Medicine Ltd.

conditioning therapy. Plasma protein binding is less than 10%, and the half-life is about 2.5–3.5 hours (range approximately 1.5–6 hours). The average adjusted clearance is about 110–115 mL/min/m^2 (range approximately 50-150 mL/min/m^2) [26]. The clearance is age dependent; children in the first 6 months of life have poorly developed hepatic function and a relatively decreased clearance, whereas at 1–4 years they have a higher clearance than adults. Second, in the age interval 1–4 years, children have a relatively increased GST activity in the intestinal tract, which may influence the initial handling of oral drug, which likely affects the bioavailability and first-pass drug elimination. These findings have led to suggestions of drug dosing that takes into account both age and body weight rather than weight/body surface area alone [27]. Parenteral BU demonstrated an improved safety profile in high-dose therapy, and in pediatric practice the formulation contributed to improved outcome when both weight and age are considered in the dosing algorithm. Alternatively, since BU does not need activation, it is a good candidate for therapeutic drug monitoring (TDM) that takes individual variability in metabolic drug handling into account. BU pharmacokinetics (PKs) are reproducible over multiple doses in an individual patient, but the area under the curve (AUC) can vary up to 2-3-fold between patients, suggesting that TDM of the parenteral product may have benefit [28].

The transition to parenteral BU applied with PK-guided TDM in high-dose therapy yields consistent dose delivery/systemic exposure, and recent clinical transplant data support the use of intravenous (IV) BU as safer and therefore preferable to oral BU in high-dose therapy. However, even though BU exhibits linear first-order kinetics, its utility and PK characteristics are influenced by numerous factors, as discussed previously. Further, BU PKs to some extent depend on the patient's underlying disease; thalassemia patients on average exhibit increased drug clearance, possibly related to increased hepatic GSTA1 activity. Finally, concomitant administration of other drugs, both chemotherapeutic agents and supportive care medications, may alter BU clearance.

Dose-limiting toxicities

Myelotoxicity is the most notable side effect of BU. This made it an almost ideal agent for pretransplant conditioning therapy. Additionally, nausea/vomiting are pronounced with oral BU since the drug is locally irritating to the gastric mucosa. When used in high-dose therapy, mucositis is common, and grand mal seizures have been reported with an incidence of 5–10% [29,30]; this mandates the use of prophylactic antiseizure medication. Phenytoin is commonly utilized, but owing to concern about an interaction between phenytoin and BU some investigators favor benzodiazepines, which do not affect BU clearance [31]. A hallmark adverse effect of high-dose BU is hepatic veno-occlusive disease (VOD), also termed sinusoidal obstruction syndrome (SOS), which may lead to multiorgan failure that commonly has a lethal outcome. The incidence increases in proportion to higher BU exposures and the risk also increases when BU is administered with other alkylating agents (e.g. CY/MEL/thioTEPA), which consume hepatic GSH. The end result can be serious/overwhelming hepatic injury if the dose and schedule of all the agents are not carefully considered. A commonly occurring adverse effect of high-dose BU is a reversible, cosmetic skin discoloration that becomes noticeable after a few weeks and which is more pronounced in individuals of a darker complexion.

Drug interactions

Other medications administered close to BU may alter the metabolic transformation of BU, GSH conjugation and outward cellular transport of (GSH-conjugated) drug, much of which is only partially known. The administration of a variety of agents in close proximity to BU may dramatically alter drug clearance; however, the mechanism(s) involved beyond those shown in Figure 19.3 are incompletely understood. Drugs that are known to, or probably, interact with BU metabolism include agents such as CY, thioTEPA, MEL, phenytoin, metronidazole, itraconazole, deferasirox, and ethacrynic acid. Caution is recommended if patients receive tyrosine kinase inhibitors, and also the mammalian target of

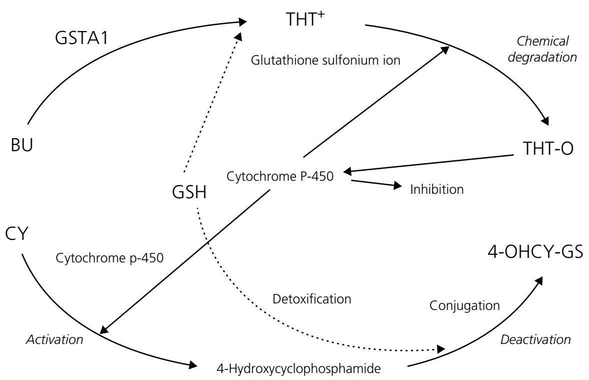


Figure 19.3 Interaction between busulfan and cyclophosphamide. Source: Hassan and Andersson 2013 [13]. Reproduced with permission of Future Medicine Ltd.

rapamycin (mTOR) inhibitors sirolimus and everolimus (observations from the M.D. Anderson Cancer Center, 2010–2013, and the Clinical Trials Network, 2011, respectively) (Figure 19.3). The end result can be excessive toxicity [19,20]. Novel regimens/combinations should be used only after careful phase I and II testing.

These findings highlight the possibility that both cytotoxic and non-cytotoxic drugs may alter BU clearance if they are used in close proximity with the conditioning therapy. Such interaction(s) may also affect both cellular resistance and corollary sensitivity to BU and other cytotoxic agents used in the treatment regimen. Even with an increased understanding of individual patients' GST and P450 isoenzyme patterns, it is recommended to use PK-based TDM whenever possible to optimize systemic BU exposure. It must be remembered that not all drug interactions are negative; this is exemplified by the synergistic cell kill that can be accomplished by an in-depth analysis of the molecular mechanisms of drug action(s) and interaction(s) together with studies of sequencing of the various agents [10,32–34].

Melphalan

Melphalan (L-phenylalanine mustard, MEL) belongs to the nitrogen mustard class of alkylating agents (for molecular structure, see Figure 19.1). These were initially explored during World War II while evaluating chemical warfare agents. During the following decade, its antitumor activity was appreciated. Finally, in 1992, the drug was formulated for IV use, making the investigation of high-dose MEL with stem-cell transplantation (SCT) feasible.

MEL was originally synthesized in 1953 [35]. The prototype bis-2-chloroethylamine mustard functionality was linked to L-phenylalanine. In water, each arm of the chloroethylamine group can form a positively-charged aziridinium ring. This unstable reactive intermediate can combine with various tissue nucleophiles (negatively charged species) to form an alkylation product. When the alkylated species is a DNA base, the second chloroethyl group can undergo the analogous reaction with the complementary DNA strand to form a "DNA cross-link." While much evidence supports DNA alkylation as the dominant antitumor mechanism, this chemistry is broadly applicable to a wide variety of tissue nucleophiles and the importance of these alkylation products to toxic and therapeutic effects is only partly understood.

MEL undergoes hydrolysis in water, and the chlorine atoms are replaced by hydroxyl groups. The resulting mono- and dihydroxy-MEL compounds are more stable, form aziridinium rings poorly, and have virtually no antitumor activity. The rate of this reaction is determined by the chloride concentration in a saline solution. In physiologic saline, MEL is completely hydrolyzed and inactivated over 8 hours [36]. In dextrose–water solutions, hydrolysis proceeds so rapidly that the latter must never be used to formulate the drug for clinical use. Further, because of hydrolysis, MEL in saline solution must be infused within 60 minutes of dilution. MEL is poorly water soluble, so its stock diluent is comprised of ethanol and propylene glycol.

Metabolism and disposition

MEL has no known metabolites, and its hydrolysis products are inactive. MEL primarily binds to plasma proteins (mostly albumin), with one-third of the binding being covalent/irreversible [36]. Radiolabeled-drug studies demonstrate that at most 15% of total drug label is excreted renally over 24 hours following dosing [36]. Known MEL hydrolysis confirms that most of the label represents inactive hydroxylated derivatives. These data strongly suggest that neither renal excretion nor metabolism play important roles in MEL disposition.

Toxicity

The dominant toxicity of MEL in conventional doses is hematologic and gastrointestinal (GI) (nausea/vomiting). Used in transplantation, GI and mucosal toxicities with frequent diarrhea dominate. High-dose MEL can cause sporadic, usually reversible, but occasionally lethal, cardiac toxicity. Renal tubular injury causing mild, asymptomatic proteinuria is common. Within 5–7 days of dosing, significant myelo- and profound immunosuppression occur.

Pharmacokinetics

Most PK studies do not differentiate between bound and unbound MEL. Taking this into account, the available data suggest that the plasma half-life ($T_{1/2}$) of free MEL is between 30 and 60 minutes and the volume of distribution is throughout total body water [37]. Representative drug clearance is 500–600 mL/min. There is at least a fourfold inter-individual variability in systemic exposure and PK parameters. The explanation for this variation is unknown. Taken together, the data suggest that tissue alkylation is the primary route of elimination of drug from the plasma. TDM of MEL is investigational. The use of oral cooling with ice slurry over 2 hours after high-dose MEL has been shown to be effective in reducing oral mucositis. This strategy is based on the short MEL half-life [38].

Cellular pharmacology

MEL enters the cell primarily by energy-dependent active transport [39]. The high-affinity L-amino acid transporter mediates most of the uptake. High extracellular concentrations of leucine or other amino acids can competitively inhibit MEL uptake.

Once inside the cell, the low chloride–high phosphate concentrations accelerate both hydrolysis and formation of reactive intermediates. In this environment, GSH conjugation becomes an important detoxification mechanism [40]. Little is known about specific mechanisms of MEL migration to the nucleus, but DNA is believed to be the primary target for therapeutic effects. Initial mono-alkylated products are primarily formed at the N^7-position of guanine or the N^3-position of adenine [41]. Whereas the rate of formation of mono-adducts is rapid, di-adduct formation (cross-links resulting from the bifunctionality of MEL) occurs over 6-18 hours. Cross-links can be either inter- or intra-strand, but most evidence suggests that inter-strand cross-links are primarily responsible for pharmacodynamic effects, and that the duration of the cross-links is predictive of these effects. In this light, the cellular effectiveness of cross-link repair is a major determinant of antitumor activity and probably also for normal-organ toxicity [42].

Drug–drug interactions/pharmacogenetics

Because of the importance of GSH conjugation for MEL detoxification, both intracellular GSH concentration and GST activity play important roles in MEL activity and toxicity. Any drug that forms GSH conjugates might deplete GSH and increase cellular MEL sensitivity. MEL produces important oral and GI mucosal toxicity in transplant doses, so in combination alkylating agent regimens the optimal dose may be lower than the 200 mg/m^2 dose commonly used for single-agent treatment. GSTA1 and GSTP1 predominate in GI mucosa, with GSTA1 dominating in the stomach and small intestine and GSTP1 dominating in the colon [43].

If high-dose MEL is combined with other agents that are nephrotoxic, caution is advised; single-agent MEL is generally not considered to be nephrotoxic, but when combined with certain nucleoside analogs, such as 2-chlorodeoxyadenosine (M.D. Anderson Cancer Center, 1996, unpublished work), or more recently with clofarabine, a significant fraction of the patients developed renal failure [44].

Drug resistance

Cellular uptake, GSH/GST activity, and DNA repair alterations are associated with tumor MEL resistance. Lowered expression of CD98, as representing the MEL carrier, has been found in MEL-resistant myeloma cells [45]. Changes in cellular GSH content have also been associated with MEL resistance [46]. GSTP1 often predominates in tumors and catalyzes the conjugation of MEL to GSH. Greater GSTP1 activity in tumors can reflect MEL resistance [47]. Finally, when compared with untreated patients who had no inter-strand cross-link repair activity, patients with myeloma having previous MEL treatment could repair 40–100% of established cross-links. This suggests augmented repair activity as a possible resistance mechanism.

Cyclophosphamide

Cyclophosphamide [(*R*,*S*)-*N*,*N*-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide) is a nitrogen mustard (Figure 19.1) that alkylates DNA. It is a prodrug that is converted to active forms with cytotoxic activity. Cyclophosphamide (CY) was approved by the US Food and Drug Administration (FDA) in 1959 as an antitumor agent.

Metabolism and mechanism of action

CY is a prodrug that is converted by hepatic cytochrome P450 (CYP) mixed-function oxidases to both active and inactive metabolites. *N*-Dechloroethylation of CY is mediated mostly by CYP3A4/3A5 [48], and yields the non-toxic 2-dechloroethylcyclophosphamide, and also chloroacetaldehyde, which is both neuro- and nephrotoxic. Oxidation at the C4-position is mediated by several CYP isoforms [49]. This oxidation step gives rise to 4-hydroxycyclophosphamide (4-OHCY), which tautomerizes to aldophosphamide; 4-OHCY may also be enzymatically converted to inactive 4-ketocyclophosphamide. Aldophosphamide may be oxidized to inactive carboxyphosphamide by aldehyde dehydrogenases (ALDH1A1 and ALDH3A1) [50] or non-enzymatically converted to phosphoramide mustard and acrolein, both of which are active metabolites. Phosphoramide mustard is a DNA alkylator that forms inter- and intra-strand cross-links between the N^7-positions of guanine similar to those of other DNA alkylating agents. Phosphoramide mustard is thought to be the primary cytotoxic metabolite. Acrolein is also cytotoxic and is considered responsible for causing hemorrhagic cystitis. This is counteracted by adding 2-mercaptoethanesulfonate (MESNA) to the treatment with high doses of CY.

The cytotoxic activity of CY and its immunomodulatory potential make it a good component of the pre-transplant regimens for hematopoietic stem-cell transplantation (HSCT). Although effective as a single agent, it is usually combined with other cytotoxic drugs or TBI. In conventional chemotherapy, R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) has a cure rate of 30–40% for aggressive non-Hodgkin lymphoma [51], but high-dose chemotherapy with CY prior to HSCT is a most effective treatment for relapsed, refractory non-Hodgkin lymphoma. High-dose CY in combination with TBI or BU with or without etoposide is considered effective conditioning therapy preceding allo-HSCT [52].

High-dose CY *after* HSCT was recently introduced as prophylaxis for graft-versus-host disease (GVHD). It is commonly administered together with tacrolimus/cyclosporine and mycophenolate mofetil. Hematopoietic stem cells possess high levels of aldehyde dehydrogensase and therefore are resistant to CY killing. Delayed T-cell engraftment and preferential killing of proliferating alloreactive, as opposed to regulatory, T-cell compartments appear to reduce GVHD activity [53]. This approach may allow the use of mismatched allotransplants with greater safety, and is being explored.

Pharmacokinetics

CY is always given intravenously in transplant doses. It has an elimination half-life ($T_{1/2}$) that ranges between 3 and 12 hours. Approximately 5–25% of the dose is excreted intact in the urine. Its elimination is dose dependent, with significant excretion of parent drug at daily doses greater than 1.5 g/m^2, and in pediatric patients where several investigators have reported up to 40% excretion of intact CY.

Adverse effects

CY, like its close relative ifosfamide, produces nausea, vomiting, alopecia, and gonadal failure. Other adverse effects include nephrotoxicity, neurotoxicity, infertility, hemorrhagic cystitis, myelo- and immunosuppression, and a potential for leukemogenesis. Hepatic injury is most commonly seen when CY is given with other (DNA-alkylating) agents, or together with TBI. High-dose CY may, through acrolein, lead to inhibition of hepatic GST activity. Acrolein has also been implicated in directly depleting hepatic GSH stores [54]. High-dose CY has been associated with (sub-)acute cardiac toxicity, cardiac ("pump") failure and arrhythmias, typically occurring at any time from about 24 hours to 2 weeks after the completion of CY administration. GSH has been demonstrated to play an important role in protection against cardiac toxicity. Acrolein-induced GSH depletion may therefore explain CY-associated cardiotoxicity [55]. The heart toxicity is dose dependent and doses above 7 g/m^2 should be avoided because of a rapidly increasing risk for cardiac complications [56].

Drug interactions

CY produces glutathione depletion and thus may potentiate other alkylating agents detoxified by this mechanism (for example, see Figure 19.3 and the Busulfan section of this chapter).

BCNU/carmustine

BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea; carmustine] is an alkylating agent used to treat several types of cancer (Figure 19.1). It has been used since 1971 to treat Hodgkin lymphoma, multiple myeloma, and brain tumors. BCNU has been popular in the treatment of brain tumors owing to its high lipophilicity and capacity to cross the blood–brain barrier. It is commonly used as part of conditioning therapy for auto-HSCT; almost 31% of high-dose auto-HSCT performed in the United States between 2009 and 2011 used BCNU in the regimen (Table 19.1). It was approved by the FDA in 1977 for the treatment of brain tumors, Hodgkin disease and other lymphomas, and multiple myeloma. Its derivative, lomustine or CCNU [1-(2-chloroethyl)-3-cyclohexyl-*N*-nitrosourea], was approved by the FDA in 1976 for the treatment of brain tumors and Hodgkin disease.

Metabolism and mechanism of action

The mechanism of action of BCNU has not been fully elucidated, but alkylation of DNA and RNA is believed to be responsible for its cytotoxicity. The drug decomposes to chloroethyl moieties which alkylate reactive sites on nucleic acids and proteins. BCNU is a monofunctional nitrosourea, but it can produce DNA cross-links. One strand is chloroethylated and displacement of the chloride ion on the other strand results in an ethyl bridge that prevents unwinding of the DNA [57]. The active form of BCNU reacts with the O^6-position of guanine producing *O*^{6}-ethylguanine, triggering mismatch repair, and induces apoptosis and senescence [58]. Apoptosis and senescence are preceded by heterochromatin organization partly contributed by decreased histone 3-acetylation and increased histone 3-trimethylation on Lys9 [59]. BCNU can also cause carbamoylation of protein lysine residues after formation of an isocyanate, but this might be a minor contributory mechanism to its cytotoxicity. BCNU kills cells in all stages of the cell cycle.

O^6-Ethylguanine is repaired by *O*^{6}-alkylguanine–DNA alkyltransferase (AT), and elevated AT levels have been linked to BCNU resistance [60]. Altered base and nucleotide excision repair may also contribute to such resistance; enhanced cytotoxicity of BCNU was observed in cell lines deficient in excision repair enzymes [61]. Chronic lymphoid leukemia (CLL) patients resistant to nitrogen mustard treatment have elevated level of DNA excision repair enzymes [62]. No correlation has been observed between the rate of repair of *O*^{6}-ethylguanine and the levels of GSH, GST activity, or P-glycoprotein expression in primary cells from CLL patients [63].

BCNU is commonly combined with etoposide, Ara-C (cytarabine), and melphalan (MEL) ("BEAM"), an effective high-dose salvage regimen used with auto-HSCT for refractory Hodgkin lymphoma and diffuse large B-cell lymphoma (DLBCL) [64].

Pharmacokinetics

BCNU is not stable in aqueous solution. Intravenous administration results in a highly variable half-life ranging from about 15 to 90 minutes. No parent product is excreted and approximately 30–80% of the degradation products are excreted in the urine within 24 hours. Its alkylating metabolites rapidly enter the cerebrospinal fluid.

Adverse effects

The major side effect of BCNU is myelosuppression, and at high doses it may cause hepatic VOD/SOS, pulmonary fibrosis, renal failure, and secondary leukemia. Other risks include infection due to myelo- and immunosuppression, thrombocytopenia, nausea and vomiting, skin flushing, redness of face, dizziness and loss of balance, and hypotension.

Drug interactions

Some of the drugs that interact with BCNU include cimetidine, digoxin, leflunomide, natalizumab, phenytoin, fosphenytoin, pimecrolimus, roflumilast, tacrolimus and trastuzumab.

ThioTEPA

ThioTEPA (TT) was synthesized in the early 1950s in the search for novel antitumor alkylating agents. The *N*-triethylenephosphoramide backbone (TEPA or T; see Figure 19.1 for structure) contains three aziridinyl three-membered rings that can hydrolyze to produce alkylating moieties similar to those of nitrogen mustard(s). TEPA was active but chemically unstable, so the more stable sulfur analog *N*-triethylenethiophosphoramide (TT) was synthesized and entered clinical use. Its dominant hematologic toxicity made it an attractive agent to explore in solid tumors and it was soon formulated for IV use. These properties made it an attractive compound for HSCT.

Chemistry

TT was synthesized to place three aziridine rings immediately adjacent to a strongly electron-withdrawing thiophosphorus functional group. In this way, electron-rich N- and O-functional groups combine with the already unstable and partially positively charged aziridine ring to form an alkylation product. This type of reaction is particularly favored when the tissue pH is lower, such as in tumors [65]. Because TT has three aziridine rings, it is capable of forming cross-links in DNA or between DNA and other cellular macromolecules. TT is converted to its oxo derivative T both as a result of hydrolysis and through CYP mediation. Both TT and T have antitumor activity, the relative amount of which varies between experimental systems.

Metabolism and toxicity

As shown in Figure 19.4, TT can be oxidized to T via CYP P450. Both T and TT are GSH-conjugated through GST. Both TT and T are active but the relative organ-specific toxicity has been incompletely studied, and it is hazardous to draw clinical conclusions based upon existing metabolic data. CYP2B6 and CYP3A4/5 are predominantly responsible for the TT conversion to T (Figure 19.4). There are at least four polymorphic alleles of CYP2B6 comprising up to 60% of a typical US population, but at most these could account for a 10–30% variation in aggregate TT and T exposure. CYP3A4 polymorphisms account for only 6% of the population and could account for 10% variability in TT plus T exposure. GSTA polymorphisms have no important effect, but GSTP, which often predominates in tumors, might result in 40–50% increased net tumor exposure in polymorphic homozygous patients with decreased conjugating capacity [66].

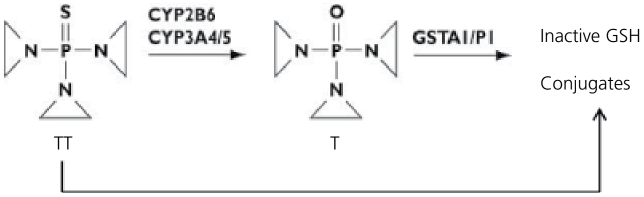


Figure 19.4 Conversion of thioTEPA (TT) to its oxo derivative TEPA (T) and their conjugation with glutathione (GSH).

TT has a broad spectrum of non-hematologic toxicity, including hepatic toxicity and oral and GI mucositis, and also central nervous system (CNS) and cutaneous toxicity [67]. CNS toxicity manifested by cortical dysfunction was dose limiting in a single-agent high-dose SCT study. TT can enhance the toxicity of (an-)other simultaneously administered alkylating agent(s) through depletion of GSH, so that (reactive intermediates of) the other agent(s) cannot be effectively detoxified.

Pharmacokinetics

There are no definitive reports of the PKs of protein-bound versus -unbound TT and T. A representative plasma half-life of total TT is 3 hours and that of T is 2–3 times longer. In most patients, the systemic exposure of T is therefore 3-4 times greater than that of TT [64]. Clearance of TT is 200–300 mL/min/m^2 and inter-patient variability is reported as 4–7-fold. Therefore, it is not surprising that sporadically serious toxicity is seen with doses above 1000 mg/m^2 (25 mg/kg). Lower doses are usually employed in high-dose combination chemotherapy. Renal excretion accounts for 10% or less of intact T and TT.

Cellular pharmacology

Cellular uptake is through passive diffusion. Once inside the cell, the drug rapidly binds to various macromolecules, and GSH conjugation accounts for a major part of the detoxification. Different tissues, organs, and tumors have differing GST subtypes, and the activity or polymorphism of these various isoenzymes can influence organ- or tissue-specific drug effects.

The movement of TT from the cytoplasm to the nucleus has not been studied. Once proximate to DNA, TT or T can form both single-stranded adducts and DNA double-stranded cross-links through the activity of the aziridine rings. Through hydrolysis, it is also possible that a single aziridine ring can form a single-stranded adduct through attack by an electron-rich DNA component [68]. Inter-strand cross-links, however, are generally considered as a major cytotoxic mechanism for all bifunctional (or, in the case of TT, trifunctional) alkylating agents. DNA adducts of TT primarily involve alkylation at the N^7-position of guanine or at the N^3- or N^7-positions of adenine [65].

Drug interactions

Because TT can react with a wide variety of tissue macromolecules containing nucleophilic sites, the administration of TT prior to a second cancer drug can produce changes in enzyme-mediated activation or metabolism of the second agent. Additionally, TT conjugation with GSH can reduce the GSH available to detoxify a second alkylating agent administered in close proximity to TT. If TT is administered prior to CY, the metabolic activation of CY by P450 is reduced [69]. This highlights the importance of paying attention to such interactions when designing new drug regimens. A "rest" day between administration of high-dose TT and other alkylating agents is often employed to allow normal tissue recovery from GSH depletion and oxidative stress. Through non-specific tissue alkylation, TT can reduce the activity of a variety of phase I and II metabolic pathways producing otherwise unanticipated changes in bioactivation or metabolism of concomitantly administered drugs.

Drug resistance

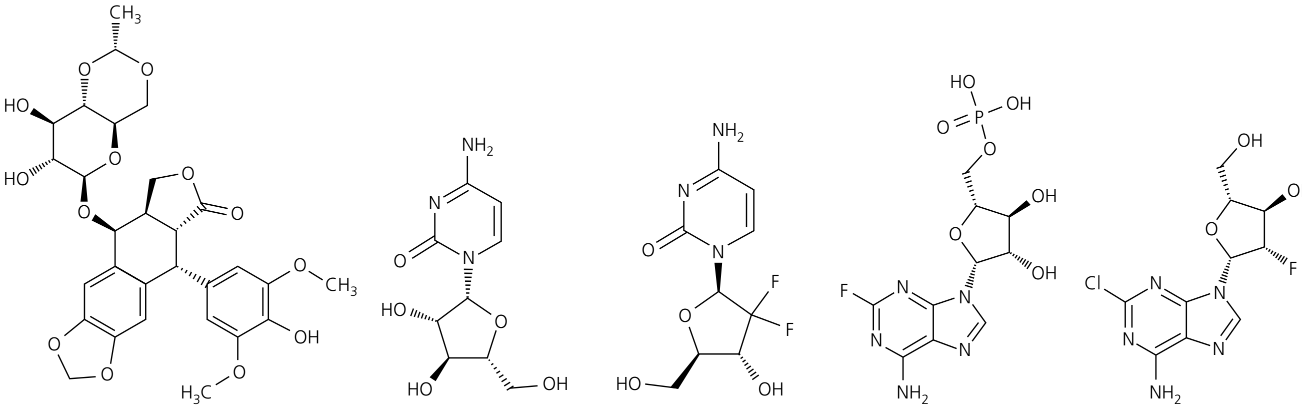
TT resistance in tumors can develop through GSTP polymorphisms. Resistance based upon alterations in GSH concentration or metabolism has been incompletely studied.

Topoisomerase inhibitors

Topoisomerases are enzymes that resolve the tension resulting from supercoiling, knotting, and catenation of DNA during replication and transcription. These enzymes bind to DNA, cut its phosphate backbone, allow the DNA to unwind, and then re-seal the break. Type I topoisomerases relax DNA tension by cutting one strand of a DNA double helix, letting it rotate around the uncut strand, and then re-ligating the cut. Type II topoisomerases cut both strands of a double helix, pass the unbroken DNA helix through it, and religate the broken strands. Topoisomerase II inhibitors stabilize the complex formed between topoisomerase II and the 5′-cleaved ends of the DNA and result in accumulation of DNA breaks that ultimately lead to DNA-damage response and trigger apoptosis. Two such agents, which are used as part of conditioning therapy, are etoposide and teniposide. One drawback of the DNA-damaging effects of topoisomerase inhibitors is the possible acquisition of secondary neoplasms.

Etoposide

Etoposide{4′-demethylepipodophyllotoxin9-[4,6-*O*-(*R*)-ethylidene]-β-D-glucopyranoside, VP-16} (Figure 19.5) or its derivative, etoposide phosphate, is a semisynthetic derivative of podophyllotoxin, a natural product from the May apple plant. It was approved by the FDA in 1983 for the treatment of testicular cancer and subsequently for the treatment of small-cell lung carcinoma in combination with other (approved) chemotherapeutic drugs.



Etoposide Cytarabine Gemcitabine Fludarabine Clofarabine

Figure 19.5 Structures of a topoisomerase inhibitor and nucleoside analogs used in HSCT.

Metabolism and mechanism of action

Etoposide is a complex molecule with a polar surface and a basic pK_a of 9.8 [70]. These properties prevent the drug from passing freely through the cell membrane. The mechanism of cellular uptake is not well defined but the human organic cationic transporter OCTN2, a high-affinity carnitine carrier, might be involved in its transport [71].

Etoposide is an active drug; its target is in the nucleus. It binds to and stabilizes the topoisomerase II-cleaved DNA complex (the cleavage complex), which is an intermediate in the removal of genomic knots and tangles by topoisomerase II. Etoposide prevents re-ligation of broken DNA strands catalyzed by the enzyme [72]. This results in accumulation of DNA breaks, which subsequently activate DNA-damage response and apoptosis. Etoposide specifically affects cells in the S and G2 phases of cell division.

The drug is primarily inactivated in the liver by 3′-demethylation mediated mainly by the cytochrome P450 system (specifically CYP3A4). Other metabolites identified in plasma and urine include *cis*-(picro)lactone, hydroxy acid derivatives, 4′-*O*-glucuronide of etoposide or agrycon, sulfate conjugates, and 3′-demethyletoposide [73].

There are two major mechanisms of resistance to etoposide: (1) increased expression of *MDR1*-encoded P-glycoprotein, multidrug resistance protein (MRP), and lung resistance protein (LRP), all of which mediate cellular efflux of etoposide [74], and (2) low expression, post-translational modifications and mutations of topoisomerase II [75]. Polymorphisms may play a critical role in the sensitivity of patients to etoposide. A genome-wide analysis of human cell lines identified 63 genetic variants that may contribute to etoposide-induced cytotoxicity [76].

Pharmacokinetics

An open two-compartment PK model of etoposide in leukemic children showed α-phase, β-phase, volume of distribution, and clearance of 1.2 h, 5.8 h, 3.1 L/m^2, and 19.5 mL/min/m^2, respectively [77]. The major metabolites were the *trans*- and *cis*-hydroxy acid derivatives in serum and urine [77]. In children with solid tumors, patients with pre-existing liver injury had a lower systemic etoposide clearance.

More recent studies showed a narrow therapeutic window and highly variable PKs of etoposide. Creatinine clearance correlates with etoposide clearance, but creatinine clearance alone is a poor predictor of etoposide systemic exposure; rather, free etoposide AUC is a better determinant of its pharmacodynamic effects [78].

Etoposide clearance and volume of distribution increase with increasing body weight in children undergoing auto-HSCT for solid tumors. Conversely, body mass index (BMI) does not seem to affect the outcome or toxicity of etoposide in acute lymphoblastic leukemia (ALL) patients [79].

Adverse effects

The more common and severe side effects of etoposide include myelosuppression, alopecia, constipation/diarrhea, secondary malignancies, cardiotoxicity, loss of consciousness, and fatigue.

Drug interactions

PK studies showed that cyclosporine treatment increased etoposide exposure by decreasing its clearance by as much as 71% [80]. Drugs that inhibit P450 CYP 3A4 (e.g., telithromycin and voriconazole) may reduce the metabolism of etoposide and increase its plasma concentration/systemic exposure.

Nucleoside analogs

Nucleoside analogs (NAs) are prodrugs which are very similar in structure to normal nucleosides (Figure 19.5). Structural modifications generally improve their aqueous solubility, stability and efficacy. The efficacy of NAs in the treatment of hematologic malignancies is partly due to the dependence of hematopoietic cells on nucleoside salvage synthesis [81]. Cells easily take them up, metabolize and incorporate them into DNA or RNA (Figure 19.6). In general, NAs are taken up by cells through nucleoside transporters. Once in the cytoplasm, they are phosphorylated to their triphosphate derivatives, considered to be the active forms that enter the nucleus and compete with normal nucleotide triphosphates in the synthesis of DNA and RNA. Their cytotoxicity is primarily attributed to inhibition of DNA synthesis and repair, which triggers DNA-damage response. Interference with DNA damage/repair in mitochondria may also mediate apoptotic cell death.

In the last decade, NAs have been increasingly used in high-dose therapy. They are typically combined with DNA alkylating agents for synergistic antileukemic effects during pre-transplant conditioning therapy. The synergistic cytotoxicity of these two classes of drugs can be primarily attributed to activation of DNA-damage response and apoptosis (reviewed in [82]) (Figure 19.7). Briefly, the initial incorporation of NAs into DNA strands inhibits DNA synthesis and repair, and also triggers histone modifications. Modified histones cause chromatin remodeling that yields better accessibility of genomic DNA to subsequent administration of DNA alkylators, resulting in propagation of DNA damage and consequent apoptosis [34]. Based on this model, chromatin modifiers are expected to improve further the efficacy of NAs and DNA alkylators. Addition of suberoylanilide hydroxamic acid (SAHA) to this combination, in fact, enhances their synergistic cytotoxicity [34,83]. Ongoing clinical trials of these drug combinations will be needed to elucidate whether the addition of agents such as histone deacetylase inhibitors and/or demethylating agents to a basic NA-alkylating agent “platform” combination will further enhance the conditioning regimen efficacy yet without jeopardizing patient safety.

Cytarabine

Cytarabine (1-β-ᴅ-arabinosylcytosine, or Ara-C) was the first analog of deoxycytidine to be synthesized and used in antitumor therapy (Figure 19.5). It is generally indicated for the treatment of acute myeloid leukemia (AML) and the blastic phase of chronic myelogenous leukemia (CML).

Metabolism and mechanism of action

Cytarabine enters the cells via the nucleoside transporter hENT1. It is phosphorylated by deoxycytidine kinase (DCK) to Ara-CMP and by nucleotide kinases to Ara-CTP. Its cytotoxicity is due to prevention of cytidylate to 2′-deoxycytidylate conversion [84], erratic coding when incorporated into DNA and RNA [85], and inhibition of DNA polymerase-α, DNA-strand elongation, and DNA repair. The prodrug is easily inactivated by cytidine deaminase (CDA) in the liver by deamination to Ara-U, which is excreted in the urine. Ara-CMP is dephosphorylated by 5′-nucleotidase NT5C2. Replacement of the 2′-OH group with fluorine yielded 2′-deoxy-2′-fluorocytidine (F-Ara-C), which exhibited 10-fold higher cytotoxicity than Ara-C in vitro, but a disappointingly low antitumor activity in vivo.

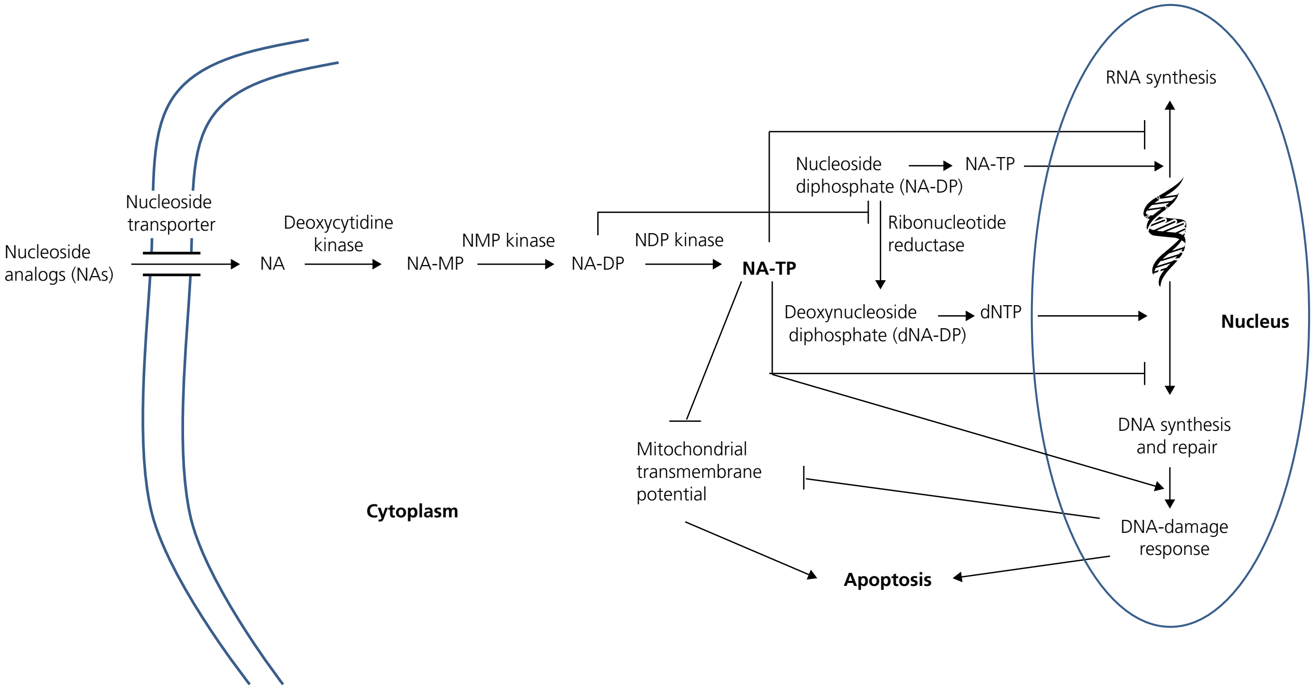

Nucleoside analogs (NAs)
Nucleoside transporter
NA
Deoxycytidine kinase
NA-MP
NMP kinase
NA-DP
NDP kinase
NA-TP
Nucleoside diphosphate (NA-DP)
NA-TP
Ribonucleotide reductase
Deoxynucleoside diphosphate (dNA-DP)
dNTP
RNA synthesis
Nucleus
DNA synthesis and repair
DNA-damage response
Mitochondrial transmembrane potential
Cytoplasm
Apoptosis

Figure 19.6 Transport, activation, and mechanisms of action of nucleoside analogs (NAs). The prodrugs are taken up by cancer cell through nucleoside transporter and activated in the cytoplasm to their triphosphate form (NA-TP) by a series of kinases. The diphosphates (NA-DPs) inhibit ribonucleotide reductase and NA-TPs inhibit DNA synthesis and repair and RNA synthesis. The inhibition of DNA synthesis and repair causes DNA breaks and activation of DNA-damage response, which results in mitochondrial-dependent and -independent apoptosis. The decrease in mitochondrial transmembrane potential caused by NA-TPs and damaged DNA releases pro-apoptotic factors to the cytoplasm. Inhibition of ribonucleotide reductase decreases the physiological dNTP pools and increases the incorporation of NA-TPs into DNA and RNA, resulting in self-potentiation.

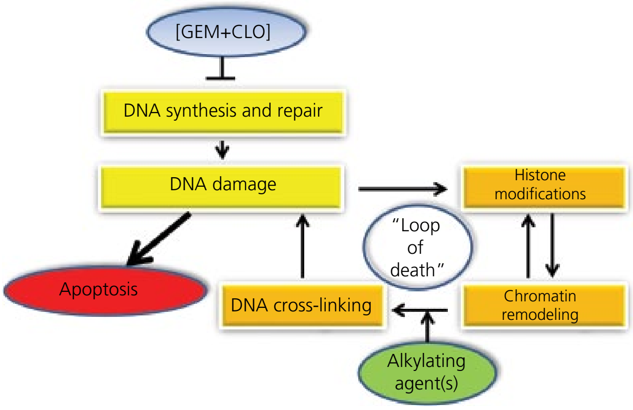

[GEM+CLO]
DNA synthesis and repair
DNA damage
Histone modifications
"Loop of death"
Apoptosis
DNA cross-linking
Chromatin remodeling
Alkylating agent(s)

Figure 19.7 Suggested mechanism of the synergistic cytotoxicity of gemcitabine (GEM) with other NAs [e.g., clofarabine (CLO)] and DNA alkylating agents (e.g., BU and MEL). The activated nucleoside analogs inhibit DNA synthesis and repair, which results in DNA damage. DNA breaks may cause histone modifications and chromatin remodeling, which may expose genomic DNA and make it more accessible to DNA alkylation. Attempts to repair DNA cross-links result in more DNA breaks and the cycle is further amplified by additional DNA alkylations. The amplified DNA damage results in cell death or apoptosis.

Pharmacokinetics

Approximately 13% of cytarabine binds to plasma proteins. Cytarabine is easily deaminated, resulting in a half-life of 10 minutes. As with several NAs, this rapid deamination becomes clinically relevant when bolus doses exceed the capacity of kinase activation, and forms the rationale for using prolonged cytarabine infusions.

Adverse effects

Cytarabine may cause leukopenia, thrombocytopenia, anemia, GI disturbances, stomatitis, conjunctivitis, interstitial pneumonitis, fever, dermatitis, palmar–plantar erythrodysesthesia ("hand–foot-syndrome"), nausea, hair loss, muscle or joint pain, and fatigue. At high doses, cytarabine may cause CNS toxicity, and also a "pulmonary leakage" syndrome that is responsive to preemptive steroid therapy.

Drug interactions

Cytarabine decreases the effect of digoxin.

Gemcitabine

In the search of more stable analogs of deoxycytidine, both H atoms at the 2′-position of Ara-C were replaced with fluorine, resulting in 2′,2′-difluorodeoxycytidine (gemcitabine, GEM; Figure 19.5). The product is considerably more resistant to deamination, and effectively impairs the growth of both solid and liquid tumors.

Metabolism and mechanism of action

GEM is hydrophilic and needs a transporter to pass through the cell membrane. It is taken up through equilibrative and concentrative nucleoside transporters hENT1, hENT2, hCNT1, or hCNT3. It is phosphorylated in the cytoplasm by DCK to dFdCMP, and this is considered to be the rate-limiting step in its activation. To a more limited extent, GEM can also be phosphorylated to dFdCMP by thymidine kinase in the mitochondria [86]. The second phosphorylation step in the cytoplasm is catalyzed by pyrimidine nucleoside monophosphate kinase (UMP/CMP kinase) to produce dFdCDP and subsequently phosphorylated by nucleotide diphosphate kinase (NDK) to dFdCTP.

GEM is recognized by several DNA polymerases as a natural substrate; it is incorporated into nascent DNA strands during synthesis, but prevents further DNA chain elongation after addition of one more deoxynucleotide [87]. This inhibition of DNA synthesis and DNA repair leads to increased DNA double-strand breaks and activation of DNA-damage response and apoptosis. GEM also inhibits RNA synthesis in a concentration- and time-dependent manner [88].

The diphosphorylated derivative dFdCDP binds to and inhibits ribonucleotide reductase (RNR), an enzyme that catalyzes the conversion of ribonucleotides into their deoxyribonucleotide forms. Inhibition of RNR decreases the NTP pools used for normal DNA synthesis, which causes enhanced GEM incorporation in the growing DNA strand. Such inhibition of RNR decreases the cellular concentration of dCTP, a feedback inhibitor of DCK, and results in the activation of the kinase, increased phosphorylation of dFdC, and greater self-potentiation of GEM.

Inactivation of GEM occurs primarily through deamination, which is catalyzed by deoxycytidine deaminase (dFdC to dFdU) and dCMP-deaminase or 5′-nucleotidase (dFdCMP to dFdUMP) [89]. dFdU is eliminated from the cell but its cellular re-entry competes with dFdC through the equilibrative nucleoside transporters, and it also modulates the phosphorylation of dFdC via DCK [90].

Pharmacokinetics

The volume of distribution (V_d) and half-life of GEM are influenced by the duration of the IV infusion. For short infusions (<70 min), V_d is 50 L/m^2 and $T_{½}$ is about 40-95 min; for prolonged infusions, V_d is up to 370 L/m^2 and $T_{½}$ is about 245–6408 min. Age and gender also affect the clearance of GEM, with women and the elderly exhibiting lower values. Both GEM and its metabolite dFdU are excreted in the urine.

Some genetic factors that affect the pharmacokinetics and pharmacodynamics of GEM have been identified. Since CDA detoxifies GEM, its activity correlates with GEM systemic exposure. Two single nucleotide polymorphisms (SNPs) in the *CDA* gene 79A>C (K27Q) (**2*) and 208G>A (A70T) (**3*) seem to correlate with a decreased enzymatic activity of CDA and cytotoxicity of GEM [91,92]. The C-allele in the 79A>C genotype is more frequent in Whites than Africans and Asians, whereas the 208G>A genotype has not been detected in Whites and African-Americans and at lower frequency in East Asian populations and some in Africans [93]. Other SNPs found associated with GEM PKs that have been found underscore the importance of studying larger patient populations.

Adverse effects

Myelosuppression is the principal dose-limiting toxicity of GEM when used as a single agent. Mild to moderate nausea, vomiting, and mild proteinuria and hematuria are commonly experienced. Fever, rash, dyspnea, edema, flu-like symptoms, infection, and alopecia occur in 10–40% of patients. More serious extrahematologic side effects, including myocardial infarction, cerebrovascular accidents, cardiac arrhythmias, and hypertension, have been experienced in ~2% of patients.

Drug interactions

GEM may increase the anticoagulant effects of warfarin analogs and enhance bleomycin toxicity. Paclitaxel increases GEM toxicity. owing to the profound effects of GEM on the various enzyme systems involved in its metabolism and on nucleoside metabolism in general, there is a significant potential for synergistic interactions with several other groups of cytotoxic agents, including, but not limited to, NAs, DNA-alkylating agents, and ionizing radiation, but so far only limited work has been carried out to exploit this systematically in a clinical setting. In particular, the inhibition of DNA repair by GEM and the activity of this drug in lymphomas make it an attractive drug for use in combination with alkylating-agents in salvage regimens for refractory Hodgkin and non-Hodgkin lymphomas [94].

Fludarabine

Fludarabine (FLU) is a monophosphorylated form of 9-β-D-arabinosyl-2-fluoroadenine (F-Ara-A), an analog of deoxyadenosine (Figure 19.5). F-Ara-A is more resistant to deactivation by adenosine deaminase compared with Ara-A and adenosine. FLU is approved by the FDA for the treatment of patients with B-cell chronic lymphocytic leukemia who are unresponsive to, or whose disease has progressed during treatment with, at least one standard DNA-alkylating agent. The wide use of FLU in non-myeloablative and in myeloablative, reduced-toxicity, conditioning therapy for allo-HSCT is attributed to both its immunosuppressive and its antitumor activities.

Metabolism and mechanism of action

FLU is negatively charged at physiological pH because of its functional phosphate group, and it cannot cross the cell membrane. Once given IV, it is converted by 5′-nucleotidases to its dephosphorylated form, which enters the cells mostly through nucleoside transporter hENT1 [95]. The prodrug is then phosphorylated by DCK to F-Ara-AMP and further phosphorylation by purine nucleotide kinase converts it to its active form F-Ara-ATP, which is incorporated into growing DNA strands and inhibits DNA synthesis. Since F-Ara-ATP inhibits DNA ligase I, an enzyme essential for joining DNA ends, both DNA replication and DNA repair are inhibited [96].

Pharmacokinetics

The limited PK data for FLU focus on its metabolite F-Ara-A. It follows linear PKs with extensive tissue distribution. FLU is primarily eliminated through the kidneys.

FLU cytotoxicity is associated with the cellular concentration of F-Ara-ATP, assayed in circulating myelo- and lymphoblasts. Infusions of 30 mg/m^2 FLU over 30 minutes result in 20–60 μM F-Ara-ATP in leukemia cells, which peaks after 4 hours [97]. The cellular concentration of F-Ara-ATP depends linearly on its plasma concentration and duration of infusion. Its cellular elimination is monophasic, with a median half-life of approximately 15 hours. When used in conditioning therapy, significant plasma concentrations have been reported for more than 48 hours after therapeutic infusions.

Adverse effects

Some serious side effects of FLU include immuno- and myelosuppression, infection, fever and chills, nausea, and vomiting. Other common adverse effects include malaise, fatigue, anorexia, weakness, and peripheral neuropathy. Doses in excess of 125 mg/m^2 daily for 4–7 days may result in lethal neurotoxicity, which can present as a Guillain–Barré-like syndrome [98].

Drug interactions

Severe toxicity may occur if FLU is combined with pentostatin, an inhibitor of adenosine deaminase. Dipyridamole may decrease the efficacy of FLU.

Clofarabine

The problems with the efficacy and stability of the first-generation purine NAs triggered the development of clofarabine (2-chloro-2′-arabinofluoro-2′-deoxyadenosine, C-Ara-FdA; CLO) (Figure 19.5). The incorporation of chlorine in the adenine ring makes it resistant to deamination and substitution with a fluorine atom in the ribose moiety makes the glycosidic bond resistant to phosphorolysis. It is approved by the FDA for the treatment of pediatric ALL in patients failing at least two prior regimens.

Metabolism and mechanism of action

CLO enters the cells through the human equilibrative nucleoside transporters (hENT1 and hENT2) and human concentrative nucleoside transporters (hCNT2 and hCNT3). It is phosphorylated by DCK in the cytoplasm and by deoxyguanosine kinase (DGK) in the mitochondria [99]. The phosphorylation of C-Ara-FdAMP to its diphosphate form by purine nucleotide monophosphate kinase determines its activation rate. It is further phosphorylated to its triphosphate form.

CLO cytotoxicity is related to its inhibition of DNA synthesis, inhibition of ribonucleotide reductase, and direct induction of apoptosis. At a ratio of C-Ara-FdATP to dATP one of >1, DNA strand elongation is significantly inhibited and causes DNA chain termination and DNA strand breaks. At lower ratios, CLO is incorporated into internal phosphodiester linkages and inhibits DNA repair. CLO can inhibit DNA synthesis by 75-95% when infused in humans at 22.5-55 mg/m^2 [100].

The diphosphate derivative of CLO inhibits RNR. This decreases the deoxynucleotide pool, enhances the incorporation of C-Ara-FdATP into DNA, and potentiates its ability to inhibit DNA synthesis. Inhibition of DNA synthesis triggers DNA-damage response, resulting in apoptosis. The direct binding of CLO triphosphate metabolites to proteins in the mitochondrial membrane lowers the mitochondrial transmembrane potential and allows the release of proapoptotic factors such as cytochrome *c* and apoptosis-inducing factor (AIF), all of which are important events in the formation of the apoptosome. C-Ara-FdATP is inactivated by cytosolic 5′-nucleotidase and the dephosphorylated metabolites are transported out of the cell.

Pharmacokinetics

The PKs of CLO are affected by age, weight, and creatinine clearance but not by gender, race, or disease type [101]. Adults experience higher CLO exposure than children given a similar dose standardized to body surface area for reasons that are unknown. In a 40-kg patient with a white blood cell count of WBC = 1 × 10^4/μL, CLO has an apparent plasma $T_{½}$ of approximately 6 hours and a volume of distribution of 210 L (72% variability) [101]. There is no reported association between genetic polymorphisms and clinical response to CLO-based therapy, but results from studies with other NAs may be applicable to CLO since similar transporters and metabolic enzymes are involved, and further investigations are warranted.

Adverse effects

Adverse reactions to CLO include myelo- and immunosuppression, infection, hyperuricemia due to tumor lysis, systemic inflammatory response syndrome, hepatic and renal impairment, and GI disorders. In several transplant regimens, CLO potentiates cutaneous toxicity and palmar–plantar erythroderma. CLO should not be used in patients who are pregnant or breast-feeding or who have serious hepatic or renal dysfunction. Some patients may experience nephrotoxicity at conventional dosing when the drug is given as monotherapy for AML. Clinical observations suggest, however, that vigorous hydration may alleviate most of the renal stress, and it is likely possible to use one such (subclinically) nephrotoxic agent without problems. If two or more such agents (e.g., CLO and Mel) are combined, however, the available clinical experience suggests that there may be an excessive risk for renal complications [44].

Drug interactions

CLO should preferably not be administered with other nephrotoxic and hepatotoxic drugs. It may enhance the effects of leflunomide and natalizumab. Tacrolimus, pimecrolimus, and roflumilast may increase the immunosuppressive effects of CLO.

Conclusion

Bifunctional DNA-alkylating agents are still a cornerstone in high-dose chemotherapy, but they are increasingly complemented with NAs, (monoclonal) antibodies, and other agents with alternative modes of action and different metabolism to improve both treatment efficacy and patient safety. Results of recent pharmacogenetic and PK investigations offer opportunities to predict and assess treatment response and side effects better based on patients' unique genotypic profiles and increasingly offer the prospect for individualizing/personalizing treatment. The several-fold variability in metabolism and disposition of cytotoxic agents, as exemplified with busulfan and cyclophosphamide, likely resides in a multitude of factors including, but not limited to, the varying isoenzyme patterns within the GST and CYP protein families, which are of importance for the metabolic handling of these drugs. Several of the (newer) agents are excellent substrates for the trans-membrane transport proteins belonging to the general classes of P-glycoproteins (MDR/MRP) and ABC transporter proteins, whereas there appears to be (at most) very modest involvement of the hepatic CYP P450 pathways in their metabolic disposition. These findings underline that personalized, optimally individualized treatment should preferably include genetic studies of both the patient and the tumor. Such genetic-based studies are over time likely to be followed by a shift to whole-genome-based investigations of drug-associated markers to follow treatment response and detect early surrogate markers of toxicity that may contribute to individualized treatment, thereby further improving the efficacy of the high-dose chemotherapy regimens.

It cannot be overemphasized that inter-individual variations in the PKs of any (antineoplastic) drug are significant. Such variability has to be countered in several ways. First, it justifies the incorporation of pharmacogenetic information about metabolic drug handling. Pharmacogenetic studies are extremely important to increase our understanding of both wanted and unwanted cytotoxic effects. Second, population-genetic studies cannot, however, replace TDM with PK-guided dose adjustments, since the concomitant use of other medications may modify cytotoxic agent PKs, thereby adding an unpredictable component that influences overall drug effect(s). Therefore, the interindividual variability in clinical PKs supports the implementation of PK-guided therapeutic drug delivery to optimize systemic drug exposure. The TDM will assist in circumventing the uncertainties contributed to by both pharmacogenetic differences and by the use of various concomitant medications. It is likely that TDM in the near future will be recognized as being important for optimizing systemic drug exposure and improving the efficacy and safety of high-dose chemotherapy for agents beyond busulfan. Third, combinations of drugs from different classes, with different mechanisms of cytotoxicity, will contribute to improving the therapeutic activity and overcome inherent and/or acquired cellular drug resistance. Healthcare providers should, however, be aware of the peril of possible drug interactions that may sacrifice therapeutic efficacy or result in excessive toxicity. In addition to studying clinical pharmacology, a detailed understanding of the mechanisms of antitumor activity and cytotoxic mode of action at the cellular and molecular level will assist in designing treatment regimens that further improve outcome. For instance, optimizing the sequence of drug exposures may significantly enhance the synergistic activity of the cytotoxic agents. Ultimately, it is through a better understanding of all aspects of the pharmacology of these agents that more effective regimes can be devised without sacrificing patient safety.

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CHAPTER 20

High-dose Preparatory Regimens

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Introduction

Treatment regimens are administered directly prior to hematopoietic cell transplantation (HCT) with two goals, which vary according to the patient's disease and the source of the graft. Since the majority of autologous and allogeneic transplant procedures are performed for the treatment of malignant diseases, the regimens must provide tumor cytoreduction and ideally disease eradication. The pharmacologic basis for this is discussed in Chapter 19. In the case of allogeneic transplants, the regimen must be sufficiently immunosuppressive to overcome host rejection of the graft. This second goal is discussed in detail in Chapter 9.

This chapter reviews high-dose chemotherapy and chemoradiotherapy regimens administered prior to autologous, syngeneic, or allogeneic hematopoietic cell infusion. Most of the high-dose regimens described have been utilized for patients with hematologic malignancies. Some high-dose regimens, however, have been developed specifically for patients with solid tumors, including sarcomas and germ-cell tumors. The overall results of HCT utilizing these high-dose treatment regimens are discussed in more detail in chapters dealing with specific diseases.

The success of HCT as curative therapy for patients with malignancy is limited, in part, by transplant-related morbidity and mortality. Even if all transplant-related problems were to be solved, however, about half (range 10–90%) of patients receiving transplants for various stages of malignant diseases would die owing to the inability of high-dose regimens to eradicate malignancy. This has led to intensive efforts to develop more effective high-dose regimens.

Evaluation of new treatment regimens

The development of new treatment regimens has, in general, required the empirical evaluation of a variety of drugs and radiation doses and schedules. Attempts have been made to systematize the development of new treatment regimens by performing phase I dose-escalation trials followed by phase II trials to estimate potential efficacy. Promising new regimens in ideal circumstances are then compared with established treatment regimens in randomized phase III trials. The methodology for trial design is discussed in more detail in Chapter 27.

Marrow ablative agents – maximum tolerated dose (MTD) when administered with HCT (Table 20.1)

Total body irradiation (Tables 20.1–20.4)

Total body irradiation (TBI) is a widely used therapy for autologous and allogeneic HCT for patients with hematologic malignancies. TBI has retained wide usage over the past 40 years because of excellent immunosuppressive properties, activity against a wide variety of malignancies even if resistant to chemotherapy, penetration of sanctuary sites such as the central nervous system (CNS) and testicles, and the relative lack of non-marrow toxicities when given at high doses.

Only a single study has evaluated TBI alone [1] while other trials have involved the concomitant administration of cytotoxic agents, usually cyclophosphamide (CY). Extensive experience has been accumulated with 10–16 Gy of TBI given as a single dose or fractionated, following or preceding high doses of CY (Tables 20.1–20.3).

A large body of experimental data has been acquired concerning methods to improve the therapeutic index of TBI [2]. Larger doses of TBI that might reduce the likelihood of relapse are limited by gastrointestinal (GI) and pulmonary toxicity, and in the long term by impaired growth and development, chronic pulmonary insufficiency, and second malignancies. In addition to the total dose, many factors, including radiation exposure rate, dose per fraction, interval between fractions, and radiation source (cobalt-60 or linear accelerator), have effects on efficacy and toxicity [2] (see Chapter 22). Many of the techniques of hyperfractionation were developed at the Memorial Sloan Kettering Center [3].

Experimental studies and clinical trials indicate that TBI administered in fractions is more tolerable than single-dose administration and that fractionation, if compensated for by increased total dose, can be given without compromising antitumor effects. Based on this principle, the use of fractionated TBI regimens has been explored in several centers, but there is a paucity of controlled studies of acute and delayed toxicities or of clinical efficacy compared with single-dose TBI. Two prospective randomized studies of fractionation versus single-dose TBI have been performed. One trial demonstrated clear superiority in event-free survival (EFS) following a fractionated regimen in patients with acute myeloid leukemia (AML) transplanted in first remission [4] (Table 20.3).

The second trial was performed in patients with a variety of hematologic malignancies undergoing autologous or allogeneic HCT. Although the patients who received hyperfractionated TBI showed a trend towards better survival and a lower rate of relapse

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Table 20.1 Maximum tolerated dose (MTD) of single-agent therapies used with hematopoietic cell transplantation

Ref.	Agent	MTD	Dose-limiting toxicities
Marrow-ablative single agents used in high-dose regimens with stem cell support			
[2]	TBI	10–16 Gy	GI, hepatic, pulmonary
[17]	Busulfan	20 mg/kg	GI, hepatic, pulmonary
[46]	BCNU	1200 mg/m^2	Pulmonary, hepatic
[59]	Melphalan	200 mg/kg	GI
[69]	ThioTEPA	1135 mg/m^2	CNS, GI
Non-marrow-ablative single agents used in high-dose regimens with stem cell support			
[71]	Cyclophosphamide	200 mg/kg	Cardiac
[72]	Ifosfamide	18–20 g/m^2	Renal, bladder, neurologic
[74]	Etoposide	2400 mg/m^2	GI
Agents used in high-dose regimens without determination of MTD with stem cell support			
[77]	Mitoxantrone	90 mg/m^2[a]	Cardiac
[80]	Cisplatin	300 mg/m^2[a]	Renal
[81]	Carboplatin	2000 mg/m^2	Hepatic, renal
[87]	Cytarabine	36 g/m^2[a]	CNS

CNS, central nervous system; GI, gastrointestinal.

[a] Maximum dose given with other agents with stem-cell support.

Table 20.2 Maximum tolerated doses of total body irradiation (TBI) given with cyclophosphamide 120 mg/kg

Ref.	Total dose (Gy)	Fraction size (Gy)	Fractionation interval (h)	Fraction No.	Days TBI
[4]	10.00	10	–	1	1
[91]	15.75	2.25	24	7	7
[113]	16.00	2	24	8	8
[11]	16.00	2	6–8	8	4
[11]	14.40 (children)	1.2	4–6	12	4
[12]	13.20 (adults)	1.2	4–6	11	4

Table 20.3 Randomized trials of total body irradiation regimens given with cyclophosphamide 120 mg/kg

Diagnosis	No. of patients	Fraction size (Gy)	No. of fractions	Total dose (Gy)	Probability of relapse	Probability of event-free survival
AML – first remission [4]	27	10.0	1	10.0	0.55	0.33
	26	2.0	6	12.0	0.20	0.54
AML – first remission [91]	34	2.0	6	12.0	0.35	0.60
	37	2.25	7	15.75	0.12	0.60
CML – chronic phase [10]	57	2.0	6	12.0	0.19	0.73
	59	2.25	7	15.75	0	0.66
Hematologic malignancies [5]	73	10.0	1	10.0	0.37	0.38
	74	1.35	11	14.85	0.23	0.45

AML, acute myeloid leukemia; CML, chronic myelogenous leukemia.

Table 20.4 Chemotherapeutic agents given with total body irradiation (TBI) and with or without cyclophosphamide

Ref.	Cyclophosphamide (mg/kg)	Total dose TBI (Gy)	Chemotherapy	Total dose
[87]	–	10–12	Cytarabine	36 g/m^2
[75]	–	13.2	Etoposide	60 mg/kg
[114]	–	9.5–11.5	Melphalan	110–140 mg/m^2
[115]	–	5 or 12[a]	Melphalan	140–180 mg/m^2
	–		Etoposide	60 mg/kg
[116]	60–120	5–12	Cytarabine	36 g/m^2
[34]	50	12	Busulfan	7 mg/kg (allografts)
	60	12		8 mg/kg (autografts)
[98]	100	12	Etoposide	60 mg/kg × 1

[a] 5 Gy at 50 cGy/min × 1 or 12 Gy in six fractions.

than patients receiving single-fraction TBI, these differences were not statistically significant [5]. Other studies suggest that fractionation decreases the incidence of idiopathic interstitial pneumonia syndrome (IPS) (Chapter 95) and cataracts (Chapter 103). Not surprisingly, a meta-analysis found that the lung dose of irradiation correlated with the incidence of interstitial pneumonitis [6]. A baseline incidence of 3–4% without radiation increased to a 50% incidence of interstitial pneumonitis with a lung dose of 10.6 Gy. Other groups have utilized reduced yet ablative doses of TBI such as 8 Gy given in four fractions with fludarabine (120 mg/kg) for patients with myeloid leukemias, with good reported efficacy and reduced transplant mortality [7].

Of interest, one center has reported results of high dose rate (30 cGy/min) single-fraction TBI (5.5 Gy) combined with CY for patients with AML undergoing allogeneic transplants from unrelated donors [8]. Transplant mortality was low (13%) for patients in first remission but 41% for more advanced disease. The leukemia-free survival at 3 years was 55% for patients in first remission, similar to that in other reports with higher total doses of irradiation. Although not proven, the antileukemic effectiveness of this lower dose appears to be a consequence of the relatively high dose rate. This same regimen, however, was associated with a high rate of graft rejection when used as a conditioning regimen for patients with chronic myelogenous leukemia (CML) transplanted from unrelated donors [9].

Studies suggest that both the antileukemic effects and normal tissue toxicities of TBI have a relatively steep dose–response curve. In studies of patients with AML in first remission or CML in stable phase, patients receiving 15.75 Gy had a markedly decreased actuarial incidence of relapse compared with patients given 12 Gy. In the CML trial the effect was profound enough to reduce relapse-free survival (RFS) [10].

In studies examining the effect of escalating doses of hyperfractionated TBI at 2.0 Gy given twice daily, the MTD was 16.0 Gy [11]. When 1.2 Gy was given three times per day, however, the MTD was only 14.4 Gy [12], suggesting that the shorter interval between doses of TBI prevented DNA repair in normal tissues.

In the past, most of the TBI regimens used in Seattle have utilized dual opposing ^{60}Co sources. This approach has the advantage of providing highly homogeneous radiation exposure and allows the patient some freedom of movement. However, it is difficult to shield organs, and radiation can be delivered only at relatively low exposure rates (8 cGy/min). Currently, all marrow transplant teams use linear accelerators to deliver TBI (see Chapter 22). With a linear accelerator, dose rates of 40 cGy/min or higher can be administered. The use of linear accelerators may have additional benefits as the radiation field can be shaped with the use of lung shielding to reduce pulmonary toxicity. Electron beam radiation can also be delivered to the chest wall and spine to compensate for the lower dose of TBI to these shielded areas [13]. Shielding of other organs, such as the liver, may be useful if specific organ toxicity limits further increases in TBI dose. Delivery of TBI with shielding of both lung and liver may be useful in patients with disease limited to bone, such as multiple myeloma, breast cancer, and Ewing sarcoma [13].

High-dose chemotherapy regimens (Tables 20.5–20.9)

There have been extensive efforts to develop non-TBI-containing transplant regimens. One of the reasons for pursuing this line of research is that many patients with Hodgkin disease or lymphoma

Table 20.5 Busulfan/treosulfan-based regimens

Ref.	Regimen	Chemotherapy	Total dose
[32]	BU/CY	Busulfan	16 mg/kg
		Cyclophosphamide	120–200 mg/kg
[117]	BU/Mel	Busulfan	16 mg/kg
		Melphalan	140 mg/m^2
[40]	BU/MEL/TT	Busulfan	12 mg/kg
		Melphalan	100 mg/m^2
		ThioTEPA	500 mg/m^2
[118]	BU/CY/MEL	Busulfan	16 mg/kg
[119]		Cyclophosphamide	120 mg/kg
		Melphalan	90–140 mg/m^2
[41,120]	BU/CY/TT	Busulfan	10–12 mg/kg
		Cyclophosphamide	120 mg/kg
		ThioTEPA	750 mg/m^2
[37]	BU/E	Busulfan	16 mg/kg
		Etoposide	60 mg/kg
[42]	BU/CY/E	Busulfan	16 mg/kg
		Cyclophosphamide	150 mg/kg
		Etoposide	30 mg/kg
[36]	BU/FLU	Busulfan	16 mg/kg
		Fludarabine	200 mg/m^2
[43]	TREO/FLU	Treosulfan	30–42 g/m^2
		Fludarabine	150 mg/m^2

Table 20.6 Nitrosourea-based high-dose chemotherapy regimens

Ref.	Regimen	Chemotherapy	Total dose
[98]	CBV[a]	BCNU	15 mg/kg
		Cyclophosphamide	100 mg/kg
		Etoposide	60 mg/kg
[121]	CBV + CPPD	BCNU	500 mg/m^2
		Cyclophosphamide	7.2 g/m^2
		Etoposide	2.4 g/m^2
		Cisplatin	150 mg/m^2
[37]		Lomustine	15 mg/kg
		Cyclophosphamide	100 mg/kg
		Etoposide	60 mg/kg
[56]	BEAM	BCNU	300–600 mg/m^2
		Etoposide	400–800 mg/m^2
		Cytarabine	800–1600 mg/m^2
		Melphalan	140 mg/m^2
[55]	BEAC	BCNU	300 mg/m^2
		Etoposide	300 mg/m^2
		Cytarabine	800 mg/m^2
		Cyclophosphamide	6 g/m^2
[122]	BAVC	BCNU	800 mg/m^2
		AMSA	450 mg/m^2
		Cytarabine	900 mg/m^2
		Etoposide	450 mg/m^2
[123]		BCNU	500 mg/m^2
		Melphalan	80–140 mg/m^2
		Etoposide	300 mg/m^2
		BCNU	500 mg/m^2
		Melphalan	140 mg/m^2
[124]		BCNU	60–100 mg/m^2
		Etoposide	2400–3000 mg/m^2
		Cisplatin	200 mg/m^2

BCNU, carmustine.

[a] Each drug given on a separate day with 1 day's rest between.

have received prior dose-limiting radiotherapy, especially to the mediastinum, which results in a high incidence of fatal IPS following TBI [14]. Chemotherapy regimens may also avoid the long-term sequelae of TBI, including cataracts, sterility, second malignancies, and growth and development problems in children. In addition, there has recently been concern about the development of myelodysplasia and AML, although this risk appears to be limited to autologous HCT, since allogeneic HCT usually eliminates damaged stem cells through the graft-versus-leukemia (GVL) reaction [15].

Table 20.7 Melphalan-based high-dose regimens

Ref.	Regimen	Chemotherapy	Total dose
[125]	MEL/E	Melphalan	140–180 mg/m^2
		Etoposide	60 mg/kg
[126]	MEL/A	Melphalan	140 mg/m^2
		Cytarabine	12 g/m^2
[78]	MEL/MITO	Melphalan	180 mg/m^2
		Mitoxantrone	60 mg/m^2
[83]	MEL/MITO/CARBO	Melphalan	160 mg/m^2
		Mitoxantrone	50 mg/m^2
		Carboplatin	1400 mg/m^2
[127]	MEL/CY/P	Melphalan	80 mg/m^2
		Cyclophosphamide	5.6 g/m^2
		Cisplatin	180 mg/m^2
[89]	MEL/MITO/TAX	Melphalan	180 mg/m^2
		Mitoxantrone	60–90 mg/m^2
		Paclitaxel	500–700 mg/m^2
[39]	MEL/FLU	Melphalan	180 mg/m^2
		Fludarabine	125 mg/m^2

Table 20.8 ThioTEPA-based high-dose regimens

Ref.	Regimen	Chemotherapy	Total dose
[128]	STAMP V	ThioTEPA	500 mg/m^2
	CTCb	Cyclophosphamide	6 g/m^2
		Carboplatin	800 mg/m^2
[129]	TTCYMITO	ThioTEPA	675 mg/m^2
		Cyclophosphamide	7.5 g/m^2
		Mitoxantrone	60 mg/m^2
[77]	TTMITO	ThioTEPA	1.2 g/m^2
		Mitoxantrone	90 mg/m^2
[130]	CYTT	ThioTEPA	700 mg/m^2
		Cyclophosphamide	7 g/m^2
[89]	CYTTP	ThioTEPA	600 mg/m^2
		Cisplatin	40 mg/m^2
		Cyclophosphamide	3.75 g/m^2

Table 20.9 Etoposide-based high-dose regimens

Ref.	Regimen	Chemotherapy	Total dose
[73]	ICE	Ifosfamide	16 g/m^2
		Carboplatin	1.8 g/m^2
		Etoposide	1.5 g/m^2
[77]	ICE	Ifosfamide	20 g/m^2
		Carboplatin	1.8 g/m^2
		Etoposide	3 g/m^2
[131]	CEP	Cyclophosphamide	4.5–5.25 g/m^2
		Etoposide	750–1200 mg/m^2
		Cisplatin	120–180 mg/m^2
[79]		Doxorubicin	165 mg/m^2
		Etoposide	60 mg/kg
		Cyclophosphamide	100 mg/kg

There are potential advantages in terms of convenience and expense of moving away from TBI, in that TBI utilizes already over-extended radiation resources, often for prolonged periods of time, and requires the skills of physicists and radiotherapists. Finally, it may be possible to develop chemotherapy regimens that are more effective than those containing TBI. It is unfortunate that there are at present few studies comparing outcomes of specific regimens used for HCT.

Alkylating agents

Alkylating agents are the major class of drug used in high-dose regimens with HCT support because they have several desirable characteristics. Many alkylating agents have marrow toxicity as the major dose-limiting factor, which allows for dose escalation when hematopoietic cells are utilized. Alkylators are not cell cycle specific, making them capable of killing non-dividing, resting tumor cells. In in vitro testing, alkylators do not generally exhibit cross-resistance and have relatively steep log-linear dose–response curves [16]. Although it is not possible to combine alkylating agents at full dose when used alone, between 50% and 70% of the single-agent MTD can be used in two to three drug combinations.

As regimens for the treatment of solid tumors evolve, there has been movement to incorporate tumor-specific drugs into the high-dose therapy setting. It is important to point out, however, that drugs such as mitoxantrone and paclitaxel, which are highly active against tumors such as breast or ovarian cancer at conventional doses, are more difficult to deliver at higher doses owing to non-hematologic toxicities, that is, cardiac or neurologic. These characteristics limit the usefulness of such drugs in high-dose therapy regimens, and their value in comparison with alkylator-based regimens remains to be proven.

Busulfan (Tables 20.1 and 20.5)

Busulfan (BU) is an alkylating agent with profound myeloablative properties and marked activity against non-dividing marrow cells, and possibly non-dividing malignant cells also. The MTD of BU, given as a single agent over 4 days followed by stem-cell support, is approximately 20 mg/kg [17]. Single-agent testing to determine the spectrum of activity in patients with malignant disease has not been extensive. However, BU is probably active in a variety of malignancies, including multiple myeloma [18], lymphoma [19], acute lymphoblastic leukemia (ALL) [19], AML [20], myelodysplasia [21], testicular tumors [22], and Ewing sarcoma [22].

One of the earlier problems with optimal utilization of BU had been the availability of only the oral form. Pharmacokinetic variability between patients is high, with a 2–3-fold difference in plasma levels between patients [23]. When given a set oral dose of BU (16 mg/kg over 4 days), children have lower mean plasma levels of BU (650 ng/mL) than adults (1050 ng/mL) [24], which may be partially compensated for by dosing children by body surface area rather than weight [24]. Variation in BU plasma levels between individuals probably contributes to the significant differences in toxicity and clinical response observed in patients receiving the same mg/kg or mg/m^2 dose. Slattery et al. demonstrated a relationship between low BU steady-state plasma concentrations and graft rejection in children [24]. There are also data suggesting a direct relationship between the severity of regimen-related toxicity (RRT), especially sinusoidal obstruction syndrome (SOS) of the liver, and high BU plasma levels [25]. This is discussed in more detail in the section on hepatic complications after transplantation in Chapter 95. In a patient accidentally overdosed with BU, hemodialysis resulted in accelerated clearance of the drug [26].

It has been shown that the steady-state BU concentration correlates with relapse in patients receiving human leukocyte antigen (HLA)-compatible transplants for CML in chronic phase [27]. Seven relapses occurred among the 22 patients with BU state concentrations below the median of 918 ng/mL compared with none among the 23 patients at or above the median ($p < 0.0003$).

Hence the average steady-state concentration of BU over a 4-day period may be a more important determinant of toxicity and efficacy than the exact mg/kg or mg/m^2 dose administered to any given patient [27]. Fortunately, within a given patient, BU is a pharmacokinetically predictable drug, and its absorption and elimination rate remain linear over a wide dose range and constant with time [24]. With repeated oral administration, steady-state BU plasma concentrations are achieved rapidly and can be predicted from first-dose kinetics. Following determination of first-dose kinetics, oral doses can be adjusted up or down to achieve the desired plasma levels. The strategy of targeted steady-state BU dosing has been used successfully to perform a dose-escalation trial in patients receiving fixed doses of CY and TBI [28]. Targeting of the steady-state plasma level of BU should increase the therapeutic index. Results of phase 2 studies indicated that targeting oral BU in patients with CML, AML, and myelodysplasia is associated with low transplant-related toxicity and a low relapse rate [29].

An intravenous preparation of BU is available, and phamacokinetic data indicate considerably less individual variation in area under the curve (AUC) values than seen with the oral preparation [30]. Intravenous BU can be given as a single daily dose over 3 hours or twice daily rather than as the usual four oral doses per day [31]. Retrospective comparisons of intravenous BU with oral BU suggest less hepatic toxicity in patients receiving the intravenous form.

A regimen of BU combined with CY has been developed that has had a wide application for the treatment of a variety of malignant and non-malignant diseases using autologous and allogeneic stem-cell support [32]. The original regimen included BU 4 mg/kg/day for 4 days followed by CY 50 mg/kg/day for 4 days. This regimen was subsequently modified by lowering the dose of CY to 120 mg/kg, with an apparent decrease in toxicity without an increase in relapses [33]. It has been reported that 16 mg/kg combined with 120 mg/kg CY results in prohibitive toxicity in older patients with multiple myeloma and breast cancer undergoing autologous or allogeneic transplantation [18]. Although these reports could be due to small numbers of patients with advanced disease, lowering the dose of BU to 14 mg/kg decreased toxicity and allowed for an increase in the dose of CY to 150 mg/kg. It has also been shown that older patients with CML or myelodysplastic syndrome tolerate targeted BU and CY 120 g/kg fairly well [34]. In patients with thalassemia, BU 14 mg/kg followed by CY 120–200 mg/kg has been a well-tolerated and effective regimen for allografting. A randomized trial comparing BU dosed at 600 mg/m^2 with BU dosed at 16 mg/kg for patients receiving transplant for thalassemia found no differences in outcome but, not surprisingly, that rejection occurred mainly in patients with low blood levels of BU [35]. Fludarabine, a nucleoside analog with significant immunosuppressive activity, has been substituted for CY in an effort to reduce RRT. In addition, fludarabine inhibits DNA repair and thus is synergistic with alkylators that cause breaks in the DNA strand. Several trials conducted in patients with myeloid leukemias undergoing allogeneic HCT indicate lower toxicity profiles with fludarabine–BU combinations [36].

Etoposide (60 mg/kg) has also been utilized to replace CY in the BU–CY regimen for the treatment of patients with AML undergoing autologous bone marrow transplantation, with encouraging disease-free survivals [37]. The major toxicities, SOS, mucositis, and skin breakdown, are similar to those described with BU + CY or BU + melphalan (MEL).

BU (16 mg/kg) and MEL (140 mg/m^2) have been combined and evaluated in patients undergoing autologous or allogeneic HCT for a variety of hematologic malignancies [38]. MEL (180 mg/m^2) has also been combined with fludarabine (125 mg/m^2) as an unrelated donor conditioning regimen for patients with hematologic malignancies [39].

A regimen of BU (12 mg/kg), MEL (100 mg/m^2), and thioTEPA (500 mg/m^2) followed by autologous HCT has been evaluated in patients with a variety of malignant diseases [40]. This regimen produces profound marrow ablation with significant mucositis and is associated with an approximately 5% treatment-related mortality due to IPS. Regimens involving BU, thioTEPA, and CY have been utilized for auto- [41] and allografting. A regimen involving BU, CY, and etoposide has been utilized prior to an autologous and allogeneic HCT [42].

Treosulfan (dihydrobusulfan) (30–42 g/m^2) has been substituted for BU in combination with fludarabine, CY, or MEL as an allogeneic preparative regimen for patients with both myeloid and lymphoid malignancies [43]. This regimen may be associated with less RRT and a lower early transplant-related mortality; however, a retrospective study comparing fludarabine, treosulfan with fludarabine, and myeloablative or non-ablative doses of BU found no significant differences in survival among patients with less advanced disease [44]. More recently, clofarabine, a second-generation analog of 2′-deoxyadenosine combining properties of cladribine and fludarabine, has been combined with BU to form an allogeneic transplant regimen in patients with refractory AML [45].

Nitrosoureas (Tables 20.1 and 20.6)

Carmustine [1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU)] is a nitrosourea commonly used as a marrow ablative agent because it is active against a variety of tumors [46]. The active metabolite of BCNU is a chloroethyl carbonium ion produced by hydrolysis. It is highly lipid soluble and has been used extensively for the treatment of malignant brain tumors. In conventional doses, BCNU is limited by delayed marrow toxicity, pulmonary fibrosis, and renal dysfunction. The MTD, as a single agent, is 120 mg/m^2 when given with autologous stem-cell infusions. The dose-limiting toxicities are pulmonary and hepatic [46]. When BCNU is used in combinations that include cisplatin and CY, there is a substantial increase in the risk of lung injury [47]. When carboplatin and CY are added to BCNU, a high rate of veno-occlusive disease has been reported [48].

BCNU and CY are often combined and may have synergistic activity. The first multidrug transplant regimen consisted of a combination of BCNU, cytarabine, CY, and thioguanine (BACT) [49]. Variations of these drugs have evolved into the three- and four-drug combinations in common use today involving BCNU.

Regimens containing CY, BCNU, and etoposide (CBV) have been evaluated extensively in patients with malignant lymphoma receiving autologous transplants [50]. The doses of drugs and the schedules are shown in Table 20.6. These regimens are often used as substitutes for TBI-based regimens for patients with lymphoma who have received prior dose-limiting irradiation [51]. Doses of BCNU exceeding 450 mg/m^2 are associated with unacceptable pulmonary toxicity [52]. Early institution of steroid therapy at the first sign of dyspnea may prevent the more serious and often fatal complication of pulmonary fibrosis [47]. Patients who receive high-dose BCNU should receive close long-term follow-up for pulmonary complications, which have been noted in adults who received large doses of BCNU as children [53]. Less frequent severe toxicities include SOS, hemorrhagic cystitis, and nephrotoxicity. Although not extensively evaluated, all of these combinations are probably sufficiently immunosuppressive to achieve allogeneic engraftment. Lomustine has been substituted for BCNU in an attempt to decrease pulmonary toxicities, but without a clear reduction in lung complications [54].

BCNU, etoposide, cytarabine, and CY (BEAC) have been extensively evaluated for patients with malignant lymphoma [55]. MEL has been substituted for CY in the BEAC regimen to create BEAM and has also been extensively evaluated in patients with malignant lymphoma [56]. Since most patients with malignant lymphoma have peripheral blood hematopoietic cells (PBHCs) collected after CY-containing regimens, the BEAM regimen may be more attractive than the BEAC regimen. Furthermore, the substitution of MEL for CY obviates the problem of cystitis and the need for mesna (sodium 2-mercaptoethanesulfonate) and/or bladder irrigation, making this a preferred outpatient regimen for patients with malignant lymphoma. Allogeneic marrow transplants also have been performed following the administration of BEAM [57].

Melphalan (Tables 20.1 and 20.7)

One of the earliest chemotherapeutic agents developed, MEL is a bifunctional alkylating agent whose structure incorporates nitrogen mustard and phenylalanine [58]. Single doses of MEL, 150–240 mg/m^2, followed by autologous stem-cell infusion, have been evaluated in patients with multiple myeloma and breast cancer with the dose-limiting toxicities being GI and hepatic [59,60]. MEL 240 mg/m^2 has been utilized alone as a conditioning regimen for patients with hematologic malignancies receiving allogeneic transplants from HLA-matched siblings [61]. MEL has been widely used in high-dose regimens for patients with breast and ovarian cancer, multiple myeloma, or lymphoma. MEL 100 mg/m^2 has been used as a reduced-intensity conditioning regimen prior to allogeneic stem-cell transplantation in patients with multiple myeloma [62]. Fludarabine has been combined with MEL to form a reduced-intensity regimen for allografting [39].

MEL is utilized alone or with TBI to treat patients with multiple myeloma [63] or leukemia. A randomized trial of single-agent MEL (200 mg/m^2) compared with reduced-dose MEL (140 mg/m^2) with TBI as a preparative regimen for patients with myeloma demonstrated a survival advantage for MEL alone [63]. As a result, single-agent MEL is by far the most commonly used conditioning regimen for patients with myeloma undergoing autologous peripheral blood HCT. Recent efforts have been made to improve on MEL monotherapy, including escalating doses to 280 mg/m^2 using palifermin or amifostine as cytoprotective drugs [64,65] or by the addition of bortezomib to MEL 200 mg/m^2 [66].

MEL is included in several of the BU- and BCNU-based regimens listed in Tables 20.5 and 20.6, and Table 20.7 summarizes additional regimens that have included MEL for myeloablation. MEL is frequently combined with BCNU (BEAM), a regimen utilized for patients with malignant lymphoma [56]. BEAM itself has been modified by substituting bendamustine for BCNU [67] or by adding fludarabine and TBI to an allograft regimen [68].

ThioTEPA (Tables 20.1 and 20.8)

ThioTEPA was first recognized for its antitumor properties in the 1950s, but because of severe myelosuppression it saw only limited use in breast and ovarian cancer. Both thioTEPA and its major metabolite TEPA (*N*-triethylenephosphoramide) are active against tumor cell lines *in vitro*. The plasma half-life of TEPA is much longer than that of thioTEPA and accounts for much of the clinical toxicity. ThioTEPA crosses the blood–brain barrier and is useful in the management of CNS disease. It is also fairly immunosuppressive and has been used in preparative regimens to reduce graft rejection in T-cell-depleted allogeneic HCT. The MTD for thioTEPA, as a single agent with stem-cell support, is approximately 1100 mg/m^2. Dose-limiting toxicities are of the CNS, GI tract, liver, and skin [69].

ThioTEPA is included in several of the above BU-, BCNU- and MEL-based regimens outlined in Tables 20.5–20.7, and Table 20.8 summarizes additional regimens that include high doses of thioTEPA.

Non-marrow ablative agents and agents for which MTD with stem-cell support is not known

Cyclophosphamide (Table 20.1)

CY alone was originally used to achieve allogeneic engraftment in patients with aplastic anemia and hematologic malignancies, but was modified in patients with malignant disease by the addition of TBI or combinations of chemotherapeutic agents. However, CY, with or without antithymocyte globulin, is still the most commonly used regimen for immunosuppression of patients with aplastic anemia prior to allogeneic transplantation. Low doses of TBI (2–5 Gy) have been added to CY as a conditioning regimen for aplastic anemia in an effort to increase immunosuppression and reduce episodes of graft rejection [70]. CY is very immunosuppressive but is not marrow ablative as the dose-limiting toxicity is hemorrhagic myocarditis. The MTD, as a single agent, is approximately 200 mg/kg, and infusion of marrow has no impact on survival at this or higher doses [71]. Stem cells are believed to be resistant to CY because they express high levels of aldehyde dehydrogenase. The major side effect, at doses of 120–200 mg/kg, is hemorrhagic cystitis, which can be diminished by bladder irrigation or the administration of mesna. While hemorrhagic myocarditis is a dose-limiting toxicity. CY can temporarily affect cardiac function leading to congestive failure. This is often first noticed by low voltage on the electrocardiogram. Both CY and its isomer ifosfamide have been extensively utilized with high-dose chemotherapy regimens for most tumor types treated with HCT.

Ifosfamide (Table 20.1)

Ifosfamide, a structural isomer of CY, has been evaluated in high-dose regimens followed by autologous stem cells as a substitute for CY. Similarly to CY, however, hematopoietic toxicity is not dose limiting even when high doses of ifosfamide are administered [72]. The MTD is 18–20 g/m^2 with the dose-limiting toxicities being renal, bladder, and neurologic, with lethargy, confusion, and seizures. Ifosfamide is always given with mesna for bladder protection. Ifosfamide is frequently combined with carboplatin and etoposide (ICE) for the treatment of lymphoma, but this regimen does not require HCT [73].

Etoposide (Tables 20.1 and 20.9)

Etoposide is a semisynthetic derivative of podophyllin. It appears to act by stabilization of the topoisomerase II–DNA complex, with subsequent DNA breaks and cell-cycle arrest. This mechanism of action may promote synergistic cell killing when combined with alkylators [37]. The MTD for etoposide, as a single agent with stem-cell support, is approximately 2400 mg/m^2. Although etoposide has substantial myelotoxicity, the dose-limiting toxicity is to the GI tract. In a phase I study with marrow support, the tempo of recovery of peripheral counts was not related to the dose of etoposide and was more rapid than recovery after TBI regimens with marrow support [74].

Etoposide is commonly used with TBI [75] in the regimens listed in Tables 20.6 and 2.7, and additional regimens are outlined in Table 20.9. It is not clear whether any of the regimens in Table 20.9 requires HCT. BU has been combined with etoposide (Table 20.5), resulting in a unique HDT regimen that is particularly active in patients with acute leukemias [37]. The major dose-limiting toxicities of this regimen are GI and pulmonary.

Fludarabine

Fludarabine, a purine analog, has been widely incorporated into non-myeloablative transplant regimens because it is profoundly immunosuppressive and can facilitate engraftment with low-intensity regimens. More recently, fludarabine has been added to marrow ablative doses of busulfan, as a substitute for CY in order to develop a high-intensity regimen that is less toxic [44]. Fludarabine and sub-myeloablative doses of MEL are also utilized as an allograft preparative regimen [39].

Mitoxantrone (Tables 20.1 and 20.7) and doxorubicin (Table 20.9)

Mitoxantrone has been utilized in a variety of high-dose regimens, but the MTD as a single agent with stem-cell support has not been reported [76]. Single-agent dose-escalation trials in patients with metastatic breast cancer have been limited by cardiac toxicity and are disappointing in terms of response rates. The highest dose of mitoxantrone administered in a high-dose combination regimen has been 90 mg/m^2 given with thioTEPA 1200 mg/m^2 [77].

Regimens including mitoxantrone are included in Tables 20.7 and 20.8. In one study, CY 7 g/m^2 and escalating doses of mitoxantrone led to unacceptable hemorrhagic cystitis despite mesna, leading the investigators to substitute MEL for CY [78].

In a similar fashion, doxorubicin, a compound structurally related to mitoxantrone, has been combined with etoposide and CY as a high-dose regimen for patients with breast cancer [79]. The MTD of doxorubicin was 165 mg/m^2, with the dose-limiting toxicity being mucositis. It is not clear that the use of mitoxantrone or doxorubicin in high-dose regimens requires stem-cell support, and it remains to be determined whether such anthracycline-based regimens are more efficacious than alkylator-based combinations. There is significant controversy as to whether high-dose regimens of any kind are useful for patients with high-risk or metastatic breast cancer.

Cisplatin (Table 20.1)

The MTD for cisplatin as a single agent with HCT has not been reported, and the highest dose given in combination regimens has been 300 mg/m^2 given with etoposide 60 mg/kg and CY 100 mg/kg [80].

Regimens including cisplatin are outlined in Tables 20.6–20.9. Although limited by nephrotoxicity and ototoxicity when utilized as a single agent, when used with other drugs the doses of cisplatin do not exceed 200 mg/m^2 and, with adequate hydration, renal dysfunction is generally avoidable.

Carboplatin (Table 20.1)

The MTD for carboplatin with HCT is approximately 2000 mg/m^2, with the dose-limiting toxicities being hepatic and renal [81]. Carboplatin in conventional doses is usually given in line with a formula based on renal function. However, Shea et al. administered carboplatin in doses of 375–2400 mg/m^2 and found a direct correlation between the dose and the measured AUC, suggesting that high doses could be based on body surface area. Carboplatin has also been combined with etoposide, CY, and escalating doses of TBI (10–12.9 Gy) for the treatment of a variety of hematologic malignancies [82]. In one study, carboplatin was administered at doses of 1000–1600 mg/m^2 in conjunction with high doses of MEL and mitoxantrone. In a retrospective analysis of this study, dosing by the Calvert formula was more predictive of grade 3–4 toxicities than dosing by body surface area. These data suggested that dosing of carboplatin to an AUC of 20 mg/mL/min rather than 1400 mg/m^2 would decrease grade 3–4 RRT in patients receiving MEL (160 mg/m^2) and mitoxantrone (50 mg/m^2) followed by PBHC infusion [83].

High-dose chemotherapy regimens including carboplatin are outlined in Tables 20.7–20.9. A phase I–II trial determined that the MTDs of carboplatin and CY administered together are 1.8 and 6.0 g/m^2, respectively [84]. Carboplatin (1500 mg/m^2) with CY (120 mg/m^2) and mitoxantrone (75 mg/m^2) appears to be an active regimen for the treatment of patients with ovarian cancer [85]. Combinations of carboplatin, etoposide, and either paclitaxel or thioTEPA are used with HCT support for the treatment of germ-cell tumors [86].

Cytarabine (Table 20.1)

Cytarabine is a cell cycle-specific antineoplastic agent that affects cells only in S-phase. Although its mechanism of action is not fully understood, its metabolite cytarabine triphosphate is believed to inhibit the action of DNA polymerase. It may also function as a false substrate when incorporated into DNA. The MTD of cytarabine when administered as a single agent with HCT has not been determined. However, in combination with other agents given in high doses, cytarabine is usually administered in doses of 3 g/m^2 for 4–12 doses at 12-hour intervals [87]. Cytarabine 12–36 g/m^2 is included in several regimens utilized for the treatment of patients with hematologic malignancies, as outlined in Tables 20.6 and 20.7.

Paclitaxel

Paclitaxel is an antimicrotubule drug that facilitates microtubule assembly from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stabilization inhibits microtubule reorganization, preventing progression to mitosis. Paclitaxel 775 mg/m^2 has been substituted for BCNU in a regimen including 5.6 mg/m^2 CY and 165 mg/m^2 cisplatin [88]. Paclitaxel 500–700 mg/m^2 has been administered with MEL (180 mg/m^2) and mitoxantrone (60–90 mg/m^2) to patients with advanced ovarian cancer (Table 20.8) [89]. Paclitaxel 150–775 mg/m^2 has also been combined with doxorubicin (165 mg/m^2) and CY (100 mg/kg) and utilized for patients with advanced breast carcinoma [90].

High-dose regimens with total body irradiation

Total body irradiation with cyclophosphamide (Table 20.2)

The use of CY with TBI is based on empirical observations. CY (120 mg/kg) was originally given prior to TBI to a patient undergoing a syngeneic marrow transplant for advanced lymphoma in 1970 in order to avoid tumor lysis and acute renal failure that had been previously observed with TBI given in a single dose of 10 Gy. CY is regularly given prior to TBI in patients receiving allogeneic or syngeneic marrow transplants with no apparent excess toxicity over that observed following TBI alone and hence has become a "standard regimen" widely used in many transplant centers. Although several agents have been added to or substituted for CY (Table 20.4), most of the TBI regimens tested over the past 20 years have included CY before or after TBI. A variety of TBI regimens have been evaluated in attempts to define an optimal dose and schedule. Table 20.2 summarizes the MTDs for several different schedules of TBI (delivered by ^{60}Co sources at dose rates of 5–10 cGy/min) given after CY 120 mg/kg.

Randomized trials involving different TBI regimens (Table 20.3)

There has been a paucity of randomized controlled trials evaluating different TBI regimens (Chapter 22). Three trials reported by the Seattle Marrow Transplant Team are summarized in Table 20.3 [4,10,91].

Total body irradiation with drugs other than cyclophosphamide (Table 20.4)

Drugs other than CY have been administered with TBI, as shown in Table 20.4, but there is no evidence that any of these agents is superior to CY when given with TBI since randomized controlled trials have not been performed. When BU was substituted for CY given with 12 Gy TBI as allograft conditioning for patients with myelodysplastic syndrome, the toxicities were considerable with a day 100 non-relapse mortality of 38% [92]. A large, retrospective analysis of patients reported to the International Bone Marrow Transplant Registry compared outcomes of two regimens used for allografting from HLA-identical siblings for patients with ALL in first or second remission [93]. All 502 patients received myeloablative doses of TBI with either CY (*n* = 298) or etoposide (*n* = 204). There were no differences in outcomes by regimen for patients in CR1 (complete remission 1); however, for patients in CR2, there was a lower risk of relapse and mortality when etoposide was used with any TBI dose when compared with CY and TBI doses below 13 Gy.

Cyclophosphamide and total body irradiation with other drugs (Table 20.4)

Other agents have been combined with CY and TBI, as shown in Table 20.5. Phase II trials suggest that many of these combinations may have better therapeutic efficacy than CY/TBI, but none of these regimens has been subjected to randomized comparison studies.

High-dose chemotherapy versus high-dose chemoradiotherapy (Table 20.10)

There have been relatively few studies comparing chemotherapy regimens with TBI-based regimens. Two randomized trials have demonstrated the equivalency of BU/CY and CY/TBI in patients with CML in chronic phase receiving HLA-matched allografts [94,95]. One study in patients receiving HLA-compatible transplants for AML showed superiority for the CY/TBI regimen due to a lower relapse rate [96]. The Southwest Oncology Group compared

Table 20.10 Randomized trials of BU/CY versus TBI ± VP16 ± CY

Ref.	Diagnosis	Regimen	No.	Survival	Relapse	Event-free survival
[96]	AML	BU/CY	51	0.51	0.34	0.47
		CY/TBI	50	0.75	0.14	0.72
[95]	CML	BU/CY	65	0.61	0.44	0.59
		CY/TBI	55	0.63	0.11	0.55
[94]	CML	BU/CY	73	0.80	0.13	0.68
		CY/TBI	69	0.80	0.13	0.71
[132]	"Advanced" leukemias	BU/CY	61	0.30	NR	0.20
		VP-16/TBI	61	0.30	NR	0.20
[101]	ALL (pediatric)	TBI/VP16/CY	22	0.45	NR	0.58
		BU/VP16/CY	21		NR	0.29

AML, acute myeloid leukemia; BU, busulfan; CY, cyclophosphamide; NR, not reported; TBI, total body irradiation.

BU/CY with etoposide/TBI in 122 patients with advanced acute leukemias or CML beyond first chronic phase undergoing matched allogeneic transplantation. There were no significant differences in toxicity, acute graft-versus-host disease (GVHD), survival, or disease-free survival in patients entered on either regimen [97]. An analysis of non-randomized trials reported by Horning et al. suggested equivalency between patients with lymphoma receiving TBI and etoposide or CBV given prior to autologous transplantation [98]. There were no differences in outcomes seen in a retrospective analysis of a BCNU/etoposide/CY regimen or TBI/etoposide/CY regimen for patients with Hodgkin disease undergoing autologous HCT [99]. A similar non-randomized analysis comparing 381 patients with AML in first remission undergoing allogeneic HCT found no differences in survival between BU/CY and CY/TBI but a lower risk of relapse with CY/TBI [100]. In children with ALL undergoing allogeneic HCT, a small randomized trial demonstrated that BU/etoposide/CY resulted in an inferior event free survival compared with a regimen of TBI/etoposide/CY [101]. In another trial of high-dose cytarabine, MEL, and either BU or fractionated TBI in children with high-risk ALL undergoing allogeneic HCT, BU was associated with fewer long-term side effects [102]. In multiple myeloma, a randomized comparison of MEL 200 mg/m^2 versus MEL 140 mg/m^2 plus 8 Gy TBI demonstrated similar event-free survivals but a superior 45-month overall survival for the MEL monotherapy group, 66% versus 46% [63].

Modified total body irradiation regimens

Because of the considerable toxicities to non-target organs associated with external-beam TBI, there has been interest in the application of technical modifications to TBI. In one early approach, external-beam TBI was given with 90% lung shielding followed by electron beam to the rib cage in order to deliver a uniform dose of radiation to all marrow-bearing areas. This modified TBI was studied in a phase I trial as the second part of a tandem transplant for patients with bone-based malignancies [13]. The MTD in this trial was 13.5 Gy. Another trial combined modified TBI (9 Gy) with BU (12 mg/kg) and CY (120 mg/kg) to treat 89 patients with multiple myeloma [103]. The transplant mortality in this study was 2%, with a complete remission rate of 48%.

More recently, studies have been conducted using image-guided tomographic intensity-modulated radiation equipment that does not require organ shielding. Simulations of dosimetry using this equipment indicate a 1.7–7.5-fold reduction in median doses to non-target organs [104]. Furthermore, this approach may allow a significant escalation of doses of TBI of up to 20 Gy to target organs such as bone.

Marrow ablation using targeted radioisotopes

Some alternatives to the external-beam delivery of radiation using radioimmunotherapy are discussed in more detail in Chapter 23. One approach to improving the cure rate of transplant conditioning regimens while simultaneously decreasing toxicities to normal organs is to replace or augment non-specific external-beam TBI with targeted radiotherapy. Radiolabeled monoclonal antibodies have the potential to focus higher doses of radiation on tumor sites than is possible with external-beam TBI and expose normal organs to lower doses of radiation. Recent evaluations of these techniques have been performed in patients with multiple myeloma, malignant lymphoma, and AML.

Bone-seeking radioisotopes

Isotopes that selectively bind to bone by themselves (yttrium-90) [105], or when complexed to bone-seeking compounds, are an attractive way to target radiation for patients with malignancies in which bone or bone marrow is the primary site of disease. Few studies have utilized radioisotopes alone for marrow ablation. Bayouth et al. performed a phase I trial of holmium-166 (^{166}Ho) complexed to the bone-seeking isotope 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid (DOTMP) in patients with multiple myeloma. The total dose of radiation adsorbed that was delivered to the bone marrow for six patients ranged from 7.9 to 41.4 Gy, and marrow ablation requiring the infusion of stem cells was achieved in two patients. In several multicenter phase I–II trials, cohorts of patients with multiple myeloma received increasing doses of ^{166}Ho–DOTMP designed to deliver 20, 30, or 40 Gy to the bone marrow. This was followed by high-dose MEL 140–200 mg/m^2, external-beam TBI (some patients), and peripheral blood hematopoietic progenitor cells [106]. There were no grade 3–4 non-hematologic toxicities within the first 60 days. After 60 days, significant hemorrhagic cystitis occurred at one center that did not use bladder irrigation. Seven patients experienced late thrombotic microangiopathy among the group receiving more than 30 Gy (or 2400 mCi dosed) to the marrow. The complete remission rate was 35%, with a median overall survival in excess of 24 months. Since two-thirds of the patients in these trials had relapsed or refractory disease, this remission rate was higher than would be expected with MEL alone.

Another bone-seeking radioisotope, samarium-153 (^{153}Sm), with less beta energy than ^{166}Ho, has been linked to ethylenediamine tetramethylenephosphonate (EDTMP), a tetraphosphonate chelate, and studied in 46 patients with multiple myeloma who received MEL 200 mg/m^2 following the isotope. Patients received individualized doses of ^{153}Sm designed to deliver 40 Gy to the bone marrow. The treatment was well tolerated and when compared with 102 contemporaneously treated patients who received MEL 200 mg/m^2 monotherapy, outcomes were better than would be expected with MEL alone [107]. The role of bone-seeking isotopes should be expanded over the next several years, and they may become a major therapeutic approach to the treatment of malignancies involving the bone marrow.

Sequential regimens with peripheral blood hematopoietic cell support

The concept of administering tandem high-dose cycles or multiple, less-intensive treatments more frequently as an alternative to single high-dose marrow ablative therapy has only recently been explored. Some groups have utilized tandem cycles of high-dose MEL 200 mg/m^2 or MEL and TBI, followed by autologous stem-cell infusion for the treatment of patients with multiple myeloma. Tandem cycles of MEL produced complete response rates of 44% in patients with myeloma. Several prospective, randomized trials of single- versus double-intensive therapy for patients with multiple myeloma have been reported [108,109]. These trials employed MEL 140–200 mg/m^2 for the first cycle and MEL + TBI or BU for the second cycle. One trial found a survival advantage for the tandem approach whereas the second study showed equivalent survival, but both trials reported better complete response rates and event-free survival favoring the tandem approach.

Tandem cycles of high-dose chemoradiotherapy with CY, etoposide, MEL, thioTEPA, and TBI have been used to treat children with stage IV neuroblastoma [110]. Tandem cycles of paclitaxel, carboplatin, and either etoposide or ifosfamide have been utilized with autologous HCT for relapsed germ-cell tumors [111]. Tandem autologous transplant, MEL (150 mg/m^2) for cycle 1 and CBV or TBI/CY/etoposide for cycle 2, has been utilized to treat patients with primary refractory or relapsed Hodgkin lymphoma [112]. It remains to be determined whether or not this approach will be superior to single high-dose ablative regimens.

Allogeneic transplantation with non-marrow-ablative regimens

There is increasing evidence that stable donor chimerism can be achieved with sub-lethal doses of TBI or chemotherapy and immunosuppressive drugs. Partial, but stable, grafts would be of benefit for patients with non-malignant diseases, such as thalassemia or sickle cell anemia, if they could be achieved without transplant-related mortality. Patients with malignancies are often considered too old for high-dose regimens or, when young enough, have organ dysfunction that precludes administration of intensive regimens prior to allografting. In contrast to non-malignant diseases, however, the successful treatment of cancers with allogeneic transplant almost certainly requires full donor engraftment in order to utilize the GVL effect. It is of major interest to develop non-toxic methods of achieving donor chimerism that would then allow the evaluation of post-transplant immunotherapy with donor lymphocytes, which would then be relied on to produce the bulk of the antileukemic therapy. Non-ablative allografting strategies are discussed in detail in Chapter 21.

Conclusion

Because of the relatively high probability of relapse in many patients receiving HCT, the further development of new treatment regimens is warranted. However, one can predict that it will continue to be difficult to identify more effective treatment regimens. Incremental improvements in treatment regimens are expected to be small and difficult to measure, and will require the study of large numbers of patients. There continues to be a great need for controlled trials to evaluate the effectiveness of specific treatment regimens for specific groups of patients. Only centers with large numbers of patients or cooperative study groups can successfully perform the trials necessary to substantiate the effectiveness of a given treatment regimen. In order to make high-dose therapies and peripheral blood hematopoietic cell support generally available, the procedure will have to be performed with low mortality and minimal morbidity in an outpatient setting. To achieve this goal without sacrificing efficacy, agents that protect normal, non-hematopoietic tissues from RRT may have to be utilized. In addition, studies utilizing targeted radioisotopes may allow further dose intensity to tumor-bearing tissues while sparing non-involved organs.

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CHAPTER 21

Reduced-intensity Allogeneic Transplantation Regimens

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Introduction

Allogeneic hematopoietic cell transplantation (HCT) has an important role in the treatment of malignant and non-malignant hematological diseases. Successful allogeneic HCT must overcome two immunologic barriers: graft-versus-host (GVH) and host-versus-graft (HVG) reactions. The conventional strategy to overcome this bidirectional barrier has relied upon three elements. First, intensive conditioning regimens are delivered with the dual purposes of immunoablation and disease eradication, which consist of otherwise supralethal doses of irradiation and/or chemotherapy. Second, donor hematopoietic cells are given to rescue patients from lethal myeloablation. Third, T-cell depletion or post-grafting immunosuppression is used to control GVH disease (GVHD) and establish long-term graft–host tolerance.

Toxicities related to the conditioning regimens are a major limitation in the application of high-dose HCT. Toxicities include pancytopenia, which sets the stage for life-threatening infections, and damage to the liver, kidneys, and lungs, which may impair the ability to deliver the doses of immunosuppression necessary for control of GVHD. For these reasons, high-dose allogeneic HCTs have been carried out in specialized hospital wards and their use restricted to relatively young patients at most transplant centers.

The concept that conditioning dose intensification was the only approach for eradicating malignancy came into question early in the history of clinical HCT. Findings in the late 1970s and early 1980s drew attention to graft-versus-tumor (GVT) effects, as evidenced by better relapse-free survival in patients with acute or chronic GVHD [1–6]. Additional evidence supporting GVT effects included lower relapse rates among patients who received unmodified allogeneic HCT compared with T-cell-depleted allografts [6], autologous grafts [7,8], or syngeneic grafts [9], and the induction of durable remissions with donor lymphocyte infusions (DLIs) in patients who had relapsed after allogeneic HCT [10–15]. These observations led to the hypothesis that GVT effects might be exploited in patients thought to be too old or medically infirm to tolerate high-dose conditioning regimens. Accordingly, several groups of investigators have developed reduced-intensity regimens that minimize the regimen-related toxicities and rely on GVT effects to treat the underlying malignancies.

The least intensive regimens rely on pre- and post-transplant immunosuppression to establish the allografts. It is important to remember that both HVG and GVH reactions are mediated by T cells of host and donor origin, respectively, when transplants are carried out across minor histocompatibility barriers. From these facts follows the hypothesis that HVG reactions can, to some extent, be controlled through novel and effective agents that at the same time serve to control GVHD. Consequently, much of the toxic high-dose pre-transplant therapy previously employed for HVG control can be replaced by relatively non-toxic immunosuppression. Once established, grafts would create marrow space through subclinical GVH reactions, and cytotoxic and myeloablative doses of total body irradiation (TBI) or busulfan (BU) would not be needed for that purpose. Ideally, control of HVG and GVH reactions results in mutual graft–host tolerance and initial mixed donor–host hematopoietic chimerism. Although mixed chimerism is likely to correct phenotypic expression of certain genetic diseases, in patients with hematologic malignancies full donor chimerism may be required to control disease through GVT effects. Thus, novel transplantation programs can be designed that are safer than high-dose regimens and have the potential to be administered in the ambulatory care setting rather than on inpatient wards. This chapter discusses the results of clinical studies with reduced-intensity conditioning for hematological malignancies, including the preclinical studies that served as their basis.

Preclinical canine studies

We developed a minimal-intensity transplantation regimen in a canine model that used a small dose of TBI (2 Gy) before and synergistic immunosuppression with mycophenolate mofetil (MMF) and cyclosporine (CSP) after dog leukocyte antigen (DLA)-identical HCT [16]. Most of the dogs so treated showed sustained marrow engraftment. Similar results were obtained when rapamycin was substituted for MMF [17]. In other studies, we substituted 4.5 Gy of irradiation targeted to cervical, thoracic, and upper abdominal lymph nodes for 2 Gy TBI and observed sustained allogeneic engraftment in non-irradiated marrow and lymph node spaces, which suggested that donor T cells were able to create marrow space for the graft to home [18].

Based on the preclinical canine studies, we successfully introduced a minimal-intensity regimen into clinical transplantation, using 2 Gy TBI ± fludarabine before and MMF and a calcineurin inhibitor after HLA-matched transplantation. Clinical results with that approach are discussed below.

Further work in the canine model has been aimed at replacing 2 Gy TBI with radiolabeled monoclonal antibodies (mAbs).

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We investigated whether radioimmunotherapy with an α-emitting radionuclide, bismuth-213 (^{213}Bi) or astatine-211 (^{211}At), could be substituted for low-dose TBI. α-Emitters were an attractive alternative to β-emitting radionuclides for targeting hematopoietic cells given that their high energy is deposited over only a few cell diameters (40–70 μm), leading to a high relative biologic effectiveness and sparing surrounding normal tissue. We have previously demonstrated in the canine model that both panhematopoietic anti-CD45 and T-cell-specific anti-T-cell receptor (TCR)αβ mAbs labeled with ^{213}Bi facilitate stable engraftment of DLA-identical marrow in the absence of other conditioning therapy [19–21]. We were not able to translate these studies to the clinic because the parent of ^{213}Bi, actinium-225 (^{225}Ac), was not readily available in quantities sufficient for scale-up to human patient trials, the cost of the isotope was prohibitive, and its half-life (46 minutes) was very short. Accordingly, we investigated the α-emitter ^{211}At, as it was significantly less expensive and its longer half-life of 7.2 hours was particularly attractive as it lends itself to better targeting of hematopoietic cells, including immune cells. Our murine studies found ^{211}At-labeled anti-CD45 mAbs to be more effective than ^{213}Bi in terms of ablating hematopoietic cells and of toxicity [22]. Dose-finding toxicity studies in dogs have been completed and DLA-identical marrow grafts successfully established using ^{211}At-labeled anti-CD45 mAbs [23]. Studies using the ^{211}At-labeled anti-TCRαβ mAbs have been initiated and preliminary results are encouraging. Selectively ablating T cells or ablating all nucleated hematopoietic cells in this way would allow transplantation to be carried out with very low toxicity, making it suitable both for patients with non-malignant and for those with malignant hematologic disorders. Furthermore, mAbs against the panhematopoietic marker CD45 would be useful in patients with major histocompatibility complex (MHC)-non-identical donors where additional host immune cells, such as natural killer (NK) cells, play a major part in graft rejection.

Based on results in both the murine and large animal models, clinical studies with β-emitting radionuclides are ongoing in clinical trials in patients (see Chapter 23). Data from ongoing preclinical studies with α-emitters will allow us to refine future approaches for our patients.

Clinical results

Several groups of investigators have explored the feasibility of reduced-intensity HCT with a variety of regimens to minimize toxicities while exploiting GVT effects. Figure 21.1 outlines the spectrum of commonly used regimens relative to their hypothetical immunosuppressive and myelosuppressive effects. In some cases, drugs were chosen for conditioning that have proven activities against the targeted malignancies for disease control while waiting for GVT effects to occur. With the minimally myelosuppressive regimens, engraftment was ensured by innovative postgrafting immunosuppression that also controlled GVHD, and disease control was more dependent on GVT effects. Other differences among the reduced-intensity regimens included the degrees of marrow aplasia and the tempo of establishing complete donor chimerism. In the reduced-intensity regimens that have more myelosuppressive doses of chemotherapy, recipients uniformly become severely hypoplastic before graft function occurs. Complete donor engraftment occurs rapidly. By contrast, recipients of a less myelosuppressive regimen show only moderate declines of peripheral blood cell counts before recoveries are seen, and all patients are initially mixed donor–host hematopoietic chimeras. In these patients, it may take 6–12 months before donor engraftment is complete.

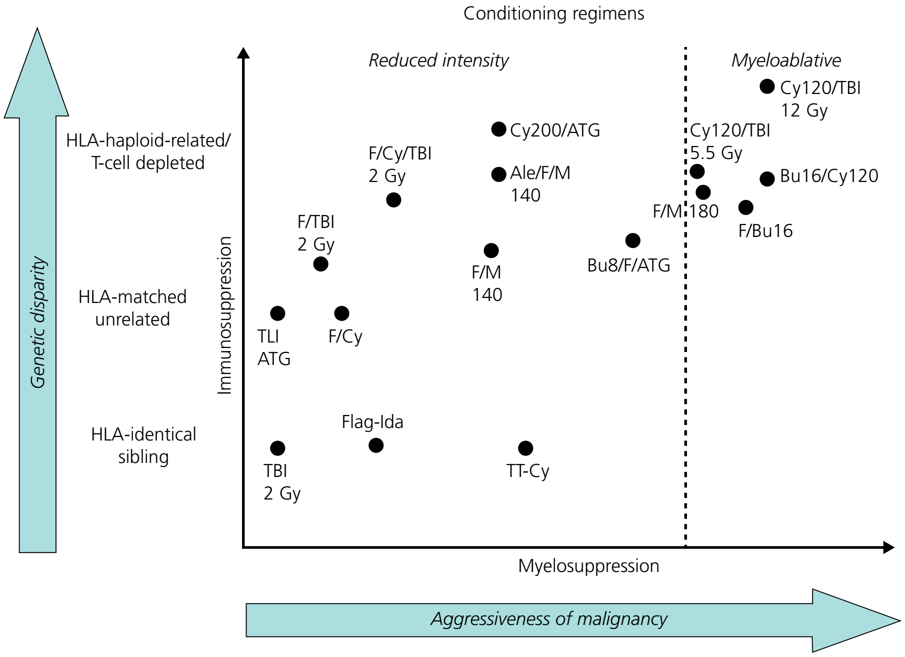

Figure 21.1 Commonly used non-myeloablative or reduced-intensity conditioning regimens in relation to their immunosuppressive and myelosuppressive properties. Ale, alemtuzumab; ATG, antithymocyte globulin; Bu8, busulfan 8 mg/kg; Bu16, busulfan 16 mg/kg; Cy, cyclophosphamide; Cy120, cyclophosphamide 120 mg/kg; Cy200, cyclophosphamide 200 mg/kg; F, fludarabine; Flag-Ida, fludarabine/cytosine arabinoside/idarubicin; M, melphalan; M 140, melphalan 140 mg/m^2; M 180, melphalan 180 mg/m^2; TBI, total body irradiation; TLI, total lymphoid irradiation; TT, thioTEPA. Source: adapted from Storb et al. 2001 [110]. Reproduced with permission of the American Society of Hematology.

In order to be able to communicate scientifically within the transplantation community and compare studies using different regimens, there was a need to define what constitutes a reduced-intensity conditioning regimen. At the 2006 Tandem meeting, a workshop was convened by the Center for International Blood and Marrow Transplantation Research (CIBMTR) to help define reduced-intensity conditioning regimens and the consensus included regimens that met the following criteria: (1) a TBI dose of ≤5 Gy if given as a single fraction or 8 Gy if given in a fractionated manner; (2) a BU dose of <9 mg/kg; (3) a melphalan dose of <140 mg/m^2; or (4) a thioTEPA dose of ≤10 mg/kg [24]. Subsequently, there was an attempt to subcategorize regimens further as non-myeloablative regimens, which had the characteristic of causing minimal cytopenias and could also be given without stem-cell support [25]. However, the field has operationally included the "non-myeloablative" regimens with other reduced-intensity regimens as one group.

The comparative benefit of one over another reduced-intensity regimen may depend on the underlying diseases, stage of diseases, and clinical status of the patients. In diseases presumed to be more sensitive to GVT effects, such as chronic myelogenous leukemia, chronic lymphocytic leukemia, myeloproliferative disorders, and low-grade lymphomas (including mantle cell lymphoma), immunosuppressive regimens may be sufficient to ensure engraftment because there is time for GVT effects to become operative. In rapidly progressive diseases or diseases where GVT is less potent or less able to keep ahead of growth of the underlying malignancy, such as high-grade lymphomas, Hodgkin lymphoma, and acute leukemias beyond first remission, a certain amount of cytoreduction may be necessary to minimize residual disease. In this chapter, we discuss strategies of reduced-intensity transplants, with a review of the initial results and current approaches, and with a focus on minimally myelosuppressive TBI-based regimens.

HLA-matched related HCT

Although many studies of reduced-intensity hematopoietic transplants have been performed in elderly and medically infirm patients unable to tolerate a high-dose preparative regimen, some studies have included younger patients who were otherwise eligible for high-dose transplants with standard eligibility criteria. These differences in patient selection generally preclude a meaningful comparison among regimens. Table 21.1 outlines the initial publications regarding reduced conditioning regimens that are currently being utilized.

Initial studies at the M.D. Anderson Cancer Center in Houston, Texas, utilized purine nucleoside analog-based regimens for the treatment of both myeloid and lymphoid malignancies. Giralt et al. [26] combined fludarabine with idarubicin and cytarabine or melphalan, or cladribine and cytarabine before HCT from HLA-identical or single HLA antigen-mismatched sibling donors for treatment of acute myeloid leukemia (AML) or myelodysplastic syndromes. Of 15 patients treated, four failed to engraft, and one treatment-related death occurred before HCT. Khouri et al. [27], also at the M.D. Anderson Cancer Center, treated 15 patients with lymphoid malignancies using a regimen of fludarabine/cyclophosphamide (CY) or fludarabine/cytarabine/cisplatin. Eleven of the 15 patients attained durable engraftment, and eight of the 11 engrafted patients achieved complete remissions. Three non-relapse deaths occurred.

Giralt et al. [28] subsequently reported on a study combining purine analogs (fludarabine or cladribine) with melphalan in patients with hematologic malignancies. It was hypothesized that these drug combinations would have enhanced abilities to achieve remissions given the antitumor effects of high-dose melphalan and the observation that purine analogs have been shown to inhibit DNA repair after alkylator-induced damage. Seventy-eight of 86 patients received fludarabine in combination with high-dose melphalan, and eight received cladribine/melphalan. The day 100 non-relapse mortality (NRM) rate was 37% for the fludarabine/melphalan combination and 88% for the cladribine/melphalan combination. Both regimens were sufficiently immunosuppressive to allow for engraftment of both HLA-matched unrelated and related and one HLA antigen-mismatched related donor grafts. At 1 year, disease-free survival was 57% in patients in first remission or chronic phase and 49% in patients with untreated first relapse or in second or later remission. Responses were seen in patients with refractory disease suggesting the beneficial effect of the cytoreductive conditioning therapy.

Slavin et al. [29] at Hadassah University in Israel used a regimen that consisted of fludarabine, BU (8 mg/kg), and antithymocyte globin (ATG) in a younger group of patients (median age 34 years, range 1–61 years) with both hematologic malignancies and genetic diseases. All patients achieved either partial or complete donor chimerism with this regimen, with four patients developing moderate to severe hepatic sinusoidal obstructive syndrome, suggesting that this regimen was still fairly toxic. The same regimen was used in another group of heavily treated high-risk lymphoma patients with grafts from HLA-matched related and unrelated donors [30]. Again, there was consistent engraftment with disease-free survival of 40% at 37 months. Childs et al. [31] at the National Cancer Institute combined CY and fludarabine to treat patients both with hematologic malignancies and with solid tumors. One of 15 patients rejected the graft, and two patients died of transplant-related causes. The observation was made that full donor T-cell chimerism preceded both acute GVHD and disease regression.

Investigators at the Massachusetts General Hospital in Boston evaluated the use of CY, ATG, and thymic irradiation (among patients without previous mediastinal radiotherapy) in patients given HLA-matched transplants [32]. Eighteen of 20 evaluable patients developed persistent mixed hematopoietic chimerism. Ten of the 20 received prophylactic DLIs to convert mixed to full donor chimerism and to optimize GVL effects. Six of eight patients receiving prophylactic DLIs converted to full donor chimerism. Transplant complications included CY-induced cardiac toxicity. In an attempt to reduce the incidence of GVHD, alemtuzumab (anti-CD52 mAb) was added to fludarabine and melphalan conditioning, in recipients of both HLA-matched sibling and unrelated donor grafts [33]. Forty-two of 43 evaluable patients had sustained engraftment. Eighteen of 31 patients studied were full donor chimeras, while 13 patients were mixed chimeras. The regimen prevented grade III–IV acute GVHD, and only two patients developed grade II GVHD.

While the chemotherapy-based reduced-intensity regimens had significantly fewer toxicities compared with conventional high-dose allogeneic HCT, patients still experienced profound pancytopenias, and most required hospitalizations of durations that were similar to those for high-dose transplants. In order to reduce further the regimen-related toxicities and thereby perform allogeneic HCT in the outpatient setting, the low-dose (2 Gy) TBI conditioning regimen developed in the dog model was evaluated in a

Table 21.1 Nonmyeloablative conditioning regimens for patients with hematologic malignancies

Transplant center [Reference]	Number of patients studied	Median age in years (range)	Donor	Stem cell source	Diagnosis	Conditioning regimen	Postgraft immuno-suppression	Rejections (%)	GVHD* (%)		Outcome(s)
									Acute grade II–IV	Chronic	
Houston [26]	15	59 (27–71)	MRD 5/6 RD	PBHC BM	AML MDS	F + I + A F + I + M 2-CDA + A	CSP + MP	27	20	0	OS: 40% DFS: 13% Median F/U: 100 days
Houston [27]	15	55 (47–71)	MRD	PBHC BM	CLL NHL	F + CY F + C + A	±T ± MTX	27	7	13	OS: 47% DFS: 33% Median F/U: 180 days
Houston [28]	86	52 (22–70)	MURD MRD 5/6 RD	BM PBHC	HM	F + M 2-CDA + M	T + MTX T + MP CSP + MP	2	40	24	2-year OS: 28% 2-year DFS: 23% 2-year NRM: 45%
Jerusalem [29]	26	34 (1–61)	MRD	PBHC	HM GD	F + B + ATG	CSP	0	38	27	OS: 85% DFS: 81% Median F/U: 240 days
Jerusalem [30]	23	41 (13–63)	MRD	PBHC	L	F + B + ATG	CSP	0	35	9	OS: 43% DFS: 43% Median F/U: 675 days
NIH [31]	15	50 (23–68)	MRD	PBHC	HM ST	F + CY	CSP	7	60	27	OS: 53% PFS: 53% Median F/U: 200 days
Boston [32]	21	44 (22–62)	MRD	BM	HM	CY + ATG ± TI	CSP	24	29	NA	OS: 52% DFS: 33% Median F/U: 445 days
Seattle [112]	426	55 (9–74)	MRD	PBHC	HM	2 Gy TBI ± F	CSP + MMF T + MMF	4	47	50	3-year OS: 51% 3-year PFS: 38%
Stanford [38]	37	52 (28–66)	MRD MURD	PBHC	HM	TLI + ATG	CSP + MMF	16	3	27	OS: 73% DFS: 62% Median F/U: 446 days
London [33]	44	41 (18–56)	MRD MURD	PBHC BM	HM	F + M + alemtuzumab	CSP ± MTX	2	5	2	OS: 82% PFS: 75% Median F/U: 270 days
Freiburg [48]	19	64 (60–70)	MRD MURD	PBHC BM	AML MDS	F + BCNU + M + ATG	CSP + MMF	0	59	65	1-year OS: 68% 1-year PFS: 61%
Baltimore and Seattle [43]	68	46 (1–71)	Haplo	BM	HM	CY + F + 2 Gy TBI	Cy + T + MMF	13	35	Cy1: 25% Cy2: 5%	2-year OS: 36% 2-year PFS: 27%
Jerusalem [45]	16	17 (8–48)	MURD	BM	HM	F + B + ATG	CSP	0	44	0	3-year OS: 75% 3-year DFS: 60%
Dresden [46]	42	47 (16–65)	MURD MMURD	PBHC BM	HM	F + B + ATG	CSP + MMF CSP ± MTX	21	26	38	OS: 36% DFS: 26% Median F/U: 390 days
London [47]	47	44 (18–62)	MURD MMURD	BM	HM	F + M + alemtuzumab	CSP	4	21	6	1-year OS: 75.5% 1-year PFS: 61.5%
Leipzig [50]	52	48 (6–65)	MURD MMURD	BM PBHC	HM	2 Gy TBI + F	CSP + MMF	12	63	25	OS: 35% DFS: 25% Median F/U: 570 days
Seattle [54]	1092	56 (7–75)	MRD MURD	PBHC	HM	2-4 Gy TBI + F	CSP + MMF T + MMF ± SIR	4	49	49	5-year OS: 41% 5-year PFS: 32% Median F/U: 5 years

2-CDA, cladribine; A, Ara-C; AML, acute myeloid leukemia; ATG, antithymocyte globulin; B, busulfan; BCNU, carmustine; BM, bone marrow; C, cisplatin; CLL, chronic lymphocytic leukemia; CSP, cyclosporine; CY, cyclophosphamide; DFS, disease-free survival; F, fludarabine; F/U, follow-up; GD, genetic diseases; Haplo, haploidentical related donor; HM, hematologic malignancies; I, idarubicin; L, lymphoma; M, melphalan; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MMURD, mismatched unrelated donor; MP, methylprednisolone; MRD, matched related donor; MTX, methotrexate; MURD, matched unrelated donor; NA, not available; NHL, non-Hodgkin lymphoma; NRM, non-relapse mortality; OS, overall survival; PBHC, peripheral blood hematopoietic cells; PFS, progression-free survival; RD, related donor; SIR, sirolimus; ST, solid tumors; T, tacrolimus; TBI, total body irradiation; TI, thymic irradiation; TLI, total lymphoid irradiation.

*GVHD before donor lymphocyte infusion.

multi-institutional clinical study [34]. TBI of 2 Gy was given as a single fraction on day 0, with postgrafting immunosuppression of CSP from day −1 to day 35, and MMF given twice per day from day 0 to 27. Granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood hematopoietic cells (PBHCs) collected over 2 days from HLA-identical related donors were used as sources of hematopoietic cells. Trial eligibility criteria required patients to be either too old to be eligible for a high-dose HCT or, if younger, to have medical contraindications to high-dose HCT. Overall, the HCT regimen was well tolerated, with the majority of eligible patients having their transplants in the outpatient clinic setting. Most patients did not develop severe neutropenia or thrombocytopenia (Figure 21.2) While all patients had initial donor engraftment, nine (20%) of the first 45 patients experienced graft rejection between 2 and 4 months after transplant, a timing that coincided with discontinuation of the MMF/CSP immunosuppression [34]. Lack of intensive preceding chemotherapy before HCT predicted rejections, and rejecting patients had low-level donor T-cell chimerism on day 28. The rejections were non-fatal, and all patients had autologous reconstitution of peripheral blood counts.

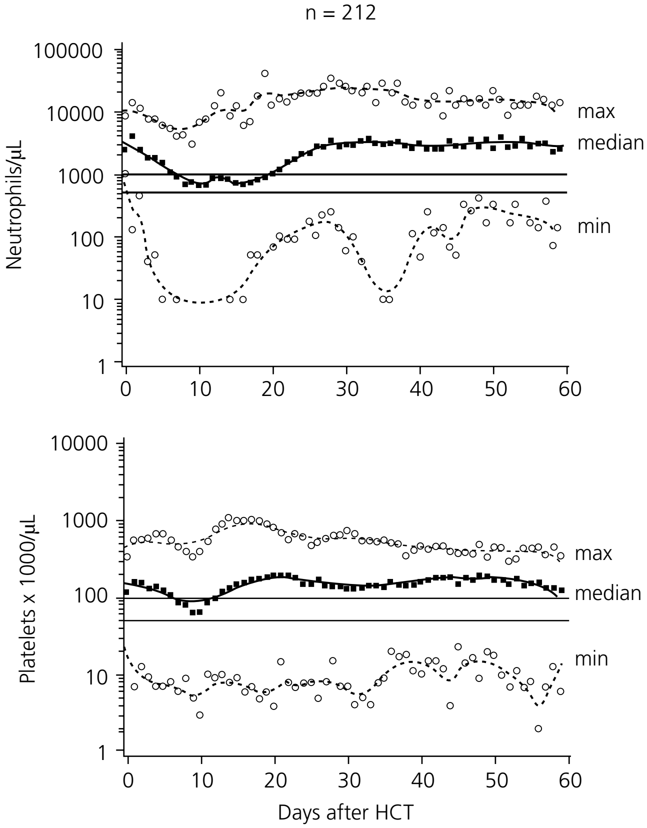

Figure 21.2 Neutrophil and platelet changes among 212 patients given 2 Gy total body irradiation ± fludarabine before and mycophenolate mofetil/cyclosporine after human leukocyte antigen-matched related HCT. Source: Storb 2002 [111]. Reproduced with permission of the American Society of Clinical Oncology.

To prevent graft rejection, the conditioning regimen was modified by adding 30 mg/m^2/day fludarabine on days −4, −3, and −2 to the 2 Gy TBI, with a subsequent reduction of the rejection rate to 3%. In a retrospective analysis of the first 176 patients with hematologic malignancies treated with non-myeloablative HCT from HLA-identical related donors, higher NRM was observed among patients conditioned with 2 Gy TBI and fludarabine (FLU/TBI) (FLU/TBI 31% vs. TBI 14% at 2 years; $p = 0.02$). For this reason, we conducted a prospective phase III randomized trial comparing 2 Gy TBI alone or in combination with fludarabine before transplantation of peripheral blood progenitor cells (PBPCs) from HLA-matched related donors [35]. Eighty-five patients with hematological malignancies were randomized to be conditioned with TBI alone ($n = 44$) or FLU/TBI ($n = 41$). All patients had initial engraftment. Two graft rejections were observed, both in the TBI group. Infection rates, NRM, and GVHD were similar between groups. Three-year overall survival was lower in the TBI group [54% vs. 65%; hazard ratio (HR) 0.57; $p = 0.09$], with higher incidences of relapse/progression (55% vs. 40%; HR 0.55; $p = 0.06$) and relapse-related mortality (37% vs. 28%; HR 0.53; $p = 0.09$), and a lower progression-free survival (36% vs. 53%; HR 0.56; $p = 0.05$). Median donor T-cell chimerism levels were significantly lower in the TBI group at days 28 (61% vs. 90%; $p < 0.0001$) and 84 (68% vs. 92%; $p < 0.0001$), as was NK-cell chimerism on day 28 (75% vs. 96%, $p = 0.0005$). This randomized trial demonstrated the importance of fludarabine in augmenting the graft-versus-tumor effect by ensuring prompt and durable high-level donor engraftment early post-transplant.

The outcomes of different durations of CSP administration were analyzed in 185 patients who received grafts from HLA-matched donors and CSP for 56, 77, or 180 days after HCT in combination with MMF for 28 days. Although the duration of CSP prophylaxis did not significantly influence the overall rate of acute GVHD (grade II–IV), chronic GVHD, or NRM, prolonged administration of CSP (180 days) was associated with a significantly decreased hazard of grade III–IV acute GVHD and an increased likelihood of discontinuing all systemic immunosuppression when compared with the shortest course of CSP (35 days) [36]. Only 3.7% of patients receiving CSP through day 180 developed grade III–IV acute GVHD.

The Stanford group took a different approach to reduce the incidence of GVHD. The reduced-intensity regimen, which was adapted from murine studies that were designed to favor the presence of a high proportion of regulatory NK/T cells [37], consisted of total lymph node irradiation (8–12 Gy total lymphoid irradiation) and ATG (thymoglobulin, 7.5 mg/kg total dose) in combination with MMF and CSP for postgrafting immunosuppression. Of the first 37 patients reported [38], two developed acute GVHD, and nine of 33 evaluable developed chronic GVHD. Further follow-up by this group and others confirmed these low rates of acute GVHD and NRM [39,40].

HLA-mismatched related HCT

The Johns Hopkins group developed a conditioning regimen for HLA-haploidentical related recipients based on a murine model whereby high-dose CY administered after HCT inhibited both graft rejection and GVHD [41]. The regimen for patients consisted of pre-transplant CY 29 mg/kg, fludarabine 150 mg/m^2, and TBI 2 Gy followed by an infusion of marrow and post-transplant CY 50–100 mg/kg, and tacrolimus and MMF (three times per day). A subsequent study expanded on the initial observations [42] in two groups of patients at two centers [Johns Hopkins and Fred Hutchinson Cancer Research Center (Seattle)], which differed in the regimens of post-transplantation immunoprophylaxis [43]. On day 3 (Seattle group) or days 3 and 4 (Johns Hopkins group), 50 mg/kg/day CY was administered. All patients ($n = 68$) received tacrolimus and MMF beginning on the day after the last dose of CY. Of evaluable patients, 13% had graft failure, and 35% and 6% of patients had grade II–IV and III–IV acute GVHD, respectively. The only difference seen between the two groups of patients was that the rate of extensive chronic GVHD in the group that received one dose of post-transplant CY was 25% compared with 5% in the group that received two doses post-transplant ($p = 0.05$). The overall and event-free survivals at 2 years after HCT were 36% and 27%, respectively. The Blood and Marrow Transplant Clinical Trials Network (BMT-CTN) conducted a multicenter phase II trial using the same regimen with two doses of post-transplant CY in patients with leukemia or lymphoma ($n = 50$) [44]. The 1-year probabilities of overall and progression-free survival were 62% and 48%, respectively, after HLA-haploidentical HCT. The 100-day cumulative incidence of grade II–IV acute GVHD was 32%. The 1-year cumulative incidences of NRM and relapse were 7% and 45%, respectively. In parallel, a concurrent BMT-CTN phase II trial using double umbilical cord blood with a similar conditioning regimen gave comparable results [44]. Currently, BMT-CTN is conducting a randomized phase III multicenter trial evaluating the relative risks and benefits of HLA-haploidentical versus double umbilical cord blood HCT using reduced-intensity regimens. Chapters 39 and 44 provide further details regarding cord blood and partially matched related donor HCT, respectively.

HLA-matched and mismatched unrelated HCT

Nagler et al. [45] reported the results of HLA-matched unrelated marrow transplantation following conditioning with fludarabine and BU (8 mg/kg) and ATG. Fifteen of 16 patients achieved 100% donor chimerism, and one patient became a mixed chimera. Seven patients developed grade II–IV acute GVHD, with one patient dying from complications related to acute GVHD. The overall and disease-free survival estimates at 36 months were 75% and 60%, respectively, with this reduced-intensity conditioning regimen in a relatively young population of patients (median age 17 years, range 8–48 years).

Using a similar regimen of fludarabine, BU, and ATG, Bornhauser et al. [46] treated 42 patients with hematologic malignancies with HLA-matched or single HLA antigen-mismatched unrelated grafts. In this older population group (median age 47 years, range 16–65 years), disease-free survival was 64% for patients with lymphoid malignancies, 38% for patients with standard-risk leukemias, and 14% for patients with high-risk diseases. Nine patients (21%) had either primary or secondary graft failures. In patients with stable engraftment, the probability of acute GVHD grade II–IV was 32%.

In a similarly aged group of patients with hematologic malignancies and using a different conditioning regimen consisting of alemtuzumab mAb, fludarabine, and melphalan, Chakraverty et al. described results in 47 patients who received HLA-matched or mismatched unrelated donor transplants [47]. Primary graft failure occurred in two patients, and three patients developed grade III–IV acute GVHD. The overall and progression-free survivals at 1 year were 75.5% and 61.5%, respectively. Bertz et al. reported on 19 patients (aged 60–70 years) with myeloid malignancies who received conditioning with fludarabine, melphalan, and carmustine before HLA-matched related ($n = 7$) or unrelated ($n = 12$) HCT [48]. With a median follow-up of 825 days, the 1-year NRM and survival rates were 22% and 68%, respectively. These results were confirmed in a subsequent update from this group on 34 patients receiving unrelated HCT using this regimen [49]. In summary, fludarabine-based chemotherapy combinations are feasible in older patients or those with comorbidities, and long-term disease control can be achieved with reduced-intensity conditioning in this population of patients.

Using the low-dose TBI (2 Gy) and fludarabine (90 mg/m^2) conditioning regimen, Niederwieser et al. [50] reported on 52 patients with advanced hematologic malignancies given unrelated grafts. It was found that 52% were HLA matched (10/10) level, 29% were one HLA antigen mismatched, and 29% had allele-level mismatches for class I HLA antigens. Durable donor engraftment was attained in 88% of patients and grade II–IV acute GVHD occurred in 63% of patients. In a subsequent study, the same group [51] reported on 89 patients with unrelated donor grafts. Eligibility criteria for this trial included serological matching for HLA-A, -B, and -C, and allele-level matching for HLA-DRB1 and -DQB1. The median patient age was 53 years (range 5–69 years). While the preferred source of stem cells was PBPCs, these were available in only 71 patients, and 18 patients received marrow. Initial donor T-cell engraftment was documented in 87% of patients, with median donor T-cell chimerism on days 28, 56, and 84 being higher in PBPC recipients than marrow recipients ($p = 0.02$, 0.01, and 0.08, respectively). By multivariate analysis, graft rejection was more frequent in marrow recipients ($p = 0.003$) and in patients without preceding chemotherapy ($p = 0.003$). Donor T-cell chimerism values of less than 50% on day 28 were highly associated with eventual graft rejection ($p = 0.001$). Preceding conventional HCT or preceding chemotherapy reduced the risk of rejection, presumably because the prior cytotoxic therapy increased pre-transplant immunosuppression of the host. With a median follow-up of 13 months (range 0.6–28 months), the cumulative probabilities of NRM were 11% at 100 days and 16% at 1 year. The Kaplan–Meier estimates for 1-year survival were 57% versus 33%, and for progression-free survival 44% versus 17% for recipients of PBPCs versus marrow, respectively. A high degree of day 28 CD3 chimerism (>50%) was associated with less relapse ($p = 0.05$). Better progression-free survival was identified in a multivariate analysis in patients with less than 5% blasts in the marrow prior to HCT ($p = 0.0001$) and those who received PBPCs as a hematopoietic stem cell source ($p = 0.006$).

The study suggested that PBPCs were the preferred source of grafted hematopoietic cells in unrelated transplantation with a fludarabine/2 Gy TBI conditioning regimen. Given the importance of postgrafting immunosuppression both to facilitate engraftment and to reduce the risk of GVHD, our attention was drawn to another finding of the study, the short (3.5-hour) serum half-life of the active metabolite of MMF, mycophenolic acid (MPA). This finding resulted in a modification of the post-transplant immunosuppression: an increase in the dosing of MMF to three times daily in the hope of minimizing the risk of late graft rejections. Among 103 patients so treated, graft rejections occurred in 5%, whereas acute GVHD remained at 53%, with 2-year NRM and overall and progression-free survival of 19%, 58%, and 49%, respectively [52]. Because three times daily compared with twice-daily MMF diminished the incidence of graft rejection, MMF should be administered three times per day in unrelated recipients receiving this regimen.

We recently published results of a three-arm, randomized, phase II study of GVHD prevention involving 208 unrelated recipients. Patients in all arms were given MMF and tacrolimus and in arm 3 sirolimus was added. There was a significantly lower incidence of grade II–IV acute GVHD ($p = 0.04$) in the MMF/tacrolimus/sirolimus arm (47%) than the reference arm (64%) consisting of tacrolimus and MMF; however, this was not significantly lower than the historical experience of 52% with MMF/CSP ($p = 0.12$) [53]. A second important finding was that MMF/tacrolimus (without added sirolimus) was no better than the historically used MMF/CSP at preventing acute GVHD among unrelated recipients. The findings from this study are the basis for the currently ongoing randomized two-arm, phase III study whereby both arms use CSP until day 96, with taper to day 150. Arm 1 is the "reference arm" in which MMF is given three times daily until day 30, then twice daily until day 150 with a taper through day 180 in an attempt to avoid late-onset acute GVHD. In arm 2, sirolimus is given until day 150 with a taper though day 180. In arm 2, in addition to CSP and sirolimus, MMF is given through day 40.

A recent publication summarized outcomes in 1092 HLA-matched related ($n = 611$) and unrelated ($n = 481$) recipients with hematologic malignancies given fludarabine and low-dose TBI before and MMF and a calcineurin inhibitor after HCT [54]. Whereas chronic GVHD and GVT effects were comparable after related and unrelated HCT, unrelated recipients experienced more acute GVHD and higher NRM (HR 1.41; $p = 0.002$).

We extended non-myeloablative HCT to patients with HLA class I mismatched donors with mismatches at the antigen level [55]. To prevent graft rejection and optimize GVHD prophylaxis, patients received CSP and MMF longer than HLA-matched recipients. Only 2 of 60 patients rejected their grafts. However, rates of acute GVHD (grade II–IV, 69%; grade III–IV, 26%) were high, with a 2-year NRM of 47%. Sirolimus has been effective as GVHD prophylaxis after HLA-matched and mismatched unrelated HCT [56–58]. Based on the observed possible superior GVHD control with a combination of sirolimus, MMF, and tacrolimus in HLA-matched unrelated donor HCT compared with MMF and tacrolimus alone, a trial using CSP, MMF, and sirolimus has been initiated.

We evaluated the pharmacodynamic relationships between MPA, the active metabolite of MMF, and outcomes in 308 patients conditioned with fludarabine/TBI who received grafts from HLA-matched related (n = 132) or unrelated (n = 176) donors [59]. All patients received post-grafting immunosuppression with MMF and a calcineurin inhibitor. Rejection occurred in nine patients, eight of whom had a total MPA concentration at steady state (C_{ss}) of less than 3 μg/mL. In patients receiving an unrelated donor graft, a total MPA of C_{ss} less than 2.97 μg/mL was associated with increased grade III–IV acute GVHD ($p = 0.05$) and increased NRM ($p = 0.007$). MPA C_{ss} was not associated with clinical outcomes in patients receiving a related donor graft. Future prospective studies are warranted to confirm whether higher initial oral MMF doses and subsequent targeting of total MPA C_{ss} to greater than 2.96 μg/mL could lower grade III–IV acute GVHD and NRM in patients receiving an unrelated donor graft.

Chimerism and engraftment kinetics

Donor chimerism after allogeneic HCT can be evaluated by several means. Currently, fluorescent in situ hybridization of sex chromosomes and amplified fragment length polymorphism analysis are the most commonly used methods (reviewed in [60] and see Chapter 24). Amplified fragment length polymorphism-based techniques utilize the polymerase chain reaction (PCR) for analysis of polymorphic DNA sequences such as variable number of tandem repeats or short tandem repeats. Additionally, real-time PCR of single nucleotide polymorphisms is being evaluated for this purpose.

Reduced-intensity conditioning usually leads to an initial state of mixed chimerism [31,61–63], although some patients given grafts after more myelosuppressive regimens achieve full donor chimerism in cellular subsets as early as day 14 after HCT [64]. Childs et al. [31] evaluated the kinetics of engraftment after conditioning with fludarabine (125 mg/m^2) and CY (120 mg/kg). Most frequently after that regimen, full donor chimerism was achieved earlier in peripheral blood T cells than in myeloid cells. The NK-cell chimerism correlated with the T-cell chimerism, whereas the B-cell chimerism was distinct from the T-cell and myeloid lineages. Bornhauser et al. observed that patients with NK chimerism below 75% in the first month after unrelated donor HCT had a higher risk of graft failure after conditioning with fludarabine 150 mg/m^2, BU 6.6 mg/kg intravenously, and ATG [46]. The kinetics of chimerism were also evaluated in patients who received conditioning with 2 Gy TBI with or without fludarabine (90 mg/m^2) [63]. Most patients remained mixed donor–host chimeras for up to 3–6 months after HCT. Patients given preceding chemotherapy or peripheral blood stem-cell grafts had the highest degrees of donor chimerism. High levels of donor T- or NK-cell chimerism (>50%) 28 days after HCT have each been associated with a reduced risk of graft rejection [34,63]. There was also an association with higher donor T-cell chimerism and an increased risk of grade II–IV acute GVHD [63]. A subsequent study in 282 patients modeling chimerism levels as a continuous linear variable confirmed that high early donor T-cell chimerism was associated with acute GVHD ($p = 0.01$), whereas high donor NK-cell chimerism had no association with GVHD ($p = 0.38$) [65]. Whereas high donor T-cell chimerism levels only trended to a lower risk of relapse ($p = 0.10$), high donor NK-cell chimerism levels were significantly associated with lower relapse risk ($p = 0.0009$).

An association between the kinetics of engraftment and GHVD was observed by Childs et al. [31]. The achievement of full donor T-cell chimerism preceded the development of grade II–IV GVHD in patients who received conditioning with fludarabine and CY. Studies by Mattsson et al. [66], by contrast, found that 82% of patients had mixed donor–host T-cell chimerism at the time of the development of acute GVHD. This is similar to the results observed after conditioning with TBI with or without fludarabine, in which 83% of the patients were mixed donor–host T-cell chimeras at the time of the development of GVHD [63]. However, when chimerism was analyzed as a continuous linear variable, patients with higher levels of donor T-cell chimerism on day 28 after HCT were at higher risk for grade II–IV acute GVHD [34,51,63], with a corresponding reduced risk of relapse in those patients who achieved full donor T-cell chimerism [63].

GVHD and GVT effects

Reconstitution of donor-derived immunity after reduced-intensity conditioning differed from what occurred after high-dose conditioning. First, as mentioned above, there was an initial state of mixed donor–host chimerism in the recipients of reduced-intensity conditioning that changed the balance of both GVH and HVG tolerance, which may affect the onset of GVHD. Second, high-dose conditioning regimens may contribute to the pathophysiology of GVHD via tissue damage causing the release of cytokines, the so-called “cytokine storm” [67]. However, the number of host-derived antigen-presenting cells may be higher after reduced-intensity conditioning, thus contributing to acute GVHD given their putative role in the initiation of GVHD [68].

Historically, the occurrence of GVHD was associated with GVT effects in patients receiving high-dose conditioning regimens [1,2]. Several retrospective analyses have been carried out to evaluate the impact of GVHD and GVT on outcomes after reduced-intensity conditioning regimens since the regimens themselves have less of a role in disease control. Most have shown lower incidences of acute and similar or lower incidence of chronic GVHD in recipients of reduced intensity as compared with high-dose HCT [69–72]. One age-matched retrospective analysis was carried out comparing the incidence of GVHD in recipients of reduced-intensity versus high-dose conditioning. The cumulative incidence of grade II–IV acute GVHD was lower in reduced-intensity HCT (64% versus 85%; $p = 0.001$), resulting in the use of less systemic immunosuppression during the first 3 months after transplant [69]. There was no difference in the cumulative incidence of chronic GVHD requiring treatment. Of note, reduced-intensity HCT was associated with a syndrome of acute GVHD occurring beyond day 100 in a subset of the related recipients, and this should be taken into consideration in the design of prospective studies comparing reduced-intensity and high-dose HCT. Martino et al. demonstrated a reduced risk of relapse in patients with AML and myelodysplastic syndrome who experienced acute and/or chronic GVHD compared with those who did not [73]. Kroger et al. found that while there was no impact of acute GVHD on the risk of relapse in patients with multiple myeloma, there was a significantly lower risk of relapse in patients who had chronic GVHD [74]. These outcomes were also observed in an

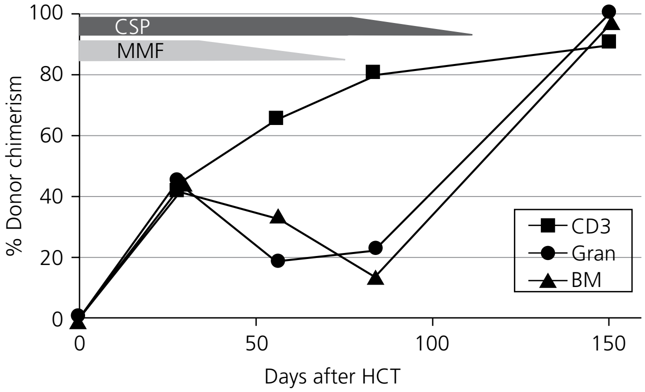

Figure 21.3 Donor chimerism (fluorescence in situ hybridization) in a patient with Philadelphia chromosome-negative myeloproliferative disorder after unrelated HCT. BM, bone marrow; CSP, cyclosporine; Gran, granulocytes; MMF, mycophenolate mofetil.

analysis of multiple myeloma patients by Crawley et al. for the European Group for Blood and Marrow Transplantation [75]. Blaise et al. performed a landmark analysis starting on day 100 in patients with AML and observed a lower risk of relapse and higher leukemia-free survival in the patients who developed chronic GVHD [76].

Figure 21.3 illustrates the donor chimerism changes in a 65-year-old patient with Philadelphia chromosome-negative myeloproliferative disorder whose disease was characterized by high white blood cell counts, very high platelet counts (>10^6/mL), and high basophil counts. The patient's T-cell chimerism increased to 80% by day 84, whereas the marrow and granulocyte donor chimerism declined from 40–45% on day 28 to 10% in the marrow on day 84. The patient's platelet and basophil counts increased to 900,000 and 1500/mL, respectively, consistent with disease progression after an initial response. CSP was rapidly tapered to induce a GVT response. The marrow and granulocyte donor chimerism rose to nearly 100% by day 150, and both basophilia and thrombocytosis resolved. The patient also developed mild signs of chronic GVHD that responded to CSP. The patient is surviving over 13 years after HCT, with complete donor engraftment and no evidence of recurrent myeloproliferative disorder. This clinical case illustrates the relationship between postgrafting immunosuppression, GVHD, and GVT effects. It also emphasizes the importance of lineage-specific chimerism evaluations in monitoring patients.

Reduced-intensity allogeneic HCT relies on GVT effects for the eradication of malignancy. An analysis was carried out in recipients of related or unrelated grafts who had received conditioning with 2 Gy TBI with or without fludarabine to obtain an estimate of relapse risk according to disease characteristics [77]. Relapse rates per patient-year at risk for the first 2 years after HCT were calculated for 29 different diagnosis and disease stages. Using this algorithm, patients were grouped into low, standard, or high relapse risk for each disease and disease stage. The lowest risk group included patients with B-cell malignancies including chronic lymphocytic leukemia and non-Hodgkin lymphoma (NHL) at any stage except aggressive NHL not in complete remission (CR). Patients with advanced myeloid and lymphoid malignancies had the highest relapse rates after reduced-intensity HCT.

The prognostic relevance of GVHD was recently evaluated in 1092 patients with advanced hematologic malignancies who had HCT from HLA-matched related ($n = 611$) or unrelated ($n = 481$) donors after conditioning with low-dose TBI ± fludarabine followed by immunosuppression with MMF and a calcineurin inhibitor [54]. The regimen avoids serious toxicities and has minimal cytotoxic antitumor effects, allowing the evaluation of both GVHD and GVT. The median patient age was 56 years (range 7–75 years) and the median follow-up time was 5 years. The cumulative incidences of grade II–IV acute GVHD and chronic GVHD were 43% and 49%, respectively, with related donors, and 59% and 50%, respectively, with unrelated donors. Grade IV acute GVHD was seen in 3% of patients. The 5-year NRM and relapse mortality were 24% and 34.5%, respectively. Respective 5-year event rates among related compared with unrelated recipients were 20.5% and 28.4% for NRM and 34.5% and 34.5% for relapse mortality. Most NRM was in patients with a history of GVHD and NRM increased in direct relation to increasing grade of acute GVHD. Serious pretransplant comorbidities and grafts from unrelated donors were the most significant factors associated with GVHD-associated NRM. There was an association of acute GVHD with NRM but not with GVT effects. Although chronic GVHD was associated with significantly lower relapse or progression rates, the high associated NRM offset this benefit. The 5-year survival ranged from 25% to 60% and was influenced by disease risk, GVHD, and comorbid conditions (Figure 21.4). Relapse rates were highest during the first 2 years after HCT, whereas rates during years 3–5 were low (Table 21.2) Comparable GVT effects among related and unrelated recipients were also observed in a CIBMTR report following high-dose allogenic HCT [78].

In a recent study of 459 patients with hematologic malignancies given either a related or unrelated HCT after minimal intensity conditioning, we explored whether peri-transplant neutrophil or lymphocyte levels predicted GVHD or GVT effects [79]. We made two key findings. First, low neutrophil nadirs in the first 3 weeks after HCT predicted increased risks of acute GVHD and NRM but had no association with relapse risk. Second, high lymphocyte counts immediately before HCT predicted reduced risks of relapse and overall mortality, without association with the risks of GVHD or NRM, suggesting that the immunologic mechanisms underlying acute GVHD might differ from those that initiate GVT effects.

Major efforts should therefore be directed to more effective GVHD prophylactic regimens in addition to strategies that control the progression of malignant disease early after transplant. Such strategies include the use of tyrosine kinase inhibitors in patients with bcr/abl-positive disease which has been shown to be effective for Philadelphia-positive acute lymphoblastic leukemia (ALL) [80]. Other newer strategies include use of peri-transplant idelalisib for chronic lymphocytic leukemia, quizartinib for AML, or brentuximab vedotin for Hodgkin lymphoma. Lastly, in order to improve patient outcomes in the high-risk group, the option of allografting should be considered earlier in the disease course when the tumor burden is lower.

Toxicities

In a retrospective cohort study, recipients of reduced-intensity conditioning ($n = 503$) had significantly reduced transfusion requirements compared with high-dose HCT recipients ($n = 1353$), with only 36% of the patients requiring platelet transfusions and 75% requiring red blood cell (RBC) transfusions compared with 99% and 96%, respectively, of high-dose HCT recipients ($p < 0.0001$) [81]. Recipients of reduced-intensity HCT who did require transfusions used significantly fewer units of platelets and RBCs ($p < 0.0001$ for both). Platelet and RBC transfusions were less frequent

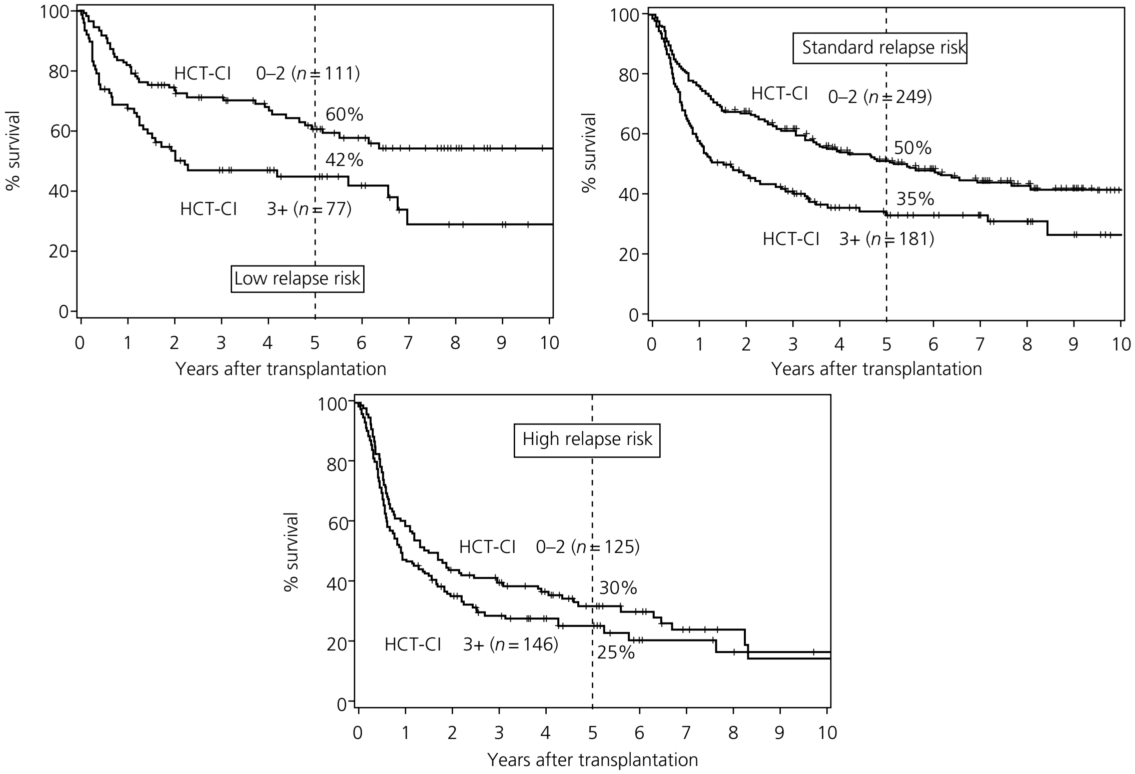

Figure 21.4 Five-year survival (%) of patients with advanced hematologic malignancies who were conditioned with fludarabine and low-dose TBI and given postgrafting MMF and calcineurin inhibitor. Survivals are shown depending on relapse risk and hematopoietic comorbidity index scores (HCT-CI).

Table 21.2 Relapse rates per patient-year among 1,092 patients

Diagnosis (No.; % related/ unrelated)	**Stage**	***n***	**Rate**	
			Years 1–2	**Years 3–5**
Low risk (243; 53/47)				
MPN	Any	18	0.10	0.00
CLL	CR	9	0.11	0.14
Waldenström	Any	10	0.13	0.06
NHL	Any stage of mantle cell and low-grade; aggressive CR	140	0.16	0.02
ALL	CR1[a]	28	0.17	0.04
MM	CR	38	0.19	0.06
Standard risk (537; 58/42)				
CLL	No CR	113	0.24	0.05
CML	CP1	24	0.24	0.00
MM	No CR	179	0.32	0.17
AML	CR[b]	191	0.33	0.02
MDS	RA/RARS	30	0.35	0.00
High risk (312; 54/46)				
NHL	Aggressive; no CR	50	0.48	0.00
AML	No CR; evolved from MDS	98	0.65	0.04
HL	After failed autologous HCT	61	0.61	0.14
MDS	RAEB; CMML; 2nd	62	0.65	0.04
CML	CP2; AP; BC	23	0.71	0.07
ALL	≥CR2; no CR	18	10.03	–

[a] 14% and

[b] 13% had minimal residual disease before HCT.

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AP, accelerated phase; BC, blasts crisis; CLL, chronic lymphocytic leukemia; CML, chronic myelocytic leukemia; CMML, chronic myelomonocytic leukemia; CP, chronic phase; CR, complete remission; HL, Hodgkin lymphoma; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; RAEB, refractory anemia with excess blasts; RARS, refractory anemia with ring sideroblasts; Waldenström, Waldenström syndrome.

Source: Storb et al. 2013 [79]. Reproduced with permission of the American Society of Clinical Oncology.

among related compared with unrelated recipients (p = 0.0001 for both). ABO-matched recipients required fewer RBC transfusions than major/directionally ABO-mismatched recipients (p = 0.006). ABO incompatibility did not affect other HCT outcomes including GVHD, rejection, relapse, NRM, or survival. Recipients of reduced-intensity conditioning had shorter periods of neutropenia than recipients of high-dose conditioning ($p < 0.0001$), and this was associated with fewer episodes of bacteremia during the first 30 days (p = 0.01) [82]. Similarly, there was a trend to less cytomegalovirus antigenemia, viremia, and disease in reduced-intensity HCT recipients compared with recipients of high-dose conditioning, with a significant reduction when more serious manifestations (cytomegalovirus viremia and disease) were combined during the first 100 days (p = 0.01) [83]. A more recent transition to a preemptive therapy strategy based on CMV viral load and host risk factors successfully prevented CMV disease without increasing the proportion of patients receiving preemptive therapy and attributable toxicity [84]. The incidence of idiopathic pneumonia syndrome was significantly lower following reduced-intensity conditioning compared with high-dose conditioning regimens (2.2% versus 8.4%; p = 0.003) [85].

Liver injury is a frequent, serious complication of high-dose preparative regimens. The frequency and severity of hepatic injury after reduced-intensity conditioning were evaluated in 193 consecutive patients [86]. No patients developed sinusoidal obstructive syndrome. About 26% of patients developed hyperbilirubinemia of 4 mg/dL or more, most commonly resulting from cholestasis due to GVHD or sepsis. Overall survival was superior for patients who had maximum bilirubin in the normal or minimally elevated (1.3–3.9 mg/dL) ranges compared with those in the elevated (≥4 mg/dL) ranges.

Incidences of acute renal failure (ARF) among recipients of high-dose and reduced-intensity conditioning were compared [87]. Severity of ARF was classified into four grades based on serum creatinine increases in the first 100 days after HCT. Recipients of reduced-intensity conditioning were significantly older and had greater pre-transplant comorbidities. Despite this, high-dose patients had greater incidences of severe ARF (73% versus 47%; $p < 0.001$) and dialysis (12% versus 3%; $p < 0.001$) compared with those receiving reduced-intensity regimens. NRM was also greater in the high-dose group at 100 days and 1 year. The incidences of chronic kidney disease after reduced-intensity HCT were analyzed in 122 patients from three centers [88]. Multivariate analysis revealed that ARF in the first 100 days was associated with chronic kidney disease. Previous autologous HCT, long-term calcineurin use, and chronic GVHD were independently associated with chronic kidney disease. Future research should focus on preventing ARF and decreasing the use of calcineurin inhibitors.

Outcomes among 1148 patients transplanted between 2003 and 2007 were compared with those among 1418 patients transplanted between 1993 and 1997 at a single center [89]. On average, the patients in the more recent cohort were older, had more comorbidities, and had more advanced disease then the older cohort. There were significant decreases in overall mortality, including mortality not preceded by relapse. This was primarily related to reduced organ damage, infection, and severe acute GVHD, which in part reflected the current use of reduced-intensity conditioning for allogeneic HCT. Improvements in overall mortality were seen in recipients of both reduced-intensity and high-dose conditioning, highlighting progress in supportive care that impacted outcomes for all patients.

Comparing morbidity and mortality: influence of pre-transplant comorbidities

Comorbidities, defined as any additional clinical entity in a patient with an index disease, affect therapeutic plans and outcomes after treatment for the index disease. With the advent of reduced-intensity conditioning regimens, HCT is being carried out in older patients and those with considerable comorbidities. Objective measures of patients' underlying health are required in order to study and disseminate transplantation approaches in an older and medically less fit population.

Data have suggested that comorbidity indices are useful tools in predicting outcomes that can be used in the design of inclusion/exclusion criteria for clinical trials. One of the indices used was the Charlson Comorbidity Index (CCI), which was originally developed in a cohort of patients at a general medical center [90]. The CCI was successfully applied to patients with hematologic malignancies after HCT following both high-dose and reduced-intensity conditioning regimens in related [91] and unrelated [92] recipients, although it had limitations. A new HCT-Comorbidity Index (HCT-CI) was developed to incorporate HCT-related comorbidities and define better previously defined comorbidities by incorporating pre-transplant laboratory parameters. The HCT-CI had better survival prediction than the CCI [92].

This tool has been validated in patients with myelodysplasia or AML, where similar outcomes have been found after high-dose and reduced-intensity conditioning after stratification of patients into low- and high-risk disease categories and low and high HCT-CI scores [93]. This suggested that patients with low HCT-CI scores could be candidates for randomized trials comparing conditioning intensity and a prospective multi-institutional phase III study is currently ongoing with the BMT-CTN in patients with myelodysplasia and AML. A prospective multi-institutional observational study was initiated at the CIBMTR to collect comorbidities from all transplant centers. The HCT-CI was a valid tool to discriminate relative risks for NRM and survival after HCT across different institutions, different conditioning intensities, and different diagnoses [94].

Long-term outcomes were investigated among patients 60 years of age and older after conditioning with low-dose TBI and fludarabine on prospective trials followed by related ($n = 184$) or unrelated ($n = 188$) donor HCT [95]. The 5-year cumulative incidence of NRM was 27% and was not different among the different age groups. Increasing age was not associated with increases in acute or chronic GVHD or other organ toxicities. Increasing HCT-CI scores were associated with worse outcomes, as were standard or high relapse risk. The 5-year overall and progression free-survivals were 35% and 32%, respectively.

Consolidative reduced-intensity allografts following planned autografts

In patients with aggressive malignancies, the GVT effect following a reduced-intensity allograft may not be sufficiently fast enough to eradicate large-volume disease. Carella et al. adopted the strategy of using debulking autologous HCT followed by reduced-intensity allogeneic HCT in patients with refractory Hodgkin and non-Hodgkin lymphomas [96] and Kroger et al. reported a similar approach in multiple myeloma [97]. Maloney et al. also published results for patients with multiple myeloma who were given a cytoreductive autograft with melphalan conditioning (200 mg/m^2) followed by an allograft from an HLA-identical sibling after conditioning with 2 Gy TBI [98]. With a median follow-up of 550 days after allograft in surviving patients, the overall survival rate was 78%. The overall disease response rate was 83%, with 57% of patients achieving complete remission and 26% partial remission. Based on this study, a phase III trial comparing tandem autologous transplantation with tandem autologous/allogeneic (auto/allo) transplantation was carried out by the BMT-CTN. A total of 710 patients were enrolled and the median follow-up was 40 months. In the standard risk patients, the 3-year progression-free survival was 43% in the auto/allo group and 46% in the auto/auto group, and overall survival also did not differ [99]. Disease relapse remains the major problem in both groups, suggesting that post-HCT maintenance therapy is needed.

Another trial based on this approach was reported by Bruno et al., who compared allografting with autografting for newly diagnosed multiple myeloma [100]. Patients ($n = 162$) were enrolled at the time of diagnosis and treated with vincristine, doxorubicin, and dexamethasone followed by melphalan and autologous PBPC infusion. Patients with an HLA-identical sibling then received 2 Gy TBI and PBPCs from the sibling donor, whereas those without an HLA-identical sibling received a second autograft. In an updated report with a median follow-up of 7.3 years (range 5.4–10.4 years), the median survival was not reached in the 80 patients with an HLA-identical sibling versus 4.25 years for 82 patients without ($p = 0.001$) [101]. Among patients who completed their treatment protocols, the median overall survival was not reached ($p = 0.02$) and the event-free survival was 3.25 years ($p = 0.02$) in the auto/allo group ($n = 58$), whereas the overall survival was 5.3 years and event-free survival was 2.75 years in the tandem autologous group ($n = 46$).

An update at a median follow-up of 8 years (range 3.9–10.6 years) of the European Group for Blood and Marrow Transplantation Non-Myeloablative Allogeneic Stem Cell Transplantation in

Multiple Myeloma (NMAM) 2000 study that prospectively compared tandem auto/allo with autologous HCT alone. Patients up to age 69 years were enrolled ($n = 357$). Patients with an HLA-identical sibling were allocated to auto/allo ($n = 108$) and those without to autologous (auto) HCT alone ($n = 249$). The progression-free survival and overall survival at 8 years were 22% and 49% versus 12% and 36% in the auto/allo versus auto group, respectively [102]. The authors concluded that long-term outcomes were better with auto/allo than auto and that a follow-up longer than 5 years is necessary for correct interpretation of the additive value of an allogenic HCT.

Reduced-intensity allografting after failed high-dose transplants

Use of conventional high-dose conditioning for allografts following failed autologous or allogeneic HCT has resulted in poor outcomes in adult patients, primarily related to early fatal regimen-related toxicities [103]. A number of investigators have therefore evaluated reduced-intensity regimens to condition patients for second HCT. Investigators in Jerusalem conditioned patients with fludarabine, BU, and ATG, with CSP as GVHD prophylaxis [104]. Among 12 relatively young patients transplanted (median age 33 years, range 8–63 years), one non-relapse death occurred and six patients were disease-free with a median follow-up of 23 months. Actuarial overall and disease-free survivals were 56% and 50%, respectively, at 34 months. Investigators at Massachusetts General Hospital described their results with 13 patients (median age 38 years) who relapsed following autologous HCT and received HLA-matched related donor allografts following conditioning with CY and ATG with or without thymic irradiation [105]. DLI was administered at 5–6 weeks post-transplant to facilitate full donor chimerism. One non-relapse death occurred, and 2-year estimates of overall and disease-free survivals were 45% and 38%, respectively.

Investigators at the City of Hope National Medical Center have described their results for 28 patients who failed previous autologous transplants or developed myelodysplastic syndrome after autografts [106]. The median interval from the failed autologous HCT and allogeneic HCT was 15 months. The median age of the patients was 47 years, and patients were treated with either fludarabine plus melphalan ($n = 24$) or fludarabine plus 2 Gy TBI ($n = 4$). Patients received HCT from either HLA-matched related ($n = 14$) or unrelated ($n = 14$) donors. The day 100 mortality and NRM were 25% and 21%, respectively. With a median follow-up of 2 years in surviving patients, the 2-year probabilities of overall survival, event-free survival, and relapse rate were 56.5%, 41%, and 41.9%, respectively.

The Seattle Consortium reported on 147 patients (median age 46 years, range 9–73 years) who failed conventional autologous ($n = 135$), allogeneic ($n = 10$) or syngeneic ($n = 2$) HCT and subsequently received HLA-matched related ($n = 62$) or unrelated ($n = 85$) grafts after 2 Gy TBI-based regimen (with or without fludarabine) [107]. The 3-year NRM was 32% in related recipients and 28% in unrelated recipients with a median follow-up of 2.2 years (range 0.23–5.3 years). The Kaplan–Meier estimates of relapse and overall survival were 48% and 32%, respectively, for related recipients, and 44% and 44%, respectively, in unrelated recipients. The best outcomes were observed in patients with non-Hodgkin lymphoma, whereas patients with multiple myeloma and Hodgkin lymphoma had the poorest outcomes due to high incidences of relapse and progression. A lower risk of relapse or progression was seen in patients in partial or complete remission at the time of HCT and in patients who developed chronic GVHD. Factors associated with better overall survival were the partial or complete remission of the underlying disease and lack of comorbidity at the time of HCT. Hence allogeneic GVT effects can be used with some success in patients for whom other treatment options have been exhausted, including high-dose therapy with hematopoietic cell rescue (either autologous or allogeneic). Outcomes using related or unrelated donors were comparable.

Thirty-eight patients underwent salvage reduced-intensity HCT after rejection (in the absence of relapse) of the first allograft [108]. Conditioning included 3–4 Gy TBI in addition to fludarabine. In 14 cases the original donor was used and in 24 cases different donors were used. Sustained engraftment was achieved in 87% of patients with rejections in five patients, of whom four had myelofibrosis. The 2-year NRM and overall survival were 24% and 49%, respectively. These data indicate that graft rejection can be overcome by salvage HCT using fludarabine and low-dose TBI.

The role of second allogeneic HCT was investigated in 90 consecutive patients with hematologic malignancies (median age 34 years, range 3–68 years) who relapsed after first allogeneic HCT and underwent second or subsequent allografts [109]. The majority of patients (89%) had relapsed following a high-dose conditioning regimen. In 15 cases the original donors were used for second HCT, and in 75 cases different donors were identified. Conditioning for second HCT included low-dose TBI (2–4 Gy) with or without fludarabine, with or without CY ($n = 49$), treosulfan, and fludarabine-containing regimens with or without low-dose (2 Gy) TBI ($n = 17$), high-dose TBI ($n = 16$), or BU ($n = 8$) based regimens. With a median follow-up of 3.2 years (range 0.6–12.8 years) among surviving patients, the 2-year Kaplan–Meier overall survival, relapse rate, and NRM estimates were 36%, 44%, and 27%, respectively. Relapse was the leading cause of treatment failure. Patients with early relapse after their first allogeneic HCT (within 6 months vs. 6–12 months vs. more than 12 months) had significantly higher 2-year relapse rates after second transplants (64%, 44%, and 31%, respectively; $p = 0.01$). This translated into overall survival differences with borderline statistical significance (21%, 24%, and 51%, respectively, $p = 0.08$). Patient age, donor type, same versus different donors for second HCT, conditioning intensity, and HCT comorbidity scores did not appear to affect outcomes in univariate analyses in this group of patients. Based on this retrospective analysis, second allogeneic HCT should be considered for post-transplant relapse, especially in patients who relapse more than 12 months after their first allografts.

Conclusion

The use of reduced-intensity or minimally myelosuppressive preparative regimens has resulted in durable allogeneic hematopoietic engraftment and the development of GVT effects in the setting of underlying malignant diseases. The regimens have been relatively non-toxic in older patients and have been successfully applied to younger patients with comorbid conditions that had excluded them from high-dose HCT. The optimal regimen may ultimately be defined by the nature of the diseases being treated. In aggressive lymphomas, acute leukemias not in remission, and other fast-growing malignancies, cytoreduction by either preceding chemotherapy with or without autologous stem-cell rescue, or the use of a reduced-intensity regimen with disease-specific chemotherapy, may be necessary to allow time for adequate GVT responses to develop. For low-grade or mantle cells lymphomas, acute leukemias in first complete remission, chronic leukemias, and other more indolent diseases, minimal reduced-intensity regimens may be sufficient for successful outcomes by allowing the donor T cells to generate sufficient GVT responses and eradicate the underlying malignancies, but here too, prevention of relapse using disease-specific approaches post-transplant is being studied.

Other current issues include minimizing GVHD, which would avoid prolonged immunosuppression, thereby reducing the risk for infections that otherwise contribute significantly to NRM in all regimens. Additionally, prolonged immunosuppression may blunt optimal GVT effects. Ultimately, reduced-intensity HCT might be used to establish mixed donor–host chimerism that can serve as a platform for adoptive immunotherapy with the infusion of tumor-specific cytotoxic T cells that would exert GVT effects without causing GVHD.

In the future, reduced-intensity transplants may become the procedure of choice also for younger patients with either malignant or non-malignant diseases. Towards that end, BMT-CTN 0901 is carrying out a randomized, phase III study of allogeneic HCT comparing regimen intensity in patients with myelodysplastic syndrome or AML. Additional phase III studies are needed to determine both immediate- and long-term outcomes in different disease categories and age groups.

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CHAPTER 22

Radiotherapeutic Principles of Hematopoietic Cell Transplantation

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Introduction

Since the initial pioneering efforts of Thomas and colleagues [1], radiation therapy continues to be an important part of conditioning regimens in patients undergoing hematopoietic cell transplantation (HCT). Irradiation is used primarily as a form of systemic therapy utilizing high-energy photons and large fields to deliver total body irradiation (TBI). In addition, total lymphoid irradiation (TLI) and locoregional irradiation directed to areas of higher tumor burden have also been combined in HCT conditioning regimens.

A primary role of TBI is the eradication of malignant cells. In patients undergoing allogeneic HCT, TBI also provides a powerful means of immunosuppression to prevent rejection of donor hematopoietic cells. TBI is often combined with chemotherapy in conditioning regimens and offers distinct advantages over chemotherapy. Unlike chemotherapy, delivery of radiation therapy to the tumor site is not dependent on blood supply or influenced by inter-patient variability of drug absorption, metabolism, biodistribution, or clearance kinetics. Radiation therapy can reach potential sanctuary sites, such as the testes and brain. Chemotherapy-resistant clones that develop may still be sensitive to irradiation.

Unlike in chemotherapy, the radiation dose can be preferentially distributed to areas of greatest tumor burden and restricted to critical normal organs. Past efforts utilized shielding to reduce the dose to critical organs such as the lung, kidney, or liver, or delivered additional locoregional radiation therapy as boost treatment to areas of greatest tumor burden. Recent advances in radiation therapy delivery systems now allow for new and novel opportunities to target radiotherapy selectively in the setting of HCT. Biologically targeted systemic radiotherapy, using radiolabeled antibodies or bone-seeking radiopharmaceuticals such as ^{166}Ho-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid (DOTMP), have been evaluated as part of conditioning regimens in clinical trials. Advances in the delivery of external beam radiotherapy using intensity-modulated radiation therapy (IMRT) coupled with image-guided radiation therapy (IGRT) now allow the radiation oncologist to "sculpt" dose distribution to the unique shape of each patient's tumor. These advances are being translated to large-field applications and are being actively investigated as a means of image-guided targeted TBI or total marrow irradiation (TMI) [2–7].

This chapter provides an overview of the principles and concepts in radiobiology and radiation physics that are important in optimizing TBI. An understanding of these principles is essential to minimize toxicities, maximize tumor cell eradication, and optimize the therapeutic ratio of this important treatment modality. Dose to tumor, dose to organs, volume of critical organ receiving a given dose, fraction size, number of fractions, time between fractions, dose rate, beam energy, uniformity of dose distribution within the target region, and reproducibility of patient alignment and set-up are all critical factors to consider in optimizing outcomes. The clinical results and normal tissue toxicities of TBI are reviewed and provide examples of how the rational application of radiobiology and radiation physics has led to improved outcomes. Finally, the rationale, methods, and clinical results of the new, targeted TBI strategies utilizing IGRT and IMRT technologies are described.

Radiobiology principles

It is now clear that fractionated TBI is superior to and associated with less toxicity than single-fraction TBI [8,9]. The issue of dose rate has not really been addressed systematically. In examining dose-rate effects, most studies have ignored the important influence of fractionation on the dose-rate effect and have also ignored the fact that dose-rate effects will be different for each different cell type irradiated. Hence the fundamental radiobiologic questions that remain in TBI are how should the treatment be fractionated and, given this fractionation, is there a dose-rate effect in the cell types of interest that is significant enough to be worth pursuing?

The fundamental response to radiation is generally described by a cell survival function. This function gives the probability, S, that a given cell will survive a dose, d, of radiation. Of course, S depends upon the type of cell, its phase in the cell cycle, and the conditions under which it is irradiated, including the type of radiation. There are many mathematical models that describe the dependence of S on d [10]. If cells were killed as a result of a single event whose probability of occurrence was dose dependent, then the cell survival curve would be exponential. However, there is a shoulder on most mammalian cell survival curves, followed by an exponential fall-off at higher doses (see Figure 22.1).

The model of cell survival, in most common clinical use today, is the linear-quadratic (LQ) model [11]. In this model, the cell survival is given by

$$S = e^{-\alpha d - \beta d^2} \tag{22.1}$$

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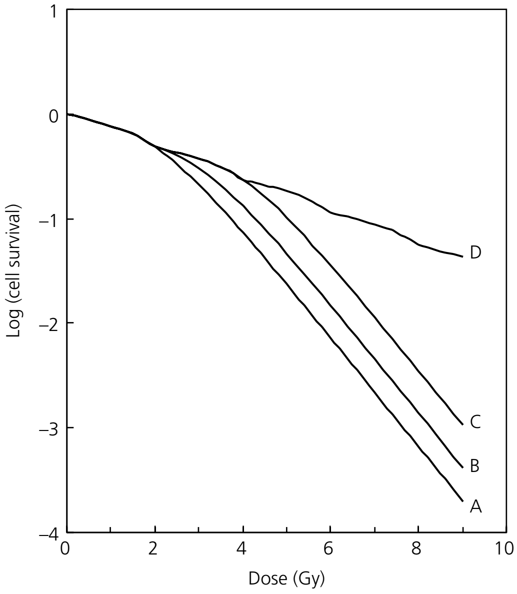

Figure 22.1 Cell survival curve for a multi-hit single target model. Curve A shows the cell survival for a single dose. Curve C shows the cell survival following a completely repaired single dose of 2 Gy. Curve B shows the cell survival after incomplete repair of the initial 2 Gy fraction. Curve D shows the cell survival for multiple 2 Gy fractions.

where the first term in the exponent can be interpreted as being the single-hit component of cell killing (e.g., double strand breaks) and the second term is the two-hit component (e.g., single strand breaks). Clearly, this model is incompatible with a model that has a linear high-dose behavior (in log$S$), but over the range of usual applicability (less than 10 Gy), the models are often indistinguishable. For N fractions, the cell survival is given by

$$S=\left(e^{-\alpha d-\beta d^2}\right)^N=e^{-\alpha D-\beta Dd} \qquad (22.2)$$

where $D = dN$ is the total dose given in N fractions of size d.

The shoulder of the cell survival curve is a result of the repair of sublethal damage (SLD) [12]. SLD consists of intracellular lesions that are not lethal by themselves, but that may combine with other such lesions to produce a lesion that leads to cell death. For example, a single strand break on the DNA would not be lethal, but two single strand breaks in close proximity would produce a lethal double strand break. Repair of SLD is thought to be complete in less than 24 hours, the normal interfraction interval in radiation oncology. However, mean repair times are generally estimated to be in the range 1–4 hours, a time that becomes important when considering treatments with multiple fractions per day (hyperfractionation). During this repair time, the shoulder on the cell survival curve is being restored, so that when repair is complete, an additional fraction has the same probability of killing a cell as the initial fraction of the same dose. If there is no repair, then the two doses add arithmetically as if they were given in one single dose, producing a much lower cell survival. If the repair is partially complete, then the cell survival is intermediate. This effect is depicted in Figure 22.1. Curve A shows a single dose cell survival curve; curve C shows the cell survival curve for a second dose following an initial dose of 2 Gy and complete repair. Curve B is the same as curve C except that the timing of the second dose is such that repair is not complete after the initial 2 Gy. Curve D shows the cell survival curve for a series of 2 Gy fractions with complete interfraction repair. If there is no shoulder on the cell survival curve, then there is no advantage to fractionation in terms of cell survival.

The same mechanism that gives rise to the shoulder on the cell survival curve is also responsible for the dose-rate effect. If the cell survival curve has no shoulder, then it is generally believed that there will be no dose-rate effect and there will also be no fractionation effect. As opposed to most normal tissues, both leukemia and lymphoma cells are usually seen to have little or no shoulder. Therefore, in HCT, a therapeutic benefit is gained by lowering the dose rate or by fractionating the dose, but not necessarily by both. The more the dose is fractionated, the more cells are killed by the single-hit mechanism, and the less impact the dose rate will have.

The ratio of the parameters in equation (22.2)22.2, α/β, has clinical implications [12]. Higher values of α/β mean that the tissue will be less sensitive to a specific fractionation schedule and more dependent on the total dose. Lower values mean that the tissue's response will be more sensitive to dose per fraction. Generally, late responses are characterized by lower values of α/β (1–3 Gy), whereas acute responses are associated with higher values (~10 Gy).

To conclude, the radiobiologic principles summarized above help to explain why fractionated TBI is less toxic than single-dose TBI for the same level of malignant cell kill. Most TBI regimens are designed to be completed in 1 week, and therefore to increase fractionation beyond five fractions one must treat with multiple fractions per day. To maximize repair of sublethal damage, an interfraction interval of at least 6 hours should be used. Not only does this make three fractions per day logistically difficult, but also the radiobiologic impact of three fractions per day may be unpredictable. However, there are a number of standard fractionation regimens that employ three fractions per day successfully. Comprehensive statistical analysis of the comparative results from one, two, and three fractions per day is not yet available.

Radiation physics and physical considerations

There are a large number of papers and reports that give details on the planning and delivery of TBI [13,14]. Discussed below are the principles that guide this treatment.

TBI treatment is the least conformal treatment that is given in radiation oncology. The primary technical concern is to deliver the desired dose to the entire patient. The most straightforward way to accomplish this objective is to irradiate the entire patient in a single radiation beam. This is achieved by using a large (approximately 4 m) source-to-skin distance (SSD) and the largest practicable field size. The large SSD reduces the dose rate to about 10% of that which is typically used in radiation therapy. The typical field arrangement is to have the patient standing on a specially designed stand, to rotate the collimator by 45° to utilize the diagonal dimension of the field, to use lead strips as compensating filters to achieve a uniform dose rate (at midline) from head to foot, to use a Plexiglas barrier close to the patient to defeat the skin-sparing effect of high-energy photons, and to block critical organs (usually the lungs, sometimes the kidneys, liver, and previously irradiated sites) to lower the dose to these tissues. The patient is treated with anterior and posterior fields, often given on alternating treatment sessions. Alternatively, the patient may be treated while lying on a gurney or a specially designed table at floor level. Treating on or near the floor is common in pediatric cases where the field size requirements are not as great. Because the SSD is

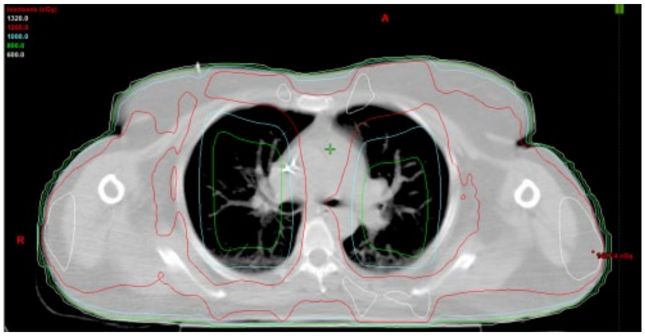

Figure 22.2 Dose distribution through T5–6 showing 6, 8, 10, 12, and 13.2 Gy isodose lines. The prescription dose was 12 Gy, with 50% attenuation lung blocks. (For a color version of this figure, please see the color plate section.)

shorter, this technique will produce a higher dose rate unless the machine's output rate can be electronically adjusted.

On modern C-arm linear accelerators, the dose rate at isocenter is adjustable. This is often used to achieve a dose rate dictated by a particular clinical trial. The selection of beam energy is not critical, with most treatment centers using photon energies between 6 and 10 MV. Photons with these energies have a skin-sparing effect produced by the buildup of forward scattered electrons until electronic equilibrium is established. (Electronic equilibrium is characterized by the same number and energy of electrons being scattered into a region as are scattered out of the region.) Because of this reduced skin dose, a beam spoiler of low atomic number material is placed close to the patient. Electrons scattered from this material to the patient increase the skin dose. This is done to achieve full dose on the skin surface, but the assumption that clonogens are always present on the skin surface has not been proven.

The dose is usually measured at several points on the patient's body with the goal of achieving a uniform dose within ±5%. To achieve this, a compensating filter is constructed of thin lead sheets on a Plexiglas tray inserted in the beam. This filter is designed to compensate both for the variations in patient thickness and for the variation in beam intensity away from the center of the beam.

Most treatment regimens now require the shielding of certain sensitive structures, with the lung being the most common of these. One reason why the AP/PA fields are often preferred is that they allow the most accurate method for supplementing the dose to the chest wall when the lung is blocked. If no lung blocking is used, then the dose to the lung is higher than the dose to midline by as much as 10%. This increase is a result of the reduced attenuation in the lung, the density of which is about one-third of that of most soft tissues. Typically, when lung blocks are used, the blocks cover the central portion of the lung, with about 1–2 cm between the edge of the lung shadow on the film and the edge of the block. It is also typical not to use full-thickness lung blocks, which have a transmission factor of 3%, but rather to use blocks that transmit 50% of the primary beam. The areas of the chest wall that are shielded by the lung blocks are then treated with supplemental electron fields whose beam energy and dose contribution are calculated to bring the dose to the pleural surface to the prescribed value. Figure 22.2 shows the dose distribution at the level T5–6 for a TBI treatment of 12 Gy total dose with 50% transmission blocks over the lungs and supplemented with 9 MeV electrons. The effect of the beam spoiler is not included.

High-dose TBI-containing regimens: clinical results

High-dose TBI schedules utilized

The first use of TBI, reported by Thomas et al. [1] in a twin HCT study, delivered the dose in a single fraction. Although engraftment was successful in both patients, relapse occurred within 12 weeks, suggesting that TBI alone was insufficient to prevent relapse. Since then, high-dose chemotherapy, usually with cyclophosphamide (CY), has been combined with TBI. A randomized trial found no difference in overall survival and remission between CY and TBI versus melphalan (MEL) and TBI [15]. Typical TBI schedules deliver a total dose of 10–14 Gy at 1.2 Gy three times daily (TID), 2 Gy twice daily(BID) or 3 Gy once daily (QD). A variety of TBI schedules have been evaluated to reduce toxicities, decrease relapse rates, and improve further the therapeutic index. Factors that are of radiobiologic importance include total dose, fraction schedule, fraction size, use of organ shielding, and dose rate. Only a few randomized trials have been performed and most trials involved changes in multiple variables, making determination of the optimal TBI schedule difficult.

Single-fraction versus multi-fraction schedules

Early studies compared fractionated with single-fraction schedules. Fractionation offered the potential to reduce organ toxicities without compromising tumor control, given the greater ability of most normal tissues to repair sublethal radiation damage compared with leukemia cells [9]. Retrospective analyses from the groups at Johns Hopkins University [16] and the Memorial Sloan Kettering Cancer Center [17] reported a reduction in interstitial pneumonitis (IP) with lung shielding and fractionated TBI (FTBI) (one fraction per day) or hyperfractionated (HFTBI) (multiple fractions per day) schedules compared with single-fraction TBI (STBI) and no lung shielding. An update of the Memorial Sloan Kettering Cancer Center experience by Shank et al. [18] reported a reduction in IP incidence from 50% with STBI to 4% with HFTBI and lung shielding.

Subsequent studies comparing single- versus multi-fraction schedules have reported mixed results [19–21]. Ozashin et al. [20] found no significant differences in relapse rate, survival, or incidence of IP or sinusoidal obstruction syndrome (SOS) in patients receiving 10 Gy STBI compared with 12 Gy (2 Gy BID) HFTBI. A report analyzing the experience of multiple institutions in the Children's Cancer Study Group [19] of patients with acute myeloid leukemia (AML) in first remission treated with 7.5–10 Gy STBI or 12–13.2 Gy FTBI (2 Gy BID or 1.2 Gy TID) reported a reduction in 2-year relapse rate from 23% to 0% ($p = 0.07$) in favor of HFTBI, but no significant differences in survival or incidence of IP. A single institution experience by Cosset et al. [22] observed no difference in relapse rate or overall survival, but documented a reduction in treatment related mortality (55% versus 26%) and a statistically significant reduction in incidence of IP (45% versus 13%) and graft-versus-host (GVHD) (77% versus 43%) with 13.2 Gy (1.2 Gy TID) HFTBI compared with 10 Gy STBI. Finally, a large retrospective analysis of the experience from 21 institutions in France [21] also reported a significant reduction in incidence of IP (37.5 % versus 1.7%) and GVHD (41% versus 22%) with a hyperfractionated schedule (10–13.2 Gy) compared with a single fraction of 10 Gy. However, no change in survival and an increase in relapse rate were observed with hyperfractionation (29% versus 16%, $0.5 < p < 0.1$).

Two randomized trials have compared single-fraction with multi-fraction TBI. A trial from Seattle [23] compared 10 Gy STBI with 12 Gy HFTBI at 2 Gy BID in patients with AML in first remission. HFTBI resulted in a reduction in idiopathic IP (4% versus 11%), a decrease in relapse rate (12% versus 22%), and a statistically significant increase in 5-year overall survival of 54% versus 33%. A more recent French study [24] randomized patients to receive 10 Gy STBI or 14.85 Gy HFTBI at 1.35 Gy TID and reported a statistically significant decrease in SOS (4% versus 14%) with HFTBI. No difference in IP incidence was observed between the two groups. Eight-year cause-specific and overall survival with HFTBI were 77% and 45%, respectively, compared with STBI with 63.5% and 38%, respectively. TBI schedule was an independent prognostic factor by multivariate analysis if cause-specific survival was the end-point.

In summary, most studies document a reduction in toxicities with fractionated or hyperfractionated TBI schedules. This is consistent with the radiobiologic principles described earlier. Many studies, including the two randomized trials, also document improved relapse and survival rates with multi-fraction as opposed to single-fraction schedules. The majority of TBI schedules used today are fractionated or hyperfractionated.

Total dose and dose escalation

Fractionated schedules and the resultant reduction in toxicities allow the possibility of increasing total dose and improving outcomes. The Seattle group carried out two randomized trials comparing CY combined with 12 Gy at 2 Gy/day or 15.75 Gy at 2.25 Gy/day. In a trial of 116 patients with chronic myelogenous leukemia (CML) in chronic phase, the higher dose resulted in a significantly lower relapse rate (0% versus 25%, $p = 0.008$), but higher treatment-related mortality rate (24% 12 Gy and 34% 15.75 Gy, $p = 0.13$), and as a result no significant change in overall survival [25]. In a separate report of 71 patients with AML in first remission, relapse rate was also decreased with the higher dose (14% versus 39%, $p = 0.06$), but these gains were offset by an increase in non-relapse mortality (38% versus 19%, $p = 0.05$), resulting in no difference in overall survival between the two arms [26].

Reports from other groups also suggest that higher doses have greater antileukemia effects. In several retrospective reports from the group in Genoa [27], relapse rate and survival were significantly decreased if the TBI dose was >9.9 Gy (3.3 Gy/day). In an analysis of the Center for International Blood and Marrow Transplantation Research (CIBMTR) and City of Hope Cancer Center databases, Marks et al. [28] reported that patients with acute lymphoblastic leukemia (ALL) beyond first remission receiving TBI–CY conditioning regimens had a lower relapse rate and increased disease-free survival if the TBI dose was ≥13 Gy.

Kal et al. [29] compared results of different TBI regimens published in three randomized trials, four studies comparing results of two to three TBI regimens, and nine studies reporting on one TBI regimen. Using linear quadratic principles, a biologically effective dose (BED) was calculated for each TBI regimen to normalize for differences in dose per fraction, number of fractions, and dose-rates of the different regimens. Higher BED values were associated with a lower relapse rate and higher disease-free survival and overall survival rates.

Other reports found no clear advantage of higher doses. A single institution retrospective analysis of 116 patients found an inverse correlation between overall survival and TBI doses of 10, 12 and 13.5 Gy given in six fractions over 3 days [30]. Phase I trials have been performed with increased toxicities but observed no obvious benefit with increasing TBI dose [31–33]. Peterson et al. [32] determined a maximum tolerated dose (MTD) of 16 Gy (2 Gy BID) when combined with CY 120 mg/kg. The primary dose-limiting toxicity was pneumonitis, followed by SOS, nephrotoxicity, and mucositis. Demirer et al. [31], also using a TBI–CY regimen, observed no additional benefit with higher total doses of 14.4 Gy at 1.2 Gy TID in patients with ALL and non-Hodgkin lymphoma (NHL). Alyea et al. [33] escalated dose in a TBI–CY regimen from 14 to 15.6 Gy. Acute GVHD and treatment-related mortality were higher at 15.6 Gy, but no improvement in relapse rate or overall survival was observed.

Other phase I trials have escalated TBI doses without concomitant chemotherapy. Results of a limited phase I TBI dose escalation trial without chemotherapy were reported in patients with NHL undergoing autologous HCT [34]; 50% lung attenuation blocks and blocks to limit the kidney dose to 15 Gy were utilized. The highest dose reached was 20 Gy (2 Gy BID), which was considered to be associated with acceptable toxicities. IP or SOS was seen in only one of nine patients. A dose escalation trial of TBI without concomitant chemotherapy was also carried out by the Seattle group [35]. Patients with primarily bone-only metastatic disease from a variety of hematologic and solid tumors first received high-dose chemotherapy [busulfan (BU), MEL, *N*-triethylenethiophosphoramide (thioTEPA), then a median of 105 days later underwent TBI as part of a tandem autologous HCT trial. Although 90% attenuation lung and liver blocks were utilized, the highest TBI dose achieved was only 15 Gy with grade 3 and 4 IP being dose limiting.

In summary, despite the use of fractionated schedules and organ shielding, escalation of TBI dose has been difficult owing to dose-limiting normal tissue toxicities, which have primarily been pulmonary. This has limited total doses of most conditioning regimens to approximately 16 Gy or less. Trials attempting to demonstrate improved relapse and survival rates with increasing TBI dose have reported mixed results, perhaps because the magnitude of dose escalation has been limited. Gains in disease control are associated with an increase in regimen-related toxicities and non-relapse mortality in some studies, resulting in no improvement in overall survival. New strategies are clearly needed to allow further dose escalation without an associated increase in side effects.

Fractionated versus hyperfractionated schedules and fraction size

Daily fractionated schedules have used fractions of approximately 2.25–4.67 Gy. Hyperfractionated schedules are usually delivered at 1.2–2 Gy fractions a minimum of 5–6 hours apart. In general, larger fractions are predicted to be associated with greater normal organ effects, potentially reducing the therapeutic index. To date, no randomized trials have been performed that specifically addressed these factors. The available clinical data suggest that larger daily fraction schedules are acceptable [36,37], although most schedules using ≥3 Gy fractions have limited the total dose to 9.9–12 Gy.

In a retrospective analysis of patients with high-risk NHL undergoing autologous HCT, Gopal et al. [38] compared groups treated to 10.2 Gy at 1.7 Gy BID with those treated to 12 Gy at 3 Gy per day. No differences in overall survival or in acute or late pulmonary complications were seen. The 3-year freedom from late pulmonary complications was 80% for the 10.2 Gy group and 70% for the 12 Gy group ($p = 0.45$). Three-year freedom from progression was significantly lower for the 12 Gy group (82% versus 31%, $p < 0.001$). Of note, lung shielding was not utilized for the 10.2 Gy group, but was utilized in the 12 Gy group after the third fraction. In addition, the total dose of 10.2 Gy is lower than what other hyperfractionated schedules deliver, raising the possibility that the two schedules did not deliver biologically equivalent doses. This may explain in part the differences observed.

Consistent with this are the results of Corvo et al. [39], who retrospectively reviewed their experience with patients undergoing unrelated donor HCT. Comparisons were made between patients treated to 9.9 Gy at 3 Gy per day and those treated during an earlier period to 12 Gy at 2 Gy BID. Lung shields were used in both groups. The actuarial 5-year transplant-related mortality rate was 31% for the HFTBI group and 41% for the FTBI group ($p = 0.1$). For the HFTBI group, there was a statistically significant improvement in the 5-year leukemia relapse rate (13% versus 31%, $p = 0.04$) and over all survival rate (68% versus 51%, $p = 0.01$). It was concluded that the improved results were primarily related to the higher total doses delivered with the HFTBI schedule.

Dose rate

TBI is delivered at relatively low dose rates, usually in the range 5–30 cGy/min. This is lower than those used with standard external beam radiotherapy to more localized sites in the body, which are typically greater than 100 cGy/min. Radiation delivered at a lower dose-rate can potentially be more organ sparing. However, dose-rate effects may be greatest and most clinically important primarily at very low dose-rates. Travis et al. [40] evaluated out to 1-year survival and histologic changes in mice receiving TBI at dose rates ranging from 1 to 25 cGy/min. A direct correlation was seen between dose rate and survival and also dose rate and histologic changes in lung and kidney. The dose-rate effects were greatest for dose rates ranging from 1 to 5 cGy/min. Consistent with this are the observations of Weiner et al. [41], who reported on 932 patients in the IBMTR database treated from 1978 to 1983 and observed a decrease in incidence of pneumonitis with lower dose rate but only in patients treated at less than 6 cGy/min.

The potential clinical benefits of using lower dose-rates are probably outweighed by other factors such as fractionation or organ shielding. Sampath et al. [42], in a multivariate logistic regression analysis of TBI in 1090 patients from 20 published studies, found an inverse correlation with pneumonitis incidence and fractionation, but no dose-rate effect. A preclinical study by Tarbell et al. [43] reported a higher radiation-related mortality rate in mice receiving STBI at a high dose rate (80 cGy/min) compared with STBI at low dose-rate (5 cGy/min). Dose-rate effects were not seen with BID or TID fractionation schedules, suggesting that fractionation was a greater factor in normal organ sparing than dose-rate effects.

In a multi-institution randomized trial from France [20], 157 patients were randomized to either higher or lower dose-rate groups. TBI was delivered either as a single fraction of 10 Gy or hyperfractionated to 12 Gy at 2 Gy BID, determined by physician preference and not randomization. The dose rate for the 10 Gy STBI group was 6 versus 15 cGy/min and for the 12 Gy HFTBI group 3 versus 6 cGy/min. No statistically significant difference in survival or incidence of IP or SOS was seen with dose rate or fractionation schedule. Of note, the highest estimated 4-year incidence of IP occurred in the single-fraction high dose-rate group (49.1%), compared with 29.5% in the single-fraction low dose-rate group. The incidence of cataracts was lower in the lower dose-rate groups (3.5%) compared with the higher dose-rate groups (14%).

A subsequent retrospective analysis of 326 patients by Belkacemi et al. [44] compared patients treated at low (<4.8 cGy/min), medium (4.8–9 cGy/min), and high (>9 cGy/min) dose rates, and also reported a statistically significant reduction in cataract incidence of 0% with lower dose rate compared with 27% with medium and 33% with higher dose-rates ($p = 0.0001$). A significant decrease in IP was also seen with a 48% incidence for the higher dose rate versus and a 19% incidence for the medium dose rate groups ($p = 0.05$). Survival and treatment-related mortality rates were not influenced by dose rate.

More recently, Carruthers and Wallington [45] retrospectively analyzed their institution's experience of 84 patients treated with 12 Gy TBI (2 Gy BID) at either 15 or 7.5 cGy/min. IP was observed in 43% treated at the higher dose rate compared with only 13% at the lower dose rate. A similar conclusion was reached by Beyzadeoglu et al. [46] in an analysis of 105 patients where IP incidence was greater in patients treated at a dose rate of >4 cGy/min.

Higher dose rates on the order of 30–90 cGy/min have been used with acceptable toxicities, but the total dose was limited to 5–5.5 Gy, making direct comparisons with other studies difficult [47,48]. Bredeson et al. [49] reported acceptable toxicity in 142 patients receiving TBI at 80 cGy/min. Although the majority received 5 Gy in a single fraction, 48 patients received 12 Gy at 2 Gy BID without lung shielding.

In summary, although the range of dose rates utilized for TBI is limited, reducing the dose rate within this range appears to result in a reduction in incidence of cataracts and, according to some reports, pneumonitis. This appears to occur without a detrimental affect on survival or relapse rates in most studies. The clinical data suggest that dose-rate effects can be seen with both single- and multi-fraction schedules, although data from animal models indicate a reduction in dose-rate effects with fractionation. Based on the available clinical data, the importance of dose-rate effects on TBI outcomes at levels greater than approximately 30 cGy/min is unknown.

Organ shielding

The primary dose-limiting toxicity of TBI is pulmonary. The use of shielding to reduce the total dose to lungs has been shown to decrease the incidence of pneumonitis and is now routinely used at many centers. Median lung doses with shielding have usually been limited to approximately 8–10 Gy. Shank et al. [18] reported a reduction in IP incidence from 50% with STBI and no lung shielding to 4% with HFTBI and lung shielding. Volpe et al. [50] reported a 3.8% incidence of lethal pulmonary complications if lung dose was ≤9.4 Gy compared with 14.3% if >9.4 Gy. Lawton et al. [51] noted lethal pulmonary events in 6/11 patients with 50% lung attenuation blocks compared with 22/107 if lung shielding with 60% attenuation blocks was used. A randomized trial was performed by Labar et al. [36] to assess the impact of lung shielding. Eighty two patients received a TBI dose of 12 Gy (4 Gy/day) and lung blocks if used were placed after 9 Gy. The incidence of IP decreased from 15% to 5% with the use of lung blocks.

Renal shielding has also reduced nephrotoxicity after TBI. The group at the Medical College of Wisconsin noted at 18 months a 29% incidence of nephropathy without shielding compared with 0% with kidney shielding in patients receiving 14 Gy HFTBI [52]. Another study explored the use of extensive lung and liver shielding in patients with multiple myeloma undergoing autologous HCT [53]. Patients first received induction with vincristine–adriamycin–dexamethasone (VAD), followed by TBI/BU/CY using 90% attenuation lung and liver blocks with electron boost to portions of the chest wall underlying the blocks. The total dose was 9 Gy at 1.5 Gy BID. Complete response rates of 48% and 41% were seen in patients with de novo and pretreated multiple myeloma, respectively. The transplant-related mortality rate was 2%. Grade III/IV mucositis was observed in 75% and three patients developed reversible SOS.

It was concluded that with lung and liver shielding, TBI can be combined with BU and CY with acceptable toxicity and encouraging response rates.

One group has reported a potential detrimental effect of shielding. A study by the Seattle group [54] found that shielding increased relapse rates in patients with refractory anemia undergoing allogeneic HCT. In this study, 14 patients were treated with CY and TBI to 12 Gy at 2 Gy BID (10 patients) or <12 Gy (four patients) utilizing 95% attenuation lung and right hepatic lobe blocks. The underlying chest wall was then boosted with electrons using an approach similar to that reported by Shank et al. [18]. When compared with historical controls, there was an increase in relapse rate (34% versus 2%, $p = 0.0004$) and a decrease in disease-free survival (38% versus 61%, $p = 0.16$). No difference in non-relapse mortality was seen. It was hypothesized that 95% shielding may have shielded malignant cells or reduced immunosuppression and graft versus leukemia effect.

High-dose TBI-containing regimens: normal tissue effects

Most of the published clinical experience using TBI is in combination with high-dose systemic chemotherapy. Some of the agents frequently administered with TBI include CY [17], etoposide (VP16) [55], MEL [56], cytarabine [57]. and thioTEPA [58]. These agents are cytotoxic and result in acute and late toxicities that are often similar to those with TBI, including nausea, vomiting, mucositis, esophagitis, diarrhea, hepatic dysfunction, pneumonitis, gonadal dysfunction, and second malignancy induction. Therefore, identifying toxicities and outcomes attributable solely to TBI is difficult and the published literature more accurately represents the clinical results with TBI-containing regimens as opposed to TBI alone.

In summaries of the HCT literature, it is very common to compare the outcomes for single-fraction with those for fractionated dose schedules without regard to the total dose or dose per fraction. In virtually every normal tissue at risk in radiation therapy, one can reduce the morbidity by increasing fractionation or, equivalently, decreasing the dose per fraction. Hence simply to ask whether fractionation ameliorates the side effects is not sufficient, since it can be assumed a priori. The more relevant questions are whether there is a demonstrable dose response, how much benefit there is to fractionation, whether there is added benefit to hyperfractionation, and what comorbidities or other treatments may impact the response.

There are numerous side effects and complications from HCT. In this section, we address primarily those complications that limit the dose of radiation that can be given. Other complications that may be due at least in part to the radiation, but either occur at a low incidence for most transplant regimens, are not associated with sufficient morbidity to limit the dose, or do not appear to demonstrate a dose response, are mentioned but are not discussed in detail.

Acute toxicities associated with high dose TBI-containing regimens

Fractionated (one fraction per day) or hyperfractionated (multiple fractions per day) TBI schedules, compared with single-fraction schedules, result in reduced acute toxicities, and are better tolerated [18,20,44]. Nausea and vomiting are the most common side effects observed, occurring in approximately 35–50% of patients [20,59,60]. In studies conducted prior to the use of modern antiemetics, Spitzer et al. [61] reported emesis in all patients after the first 1.2 Gy fraction of hyperfractionated TBI, but a decline with subsequent fractions. Buchali et al. [59], using a 12 Gy hyperfractionated regimen, reported an increasing incidence of emesis with each 1.5 Gy fraction up to the third fraction, with a constant incidence with subsequent fractions. Antiemetics such as ondansetron and granisetron can significantly reduce the incidence and severity of emesis [62,63].

Transient parotiditis occurs in approximately 10–30% of patients undergoing fractionated TBI with a higher incidence (25–75%) associated with single-fraction schedules [59,60]. The onset is usually after the first fraction, with resolution over the next 24–48 hours. Xerostomia and xerophthalmia can occur in approximately 15–30% of patients [59,60].

Mucositis is also observed in approximately 40–100% of patients in the days after completing TBI and can be influenced by the chemotherapy used. For example, mucositis is a primary acute toxicity with etoposide-containing regimens [55]. Mucositis observed with TBI combined with high-dose MEL limited the feasibility of this combination [64]. Diarrhea can also occur in the days after completing TBI in 10–35% of patients. Other acute side effects include fatigue, decreased appetite, erythema, esophagitis, and alopecia.

More recent studies provide some indication of the acute toxicities primarily related to TBI alone. Zaucha et al. [35], in a phase I dose escalation trial, treated patients with a tandem autologous HCT approach, first with high-dose BU, MEL, and thioTEPA, followed a median of 105 days later by TBI to a total dose of 12, 13.5, or 15 Gy at 1.5 Gy BID. Acute toxicities were primarily grade 1–2 (by Bearman regimen-related toxicity scale [65]) with mucositis occurring in 100%. Gastrointestinal, skin, renal, and hepatic grade 1–2 acute toxicities occurred in 34, 19, 9, and 9%, respectively. In a limited phase I dose escalation study, McAfee et al. [34] reported mucositis in 88% and nausea in one-third of patients receiving 16, 18, or 20 Gy TBI alone at 2 Gy BID.

GVHD is known to increase the risk of some of these complications, and can occur with both TBI- and non-TBI-containing conditioning regimens. In some clinical studies TBI does not appear to increase the risk of GVHD over non-TBI conditioning regimens [66–68], although one randomized trial reported a greater incidence of acute GVHD with TBI–CY versus BU–CY [69]. Development of GVHD is probably related more to the intensity of the regimen rather than to TBI itself. In experimental models, the incidence of GVHD is increased with higher TBI doses [70] or with the addition of CY to TBI [71]. The initiating event is thought to involve damage to the intestinal mucosa by the conditioning regimen, resulting in release of lipopolysaccharide into the circulation, release of TNF-α, and subsequent increase in expression of host major histocompatibility antigens, enhancing recognition by donor T cells.

Lung: interstitial pneumonitis

IP is a clinically important complication of TBI in patients undergoing HCT. Kim et al. defined IP as "a condition in which a patient develops interstitial pulmonary infiltrates on chest X-ray with or without patchy alveolar infiltrate. The patient produces scanty non-purulent sputum which is negative in culture for bacterial and fungal infection" [72]. Experimental and clinical data have confirmed that fractionated TBI is less toxic to the lung than single-dose TBI [16,18,22]. Fractionated doses above 15 Gy [73] are associated with higher incidences of IP. IP also occurs in conditioning regimens that do not include TBI at all. BU alone is associated with a crude incidence of IP of 4–10%. It is known that chemotherapy, particularly using alkylating agents given with TBI as part of the bone marrow transplantation (BMT) procedure, contributes to pulmonary

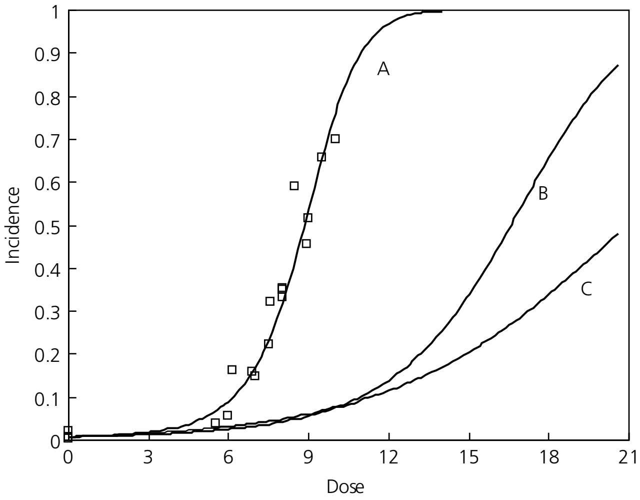

Figure 22.3 Dose response for IP. Curve A is the single-fraction dose response with data from Sampath et al. Curve B is the five-fraction dose response. Curve C is the dose response for 2 Gy per fraction.

toxicity. This makes it difficult to distinguish TBI toxicity from other etiologies of lung damage [38].

To investigate the radiation dose response and the other factors that may affect the incidence of IP, Sampath et al. reviewed the literature, selecting papers that included sufficient data for a multivariate analysis (MVA) including dose, dose rate, chemotherapy regimen, and other variables [42]. The final number of articles included was 20, with a total of 26 TBI/chemotherapy conditioning regimens. Five regimens had no TBI and four had no chemotherapy (upper half body treatments). These figures reflect only those regimens in which patients received *daily* fractionation. The data for multiple fractions per day had too much dispersion to be fitted by logistic regression. Figure 22.3 shows the dose response obtained from MVA, illustrating the dose-response functions for one fraction, five fractions, and for daily fractions at 2 Gy per fraction. These dose response functions reflect the response to radiation only. The addition of CY and many other chemotherapeutic agents is associated with higher rates of IP. The findings of Sampath et al. indicated that there was no dose-rate effect for IP on MVA. The odds ratio for 120 mg/kg of cyclophosphamide was 5.9. The α/β ratio (from the LQ model) was 2.8 Gy.

The low value of the α/β ratio indicates that IP is significantly diminished with increasing fractionation. However, direct analysis of data from hyperfractionation regimens has not been possible. This could be due to the complex repair of sublethal damage to the lung. There appears to be a slow repair component that would at least partially offset the beneficial effects of hyperfractionation [74].

Other pulmonary side effects include idiopathic pneumonia, radiation fibrosis, and diminished lung function.

Renal toxicity

Renal damage is expressed up to about 2 years after treatment. It is characterized by reduced filtration rates, increased BUN and creatinine, proteinuria, hypertension, and anemia. Ozsahin et al. found no difference in renal toxicity among groups in a randomized trial comparing 10 Gy single fractions to 12 Gy in six fractions and using two different dose rates [20].

In a study combining literature reports of renal damage after TBI, Kal and van Kempen-Harteveld reported their findings regarding the dose response after assuming an α/β ratio of 2.5 Gy and a repair half-time of 2 hours [75]. However, fractionation parameters were not analyzed, but rather assumed, and no accounting was made for regimens in which highly nephrotoxic drugs were used. Using 24 regimens from 12 studies, Cheng et al. [76] investigated the impact of total dose, dose rate, dose per fraction, number of fractions, fractions per day, interfraction interval, age (pediatrics versus adults versus mixed), and chemotherapy on renal toxicity following HCT. A dose response was seen in the adult population but not in the pediatric population. Dose rate was significant but only below 10 cGy/min. The commonly used schedule of six times 2 Gy is associated with a renal toxicity rate of about 10%. Teniposide and cyclosporine were associated with relative risks of 10.5 and 5.9, respectively, in pediatric patients; fludarabine had a relative risk of 6.2 for adults Although Kal and van Kempen-Harteveld [75] estimated the tolerance dose for the kidney in TBI to be approximately 16 Gy, Cheng et al. found the dose associated with 10% incidence of late renal toxicity to be 10–12 Gy [76].

Sinusoidal obstructive syndrome

Sinusoidal obstructive syndrome (previously termed veno-occlusive disease) is a dose-related and potentially fatal complication of the HCT conditioning regimen. It is much more common in regimens where the TBI is replaced with BU [66–68]. However, even with TBI regimens, the incidence can be up to 10%. Although a lower incidence is generally found with fractionated radiation [24], the dependence on radiation dose is modest. Only one case (11%) was seen in nine patients treated with doses of 16–20 Gy in 2 Gy fractions in a phase I dose escalation study [34], and Ozsahin et al. found six cases in 100 patients treated with 12 Gy in six fractions [20]. There appears to be no dose-rate dependence for fractionated treatment, although there is some evidence for a single-fraction dose-rate effect below 7 cGy/min [77].

Cataract formation

Cataract formation occurs up to 10 years after HCT, and the cumulative incidence can reach 100% [78]. A dose response for cataract formation was shown by van Kempen-Harteveld et al. in a multi-institutional study [78]. (Although they assumed a dose-rate effect, statistical analysis presented in their paper argues against it.) They reported an α/β ratio of 0.75 Gy, indicating that cataractogenesis should be very sensitive to fractionation. In addition, treatment with steroids (for GVHD) and heparin shifted the dose–response curve to the left, but they did not determine relative risks associated with these drugs. This study has the advantage that incidence of cataracts was determined using actuarial methods rather than crude incidence. Because of severe censoring, using crude incidence may not produce reliable results. In a different study, van Kempen-Harteveld et al. showed that shielding the lens in the anterior field reduced the incidence and severity of cataract formation while increasing the latency without increasing central nervous system (CNS) recurrence [79].

For CML, Socié et al. reported a hazard ratio of 2.3 for TBI-associated cataractogenesis compared with BU regimens in a summary analysis of four randomized trials [68]. However, in AML, there was no difference in the two conditioning regimens. Also, chronic GVHD was associated with a hazard ratio of 2.6, but only for CML. Belkacemi et al. [44] and Ozsahin et al. [20] found a protective effect for dose rates lower that 4 and 6 cGy/min, respectively.

Second malignancy induction

There is no question that ionizing radiation can induce carcinogenic transformations. However, the risk of second solid tumors resulting from TBI has been overstressed in the literature. In a study of more than 19,000 HCT patients, an excess of 50 cancers were seen [80]. Even if every one of these was attributable to radiation, the absolute incidence is less than 0.3%. In fact, in MVA of these data, only fractionated radiation to TBI doses higher than 14 Gy and limited-field irradiation were associated with a significantly increased incidence of invasive solid tumors. In two separate single-institution analyses of over 4200 patients, TBI was not significantly related to induction of second solid tumors on MVA, despite the inclusion of basal cell cancers, accounting for nine out of the 46 solid malignancies [81,82]. It is clear that the incidence of radiation-induced solid malignancies from TBI, although occurring, is just barely above the background and can be detected only in populations on the order of 10^4 or higher. The risk is apparently higher among children than adults [83].

There is continued debate as to whether TBI contributes to the risk of developing MDS after autologous HCT. In addition to TBI, other possible contributing factors include the amount and type of pretransplant chemotherapy, pretransplant radiotherapy, the specific conditioning regimen, stem-cell priming regimens, source of stem cells, selection criteria used for TBI, and length of follow-up between TBI- and non-TBI-treated cohorts. These factors are difficult to control for in any retrospective analysis. A number of retrospective studies have reported TBI as an independent risk factor for MDS in patients with NHL [84,85] and Hodgkin lymphoma [86]. However, Krishnan et al. [87], in a retrospective analysis of 218 patients at City of Hope Cancer Center, did not find TBI to be an independent risk factor. Only priming with etoposide was a risk factor. Harrison et al. [88], in a case-controlled study of 595 patients with Hodgkin disease, found extent of pretransplant therapy and pretransplant exposure to lomustine or the combination of mechlorethamine, vincristine, procarbazine and prednisone to be risk factors, but not autologous HCT.

Other late effects of TBI may include growth retardation, neurocognitive effects, gonadal dysfunction, and late cardiomyopathy. Because of the highly censored population and the possibility of competing risks, analysis of the contribution of TBI to very late effects is extremely difficult.

High-dose TBI versus non-TBI regimens

Multiple randomized trials and non-randomized studies have been performed comparing TBI- versus non-TBI-containing high-dose conditioning regimens. Most studies have compared TBI–CY with BU–CY regimens. Results of select randomized trials are summarized in Table 22.1.

A number of randomized trials demonstrate an advantage for TBI-containing regimens. Blaise et al. [89] randomized patients with AML in first complete remission to TBI–CY (120 mg/kg) or

Table 22.1 Select randomized trials comparing TBI- with non-TBI-containing high-dose HCT conditioning regimens

Study (first author, year)	Patient No. Disease type	Randomization	TBI dose and schedule	Relapse rate	Survival	Toxicities
Blaise 1992 [89]	101 AML CR1 Allogeneic HCT	TBI–CY BU–CY	12 Gy 2 Gy BID Most patients	14% TBI–CY 34% BU–CY ($p < 0.04$)	2 year DFS: 72% TBI–CY/47% BU–CY ($p < 0.01$) OS: 75% TBI–CY/51% BU–CY ($p < 0.02$)	TRM: 8% TBI–CY/27% BU–CY ($p <0.06$)
Dusenbery 1995 [66]	35 AML CR1 and >CR1 Autologous HCT	TBI–CY BU–CY	13.2 Gy 1.65 Gy BID	2 year 43% TBI–CY; 70% BU–CY ($p = 0.17$)	2 year DFS: 50% TBI–CY/24% BU–CY ($p = 0.12$) If >CR1 DFS: 42% TBI–CY/9% BU–CY ($p = 0.06$) OS: 46% TBI–CY/35% BU–CY ($p = 0.41$)	IP no difference SOS: 0/18 TBI–CY; 3/17 BU–CY
Ringden 1994 [91]	167 AML, ALL, CML, NHL CR1, CP1, advanced Allogeneic HCT	TBI–CY BU–CY	11.3–12 Gy 3–7 fractions		3 year DFS: 67% TBI–CY/56% BU–CY Difference in advanced disease patients 49% TBI–CY/17% BU–CY ($p = 0.005$)	TRM: 62% BU–CY/12% TBI–CY ($p = 0.04$) IP no difference, but significant increase in SOS, cystitis, seizures, obstructive bronchiolitis with BU–CY
Bunin 2003 [93]	43 ALL CR1, CR2, CR3 Allogeneic HCT	TBI–CY-VP16 BU-CY-VP16	12 Gy 2 Gy BID		3 year DFS: 58% TBI–CY–VP/29% BU–CY–VP ($p = 0.03$) OS: 67% TBI–CY–VP/47% BU–CY–VP ($p = 0.04$)	
Clift 1994 [69]	142 CML CP Allogeneic HCT	TBI–CY BU–CY	12 Gy 2 Gy QD	3 year 0.13 BU–CY; 0.13 TBI–CY ($p = 0.43$)	3 year EFS: 0.7 TBI–CY/0.66 BU–CY ($p = 0.36$) OS: 0.8 TBI–CY/0.8 BU–CY	Non-relapse mortality no difference 0.24 TBI–CY/0.18 BU–CY Hospital LOS, aGVHD, elevated creatinine greater with TBI–CY
Devergie 1995 [95]	120 CML CP Allogeneic HCT	TBI–CY BU–CY	10 Gy single fraction; 11–12 Gy Most 2 Gy BID	4.4% BU–CY 11.1% STBI 31.3% HFTBI ($p = 0.039$)	5 year DFS no difference 55% TBI–CY/59.1% BU–CY 5 year OS no difference 62.9% TBI–CY/60.6% BU–CY	TRM: 29% TBI–CY/38% BU–CY
Blume 1993 [97]	122 AML, ALL, CML >CR1 or >CP1 Allogeneic HCT	TBI–VP16 BU–CY	13.2 Gy 1.2 Gy TID		No difference between arms	No differences between arms

OS, overall survival; DFS, disease-free survival; EFS, event-free survival; CR1, first complete remission; >CR1, beyond first complete remission; CR2, second complete remission; CR3, third complete remission; CP1, first chronic phase; >CP1, beyond chronic phase; TRM, treatment-related mortality; SOS, sinusoidal obstructive syndrome; IP, interstitial pneumonitis; LOS, length of stay.

BU (16 mg/kg)–CY (120 mg/kg). A statistically significant improvement in 2-year disease-free survival (72% vs. 47%), overall survival (75% vs. 51%), relapse rate (14% vs. 34%), and transplant-related mortality (8% vs. 27%) was observed in favor of TBI–CY. In MVA, BU–CY was associated with higher relapse rates and lower survival. A subsequent update with a median follow-up of 10.8 years continued to demonstrate an advantage to TBI–CY [90].

Dusenbery et al. [66] also observed a possible advantage to TBI–CY (120 mg/kg) compared with BU (16 mg/kg)–CY (200 mg/kg) in patients with AML. For patients in first complete remission there was no difference in estimated 2-year disease-free survival. However, for patients beyond first complete remission, a trend towards improved disease-free survival was observed with TBI–CY (42% vs. 9%, $p = 0.06$). Acute toxicities and IP incidence were similar for the two regimens. None of the 18 TBI–CY patients developed SOS, compared with 3 of 17 receiving BU–CY.

The Nordic Bone Marrow Transplantation Group also demonstrated an advantage for TBI–CY over BU–CY, especially in patients with advanced disease [91,92]. Patients with AML, ALL, and CML in first remission, first chronic phase, and in more advanced phases were entered. There was no significant difference in 3-year disease-free or overall survival between the two arms when looking at the entire population. However, for patients with high-risk disease (beyond first remission or first chronic phase), TBI–CY compared favorably with BU–CY, resulting in a higher disease-free survival (61% vs. 18%, $p = 0.005$) and overall survival (66% vs. 21%, $p = 0.002$). In addition, TBI–CY was associated with a significantly lower incidence of SOS, obstructive bronchiolitis, hemorrhagic cystitis, seizures, and transplant-related mortality compared with BU–CY. It was concludes that TBI–CY is the treatment of choice, especially in patients with advanced disease. A later update with 5–9 years follow-up reached similar conclusions [90].

Bunin et al. [93] randomized children with AML to TBI or BU (16 mg/kg) combined with CY (120 mg/kg) and VP16 (16 mg/kg). Event-free survival at 3 years was 58% for the TBI group and 29% for the BU group ($p = 0.03$). The differences were primarily seen in those receiving HCT from unrelated donors.

Other randomized studies have not observed a significant advantage for TBI- over non-TBI-containing regimens. The Seattle group [69,94] reported comparable efficacy and non-relapse mortality between TBI–CY and BU–CY for patients with CML in chronic phase. BU–CY was better tolerated with a shorter length of hospital stay and lower incidence of acute GVHD. A multi-institution randomized study from France also compared TBI–CY with BU–CY in patients with CML in chronic phase [95], and observed comparable efficacy and transplant-related mortality between the two arms. Finally, a randomized trial performed by the Southwest Oncology Group compared TBI–VP16 with BU–CY and found no difference in efficacy in patients with AML, ALL, and CML beyond first complete remission or chronic phase.

Hartman et al. [96] performed a meta-analysis of five published randomized trials [69,89,91,95,97]. Survival and disease-free survival were better with TBI-based regimens compared to BU–CY but the differences were not statistically significant. A significantly greater incidence of SOS was observed with the BU–CY regimens. It was concluded that TBI regimens were at least as good as BU–CY regimens. Recently, Gupta et al. [98] performed a meta-analysis of seven randomized HCT trials involving 730 patients with leukemia randomized between BU–CY and TBI–CY. TBI–CY was associated with a modest but non-significant reduction in all cause mortality and relapse rates.

Socié et al. [68] analyzed the long-term outcome of 316 patients with CML and 172 patients with AML originally entered in four of the randomized trials described above [69,89,91,95]. Mean follow-up on the study was over 7 years. For patients with CML, there was no significant difference in 10-year overall survival or disease free-survival. For patients with AML, a trend favoring TBI regimens was observed with projected 10-year overall survival (63% vs. 51%, $p = 0.068$) and disease free survival (57% v . 47%, $p = 0.051$). Disease-free survival rates were similar if analysis was limited to AML in first remission. Late complications were similar for the two regimens, with the exceptions of a higher incidence of cataracts with TBI–CY and permanent alopecia with BU–CY.

Multiple non-randomized studies in acute and chronic leukemia have also compared TBI and non-TBI regimens, with several trials demonstrating superior efficacy with TBI regimens. Table 22.2 summarizes results from selected trials.

A limited number of studies have also compared TBI and non-TBI high-dose regimens in patients with multiple myeloma and reported no advantage to TBI [64,99]. The French Myeloma Intergroup performed a prospective randomized trial [64]. Patients with newly diagnosed multiple myeloma were randomized after VAD chemotherapy to 8 Gy TBI (2 Gy QD) and MEL (140 mg/m^2) versus MEL (200 mg/m^2). Median duration event-free survival was similar (21 vs. 20.5 months). In an analysis of the Spanish Registry for Transplantation in Multiple Myeloma [99], similar efficacy and treatment-related mortality (TRM) rates were observed for the three most common regimens used: TBI (8–12.5 Gy)/MEL (140 mg/m^2), MEL alone (200 mg/m^2), and BU (12 mg/kg)–MEL (140 mg/m^2).

In summary, several reports, including at least four randomized studies, demonstrate or suggest increased efficacy and/or decreased toxicity with TBI-containing compared with non-TBI-containing high-dose conditioning regimens, usually BU–CY, particularly in patients with acute leukemia. Most of these studies were carried out prior to patient-specific dosing of BU. Other studies demonstrate comparability between the two approaches. With respect to toxicity and treatment-related mortality rates, high-dose chemotherapy alone regimens, such as BU–CY, offer no clear advantage over TBI regimens and can be associated with a higher rate of SOS, seizures, obstructive bronchiolitis, and hemorrhagic cystitis [89,91].

TBI and reduced-intensity conditioning (RIC) regimens

Older patients or patients with comorbidities are often not able to tolerate standard myeloablative, full-intensity TBI-containing regimens. In some centers, full-intensity TBI-containing regimens are limited to patients younger than approximately 50–55 years of age. As a result, non-myeloablative and RIC regimens are being actively investigated. These regimens are usually associated with fewer acute toxicities, and are primarily used as a method of immunosuppression to allow engraftment of donor cells and the subsequent graft-versus-tumor (GVT) effects.

Both TBI-containing and non-TBI-containing regimens are being investigated. Low to intermediate TBI doses have been used. McSweeney et al. [107], using 2 Gy TBI combined with cyclosporine and mycophenolate mofetil in 45 patients, reported reduced regimen-related toxicities compared with standard high-dose regimens, a survival rate of 66.7%, and non-relapse mortality of 6.7%. However, non-fatal graft rejection was seen in 20%. As a result, the purine analog fludarabine (FLU) was added to the regimen to

Table 22.2 Select non-randomized studies comparing TBI- with non-TBI-containing high-dose HCT conditioning regimens

Study (first author, year)	Patient No. Disease type	Study design	TBI regimen	Non-TBI regimen	Relapse rate	Survival	Toxicities	Study conclusion
Inoue 1993 [100]	123 AML CR1 Allogeneic HCT	Japanese BMT registry analysis	TBI–CY ± other chemotherapy 10–13.2 Gy, most 12 Gy at 2 Gy BID	BU–CY ± other chemotherapy	2 year 16% TBI/39% non-TBI ($p = 0.197$)	2 year OS 77% TBI 51% non-TBI ($p = 0.001$)	IP no difference 21% TBI 24% non-TBI ($p = 0.08$)	TBI regimens superior
Ringden 1996 [67]	921 AML, ALL Most CR1 Allogeneic, autologous HCT	Matched-pair analysis EBMT database	TBI–CY	BU–CY	2 year No difference except ALL intermediate risk autologous HCT 82% BU–CY/62% TBI–CY ($p = 0.002$)	2 year DFS No difference except ALL intermediate risk autologous HCT 14% BU–CY v 34% TBI–CY ($p = 0.002$)	Increased SOS ($p < 0.05$) and cystitis ($p < 0.001$) with BU–CY	No difference except TBI better for intermediate-risk ALL
Davies 2000 [101]	627 Pediatric ALL CR1, CR2, >CR2 Allogeneic HCT	IBMTR database analysis	TBI–CY	BU–CY	35% TBI–CY/41% BU–CY ($p = 0.07$)	3 year DFS 50% TBI–CY/35% BU–CY ($p = 005$) 3 year OS 55% TBI–CY/40% BU–CY ($p = 0.003$)	TRM 15% TBI–CY/23% BU–CY ($p = 0.02$)	TBI–CY superior
Granandos 2000 [102]	156 ALL CR1, CR2, >CR2 Allogeneic, autologous HCT	Two-institution retrospective	TBI–CY 12 Gy 3 Gy QD or 2 Gy BID	BU–CY	3 year 47% TBI–CY/71% BU–CY ($p = 0.01$)	6 year EFS 43% TBI–CY/22% BU–CY ($p = 0.01$)	TRM 17% TBI–CY/22% BU–CY ($p = 0.24$)	TBI–CY superior
Gutierrez-Delgado 2001 [103]	351 Follicular lymphoma CR1, CR2, relapse Autologous HCT	Single-institution retrospective	TBI–CY–VP16 12 Gy 1.5 Gy BID	BU MEL ThioTEPA	5 year 49% TBI–CY–VP16/42% BU/MEL/thioTEPA	5 year EFS 32% TBI–CY–VP16 34% BU-MEL–thioTEPA	TRM 16% TBI–CY–VP16 21% BU-MEL–thioTEPA	No difference
Litzow2002 [104]	581 AML CR1 Allogeneic HCT	IBMTR analysis	TBI–CY 12 Gy	BU–CY	5 year 12% TBI–CY; 19% BU–CY ($p = 0.042$)	No difference 5 year OS 60% TBI–CY/55% BU–CY ($p = 0.579$) LFS 58% TBI–CY/54% BU–CY ($p = 0.438$)	CNS relapse 0% TBI–CY/3% BU–CY SOS 6% TBI–CY/13% BU–CY (p= 0.009) TRM no difference 30% TBI–CY/27% BU–CY	Decreased relapse with TBI–CY but no difference in LFS and OS
Mattsson 2003 [105]	180 AML, ALL, CML, MDS Allogeneic HCT	Single-institution retrospective	TBI–CY 10–12 Gy	BU–CY	444% FTBI; 39% STBI; 16% BU–CY ($p = 0.01$)	3 year OS 77% BU–CY 55% STBI 59% FTBI ($p = 0.04$)	TRM no difference	BU–CY superior
Scott 2004 [106]	128 MDS, AML Allogeneic HCT	Single-institution retrospective	TBI–BU 12 Gy 2 Gy BID	BU–CY	3 year 26% TBI–BU 44% BU–CY	No difference DFS 24% TBI–BU 19% BU–CY	3 year NRM 50% TBI–BU 37% BU–CY	BU–CY less toxic but with increased relapse rate

OS, overall survival; DFS, disease-free survival; EFS, event-free survival; LFS, leukemia-free survival; CR1, first complete remission; >CR1, beyond first complete remission; CR2, second complete remission; CR3, third complete remission; CP1, first chronic phase; >CP1, beyond chronic phase; TRM, treatment-related mortality; NRM, non-relapse mortality; SOS, sinusoidal obstructive syndrome; IP, interstitial pneumonitis; LOS, length of stay; STBI, single-fraction TBI; FTBI, fractionated TBI; EBMT, European Group for Blood and Marrow Transplantation.

increase immunosuppression and significantly reduced the rate of graft rejection [108].

Sorror et al. [109] retrospectively compared myeloablative regimens with RIC regimens (TBI 2 Gy with or without FLU) in chronic lymphoid leukemia (CLL) and lymphoma and found lower non-relapse mortality and higher overall survival rates with RIC regimens in patients with comorbidities. In patients without comorbidities, non-relapse mortality rates and overall survival rates were comparable between RIC and myeloablative regimens.

A large, multi-center, retrospective analysis of 834 patients who received related or unrelated donor transplantation following 2 Gy TBI alone or with FLU reported a 3-year relapse incidence of 25, 40, and 57% for low-, standard-, and high-risk groups, respectively [110]. It was concluded that this regimen was best for patients with low tumor burden and slow-growing tumors. Patients with high-risk disease and greater tumor burden may benefit from cytoreductive therapy before undergoing this RIC regimen.

Intermediate TBI doses of 4–9 Gy have also been evaluated as part of reduced-intensity regimens with reported success [111–115]. For example, Russell et al. [116], in a retrospective analysis, compared outcomes in AML patients between FLU, IV BU, and TBI 400 cGy (200 cGy/day × 2) (90 patients) and FLU–BU alone (89 patients). The addition of TBI resulted in a decrease in relapse rate and an increase in disease-free survival and overall survival rates, with the difference reaching statistical significance for the high-risk group. Non-relapse mortality was the same between groups.

Belkacemi et al. [117] retrospectively analyzed the European Group for Blood and Marrow Transplantation (EBMT) experience of 130 patients administered reduced-intensity regimens. A TBI dose of 2 Gy was administered in 78% and 4–6 Gy in 22%. The median dose rate was 14.3 cGy/min (range 6–16.4 cGy/min). TBI parameters did not have a statistically significant impact on outcome, possibly owing to the small number of patients in each subset. TRM incidence was 43% for dose rates ≥14.3 cGy/min and 29% for dose rates <14.3 cGy/min ($p = 0.09$).

The group at the M.D. Anderson Hospital in Houston combined 9 Gy TBI (3 Gy QD) with a reduced-intensity regimen of FLU (120 mg/m^2) and MEL (140 mg/m^2) in 29 patients with advanced pediatric hematologic malignancies [114]. The TBI dose used is close to that used in some high-dose regimens [27] and therefore begins to blur the distinction between myeloablative and reduced-intensity regimens. However, assuming an α/β ratio of 2.8 Gy based on lung toxicity [42], this regimen is the biologic equivalent of only 10.9 Gy when compared with TBI schedules delivered at 2 Gy per fraction, which usually go to a total dose of 12–16 Gy. Grade 1–2 oral mucositis and diarrhea were the most common toxicities. Although 21 donors were unrelated, only one patient had graft failure. The TRM rate was 24%. It was concluded that this regimen was fairly well tolerated, but also pointed out that the same regimen was not tolerated in adults in a separate trial forcing study closure.

In summary, low to intermediate doses of TBI have been successfully integrated into RIC regimens with encouraging results and continue to be actively investigated, especially in older patients and those with comorbidities unable to tolerate myeloablative regimens. The optimal TBI dose in RIC regimens remains to be determined. Compared with myeloablative regimens, RIC regimens result in lower TRM rates, but higher relapse rates, particularly for patients with high-risk disease. Efforts to improve results using higher TBI doses will prove challenging in this patient population owing to increased treatment-related morbidities. More targeted forms of radiotherapy delivery associated with lower toxicity are now being investigated as part of RIC regimens [6,118].

Total lymphoid irradiation (TLI)

TLI has been used instead of TBI for patients undergoing allogeneic HCT with malignant and non-malignant conditions such as aplastic anemia and thalassemia [119–121]. TLI provides the needed immunosuppression with reduced dose to critical organs compared with TBI. Mantle and inverted Y fields, which cover the major central lymphoid regions, are often utilized. As with TBI, TLI is often combined with chemotherapy such as CY or BU–CY. TLI (approximately 6–8 Gy) has also been combined with standard doses of TBI in an effort to increase immunosuppression and engraftment further, especially in patients receiving T-cell-depleted donor marrow [122,123].

The group at Stanford University has evaluated the use of fractionated non-myeloablative TLI and antithymocyte globulin (ATG) as an immunomodulatory strategy to reduce the incidence of acute GVHD and promote tolerance in patients receiving allografts [124,125]. This novel approach takes advantage of the immune system's regulatory T cells and the relative radioresistance of regulatory natural killer T (NKT) cells compared with conventional T cells [126]. NKT cells prevent acute GVHD by inhibiting proliferation and cytokine secretion of CD4$^+$ and CD8$^+$ donor T cells without affecting GVT activity [127]. Secretion of interleukin-4 by NKT cells appears to be involved [128]. Initial preclinical studies in mice demonstrated that pre-transplant non-myeloablative doses of TLI resulted in chimerism and tolerance to allogeneic bone marrow and skin grafts without the development of GVHD [129]. In larger animal models, prolonged tolerance to mismatched heart allografts was observed when lower doses of pre-transplant TLI combined with antithymocyte serum was administered [130]. TLI and antithymocyte serum delivered in the post-transplant setting also resulted in donor-specific tolerance of mismatched heart transplants if same donor bone marrow was also infused immediately after TLI [131].

TLI strategies developed in preclinical models have demonstrated promise in the clinic. In an initial report, 37 patients with lymphoid malignancies or acute leukemia received 8 Gy fractionated TLI (80 cGy × 10 fractions over 11 days) and ATG followed by HLA-matched mononuclear cells from related and unrelated donors. Only 2 of 37 had acute GVHD. Seven of 33 had extensive chronic GVHD. Of 18 patients with lymphoid malignancies and measurable disease at the time of transplant, 12 achieved complete remission without acute GVHD. An increase in the median percentage of NKT cells and a marked reduction in the response to alloantigenic stimulation of donor CD4$^+$ T cells were also observed. In an update of 111 patients (64 lymphoid and 47 myeloid), acute GVHD (grades 2–4) were 2% and 10% in patients receiving related and unrelated donor grafts, respectively [132]. Cumulative chronic GVHD was 27%. About 69% of patients were taken off immunosuppressive drugs (1–7 years). Consistent with observations from preclinical studies, there was a median decrease of CD3$^+$, CD4$^+$, and CD8$^+$ T cells of 191-, 180-, and 268-fold, respectively, but only an 8.6 - fold decrease in NKT cells. Overall survival and event-free survival at 36 months were 60% and 40%, respectively. Outcomes were best in patients with NHL in complete remission (CR) or partial remission (PR), AML in CR1 or CR2, and those who achieved complete donor chimerism.

Tolerance induction after TLI and ATG has also been demonstrated in kidney transplant recipients. Sixteen patients were conditioned with TLI (80 or 120 cGy × 10 fractions) and ATG in the first 10 days after kidney transplantation followed by infusion of donor CD34$^+$ hematopoietic progenitor cells and 1×10^6/kg T cells on day 11. Fifteen patients developed chimerism without GVHD and eight were withdrawn from antirejection immunosuppressive drugs for 1–3 years. Higher ratios of CD4$^+$CD25$^+$ regulatory T cells and NKT cells compared with conventional naïve CD4$^+$ cells were observed in all patients [133].

Involved field radiotherapy

Involved field radiotherapy (IFRT) to sites of initial involvement is often utilized in patients with Hodgkin and non-Hodgkin lymphoma undergoing HCT. This is based on the fact that locoregional disease, particularly if bulky on initial presentation, can often be a site of relapse after systemic therapies. Most patients who relapse after high-dose regimens recur in sites of initial disease [134–136]. IFRT to involved sites will reduce relapse rates. For example, Poen et al. [134] reported on 100 patients with Hodgkin disease transplanted at Stanford. Of the 32 patients who relapsed, 22 relapsed at sites involved prior to transplant. In the 49 patients who received IFRT, 3-year freedom from relapse was 100% compared with 67% ($p = 0.04$) for the 13 who did not receive IFRT. Moskowitz et al. [137] combined 18 Gy IFRT and 18 Gy TLI prior to high-dose chemotherapy and autologous HCT in patients with refractory of relapsed Hodgkin disease. Event-free survival was 68% and only 18% relapsed in previously irradiated areas. Similar findings were seen in patients with NHL undergoing HCT [136]. Hoppe et al. [138] delivered IFRT prior to autologous HCT in 164 patients with relapsed or refractory diffuse large-cell lymphoma. Of 67 patients who relapsed, only 10 relapsed completely in the IFRT field. The group at the University of Rochester transplanted 164 patients with refractory or recurrent large B-cell NHL, of whom 85 received IFRT after autologous HCT, and noted a 10% reduction in local failure rate and a significant increase in overall and disease-specific survival in the IFRT group.

Other studies have cautioned against combining TBI with IFRT to the mediastinum and thorax owing to an increased risk of IP [139]. Friedberg et al. [140], in a large retrospective analysis of 552 patients with NHL undergoing TBI and high-dose chemotherapy conditioning regimens prior to autologous HCT, compared outcomes between the 152 patients who received IFRT prior to conditioning regimen with the 400 patients who did not. IFRT resulted in a significantly higher rate of secondary myelodysplasia (12.5% vs. 5.8%) and, in those receiving IFRT to the mediastinum and axilla, a significant increase in late respiratory death (25% vs. 5%). There was no difference in progression-free survival or acute treatment-related mortality with IFRT. However, Song et al. [141], in a phase I study of 21 patients with lymphoma, observed no significant pulmonary toxicity with IFRT given 1 week prior to 12 Gy TBI. Others have successfully combined TLI with TBI with no reported significant additional toxicity [122,123].

Targeted total body irradiation and future directions

A dose–response relationship has long been recognized for many types of hematologic malignancies [142–144]. TBI dose escalation in patients with AML and CML has resulted in lower relapse rates [25], but these were accompanied by an increase in treatment-related complications resulting in no change in overall survival [26,145]. New strategies are needed to allow further dose escalation without associated increases in short- and long-term side effects. Various approaches are currently being actively explored to deliver a more targeted form of TBI, one that would preferentially deposit higher doses to areas of greatest tumor burden to maximize disease control, while simultaneously reducing the dose to critical normal organs to reduce toxicities.

Biologically guided systemic radiotherapy, using radiolabeled antibodies or bone-seeking radiopharmaceuticals such as ^{166}Ho-DOTMP, are currently undergoing evaluation as part of HCT conditioning regimens. This area is covered in more detail in Chapter 24 and has been reviewed elsewhere.

Recent technologic advances in radiotherapy systems now allow for the delivery of a more targeted form of TBI. The TomoTherapy HiArt System® was the first system to be used to deliver targeted TBI. Tomotherapy uses a 6 MV linear accelerator mounted on a computed tomography (CT) ring gantry that rotates around the patient as the patient translates through the ring. The maximum target size is approximately 60 cm in width by approximately 160 cm in length [146]. This allows for the delivery of conforming dose distributions to large, complex target shapes while simultaneously reducing the dose to critical normal organs [147], making it suited for the delivery of conformal targeted TBI. More recently, other groups have reported on the potential use of other volumetric arc-based image-guided IMRT systems to deliver targeted TBI [148–150]. This approach has also been referred to as total marrow irradiation (TMI) or total marrow and lymphoid irradiation (TMLI).

The group at City of Hope Cancer Center transplanted the first patients utilizing tomotherapy to deliver 10-17 Gy in patients with multiple myeloma and acute leukemia [2–7,151–154]. Figure 22.4 shows the typical conformal dose distribution pattern that is achieved to the designated target structure, with simultaneous reduction of dose to critical organs. Table 22.3 compares the median doses for various normal organs delivered through standard TBI to 12 Gy with lung shielding versus TMI to 12 Gy to the skeletal bone. Figure 22.5 shows normal organ dose–volume histogram plots for standard TBI versus TMI in the same patient. Depending on organ site, a reduction in median organ doses of approximately 15–80% and volume of organ receiving full dose compared with standard TBI has been reported, predicting reduced short- and long-term toxicities. The system can also be used to reduce differentially the radiation dose to organs or any other user-defined avoidance structure, depending on the radiosensitivity of the organ, and also to increase the dose to a particular target region depending on the tumor burden and clinical situation.

A phase I trial evaluated TMI in patients with Durie–Salmon Stage I–III multiple myeloma [2,5,155] responding after first-line therapy. Patients underwent tandem autologous HCT, with MEL (200 mg/m^2) followed by TMI. The TMI dose was escalated from 10 to 18 Gy at 2 Gy BID. The TMI target structure was bone (Figure 22.4). This trial allowed the evaluation of baseline toxicities associated with TMI without concurrent chemotherapy. Dose-limiting toxicities were not observed until a TMI dose of 18 Gy (one patient with reversible grade 3 pneumonitis, congestive heart failure, and enteritis and one patient with grade 3 hypotension), establishing the MTD at 16 Gy.

A subsequent pilot trial evaluated TMLI 12 Gy (1.5 Gy BID) combined with the reduced-intensity regimen of FLU (25 mg/m^2/d × 5 days) and MEL (140 mg/m^2) followed by allogeneic HCT in patients with advanced hematologic malignancies (primarily AML) who were over age 50 years or with comorbidities making them

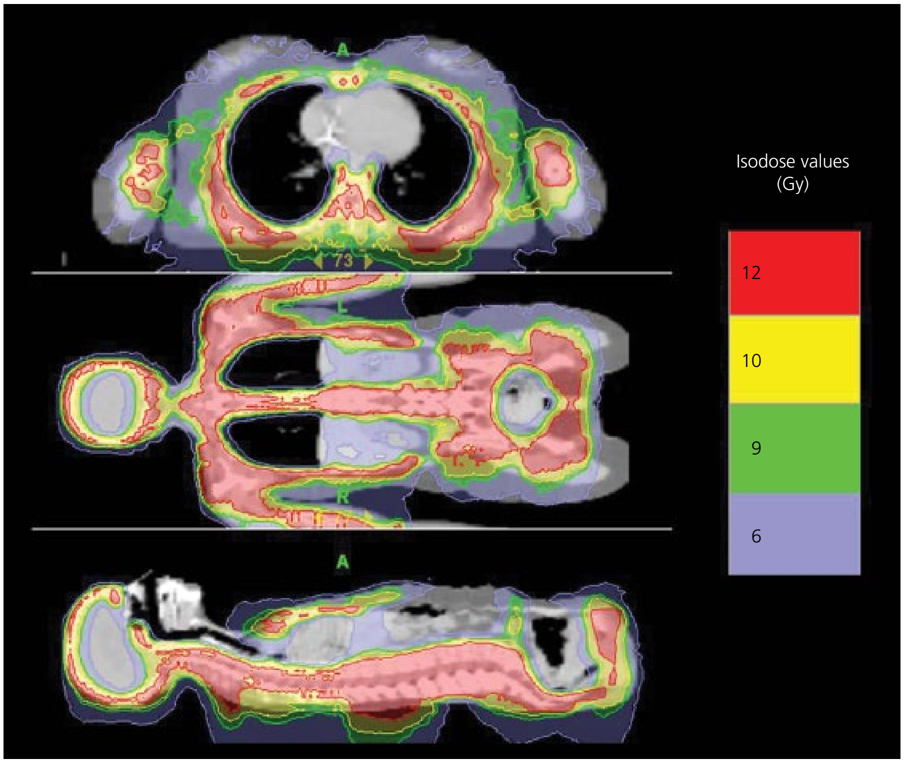

Figure 22.4 Color wash demonstrating typical dose distribution in a patient treated with targeted TBI using tomotherapy. The target structure is skeletal bone. Source: Wong et al. 2006 [2]. Reproduced with permission of the American Society for Blood and Bone Marrow Transplantation. (For a color version of this figure, please see the color plate section.)

Table 22.3 Median dose to normal organs with TMI using tomotherapy compared with standard TBI to deliver 12 Gy

Organ at risk	Median dose (Gy)		TMI/TBI median dose
	TMI	TBI	
Bladder	7.5	12.3	0.61
Brain	7.1	12.2	0.58
Breast	7.7	12.4	0.62
Esophagus	4.9	11.7	0.42
Orbits	6.0	12.0	0.50
Heart	6.1	11.5	0.53
Lens	2.3	10.5	0.22
Liver	6.9	11.7	0.59
Left kidney	7.4	11.9	0.62
Right kidney	6.9	11.9	0.58
Left lung	6.3	9.0	0.70
Right lung	6.4	9.7	0.66
Optic nerve	6.4	12.3	0.52
Oral cavity	2.5	12.5	0.20
Ovary	7.0	12.5	0.56
Parotids	4.6	13.1	0.35
Rectum	4.8	12.6	0.38
Small intestine	5.0	12.5	0.40
Stomach	4.6	11.5	0.40
Thyroid	4.4	12.6	0.35

Standard TBI utilized 10 MV photons to deliver 12 Gy; 50% attenuation blocks were used to shield the lungs. Electrons were used to deliver 6 Gy to the rib cage underlying the lung blocks. Data are averages of comparison plans from six patients [3].

ineligible for standard TBI myeloablative regimens [6]. The treatment-related mortality rate at 1 year was 19%, which compared favorably with the 30-40% rates reported for FLU and MEL alone in a similar patient population [156–159].

Phase I trials have also evaluated TMLI with higher intensity chemotherapy conditioning regimens. Twelve patients with high-risk (relapse or induction failure) acute leukemia less than age 55 years and not eligible for other intent-to-cure standard HCT regimens underwent allogeneic HCT with a conditioning regimen of TMLI, etoposide (VP16, 60 mg/kg), and CY (100 mg/kg). The TMI dose ranged from 12 to 15 Gy (1.5 Gy BID). Nine patients had persistent marrow blasts (10–98%) and five had circulating blasts (24–85%). Eleven of 12 achieved complete remission at day 30. The non-relapse mortality (NRM) cumulative incidence at day 100 was 8% [4]. Dose escalation on this trial continues. A phase I trial of TMLI with BU and VP16 in 20 patients with AML in relapse or with induction failure demonstrated that TMLI dose escalation was not feasible, with dose-limiting toxicities seen at 13.5 Gy [4]. Table 22.4 summarizes the toxicities of TMI trials at City of Hope Cancer Center.

Other groups have also evaluated TMI and TMLI containing regimens. Hui and colleagues at the University of Minnesota [160] reported on the first patient treated as part of a phase I autologous HCT trial in Ewing sarcoma using TMI to 6 Gy (2 Gy per day), followed by BU (targeted, 4 mg/kg days –8 to –6), MEL (50 mg/m^2 days –5 and –4), and thioTEPA (250 mg/m^2 days –3 and –2). The regimen was well tolerated with only nausea and vomiting observed. Sheung et al. [161] reported on three patients conditioned with 8 Gy TMI at 2 Gy per day (days –6 to –3) combined with MEL at 140 m/m^2 (day –2) prior to autologous HCT and observed only a single event of grade 3 toxicity (mucositis). Corvo et al. [162] demonstrated the feasibility of adding a 2 Gy TMI boost to bone marrow and spleen after standard TBI 12 Gy and CY in patients with AML and ALL undergoing allogeneic HCT. Other centers in Europe, North America, and Asia have also initiated trials but the early experience to date remains unpublished.

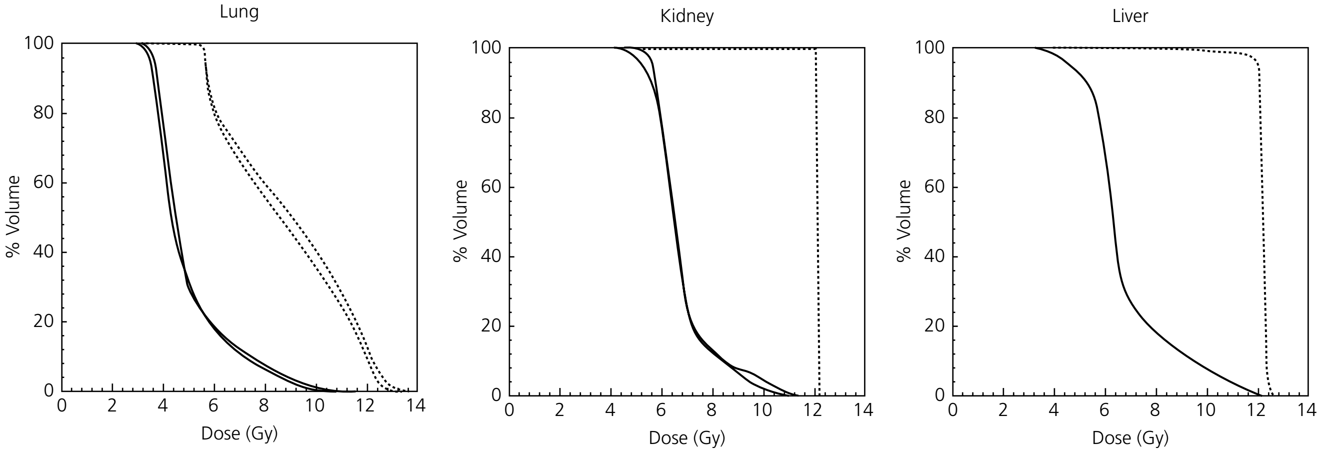

Figure 22.5 Comparison of dose–volume histogram (DVH) plots for lungs, liver, and kidneys with TMI versus standard TBI to 12 Gy. TBI and TMI plans were performed on the same patient data set. Standard TBI utilized 10 MV photons to deliver 12 Gy; 50% attenuation blocks were used to shield the lungs. Electrons were used to deliver 6 Gy to the rib cage underlying the lung blocks. With standard TBI (dashed lines) close to 100% of the liver and kidneys receive the full dose of 12 Gy. Lung shielding results in reduced doses to the lung, although 100% of the lung still receives approximately 6 Gy. With TMI, the DVH plots (solid lines) demonstrate reduced doses to a large percentage of each organ compared with TBI.

Table 22.4 Summary of toxicities observed on TMI trials at City of Hope Cancer Center

Parameter	CY–VP16–TMI [4]	BU–VP16–TMI [4]	FLU–MEL–TMI [4,6]	MEL → TMI [4,5]
TMI dose (Gy)	12.0, 13.5, or 15.0	12.0 or 13.5	12	10, 12, 14, 16, or 18
Target organs	Bone, major lymph node chains, spleen, liver, brain, testes	Bone, major lymph node chains, spleen, liver, brain, testes	Bone, major lymph node chains, spleen If ALL: brain, testes	Bone
Chemotherapy	Cyclophosphamide (CY) + etoposide (VP16)	Busulfan (BU) + etoposide (VP16)	Fludarabine (FLU) + melphalan (MEL)	Melphalan (MEL)
Type of transplant	Allogeneic	Allogeneic	Allogeneic	Tandem autologous
No. of patients	12	20	47	31
Diagnosis	AML, ALL, mixed lineage	AML	AML, ALL, NHL, MDS, CML	MM
Median age (range) (years)	33 (21–54)	41 (23–52)	55 (12–68)	53 (35–66)
Day 30: grade 3 toxicities (NCI CTC)	Nausea 4 (33%) (3 at 15 Gy) Vomiting 1 (8%) (1 at 15 Gy) Mucositis 7 (58%) (6 at 15 Gy) Esophagitis 0 Diarrhea 0	Nausea 9 (45%) Vomiting 1 (5%) Mucositis 17 (85%) Esophagitis 1 (5%) Diarrhea 3 (15%)	Nausea 26 (55%) Vomiting 13 (28%) Mucositis 28 (60%) Esophagitis 2 (4%) Diarrhea 27 (57%)	Nausea 3 (10%) (2 at 18 Gy, 1 at 16 Gy) Vomiting 0 Mucositis 0 Esophagitis 0 Diarrhea 1 (3%) (1 at 18 Gy) Pulmonary 1 (6%) at 18 Gy DLT Hypotension 1 (6%) at 18 Gy DLT
Day 30: NCI CTC v3 grade 4 or Bearman grade 3 toxicities	None	Mucositis 1 (5%) at 13.5 Gy Ileus and SOS 1 (5%) at 13.5 Gy	Pulmonary 5 (11%) Renal 4 (9%) Bladder 1 (2%) CNS 1 (2%) Cardiac 2 (4%)	None
Non-relapse mortality at 100 days	1 (8%) (at 15 Gy)	4 (20%) (2 at 13.5 Gy)	7 (15%)	None

In summary, strategies to deliver a more targeted form of TBI are being actively investigated through the use of newer image-guided IMRT radiotherapy delivery systems. The potential advantages are the ability to reduce normal organ doses, reduce toxicities, and broaden the range of patients able to undergo radiation conditioning regimens, such as older patients or those with comorbidities. This approach also offers the opportunity to escalate target dose with acceptable toxicities in an effort to improve outcomes.

Key points

- TBI remains an important component of HCT conditioning regimens owing to its immunosuppressive and antitumor effects.
- Fractionated TBI is superior to single-fraction TBI schedules owing to reduced normal tissue toxicity.
- Dose-rate effects in normal tissues decrease with fractionation. Dose-rate effects are minimized with fractionated schedules.
- TBI or TLI is also an important part of many reduced-intensity regimens. Fractionated TLI demonstrates promise as a method to reduce the incidence of GVHD and induce tolerance to allografts.
- Evaluation of image-guided intensity-modulated radiotherapy delivery systems as a method to deliver a more targeted form of TBI is ongoing. This allows for the potential to dose escalate to tumor or selected target regions, with simultaneous reduction in dose to critical structures, which could improve the therapeutic index of TBI.

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CHAPTER 23

Radioimmunotherapy and Hematopoietic Cell Transplantation

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> The odds of hitting your target go up dramatically when you aim at it.
> Anonymous

Background

For over half a century, minimal attention was paid to German pathologist Paul Ehrlich's prescient notion for utilizing target specific antisera as a form of cancer therapy [1]. While in the late 1890s Hericourt and Richet reported temporary responses in patients treated with antisera raised against human osteogenic sarcomas, and Madame Marie Curie and colleagues identified a potential role for the α particle-emitting radionuclide radium as an intravenous therapeutic [2], few other studies advanced the field of targeted radiation therapy for over 50 years. In 1949, Eisen's report of a technique for linking radioactive iodine to antibodies without impairment of antibody binding [3] paved the way for Pressman and Korngold's discovery that radioiodinated tumor-specific antibodies would preferentially accumulate in target tissues [4]. In the 1960s, Bale et al. demonstrated that ^{131}I-labeled antibodies could be effective as therapeutic agents [5]. Also in the 1960s, radioiodinated antifibrinogen was shown to provide some symptomatic improvement in a variety of tumor types [6]. In the 1970s, Ettinger et al. demonstrated significant responses in patients with hepatoma treated with ^{131}I-labeled anti-CEA or antiferritin antibodies in combination with external beam radiation and doxorubicin chemotherapy [7]. Lenhard et al. subsequently showed that ^{131}I-labeled polyclonal antiferritin antisera could produce objective remissions in patients with hepatomas and also up to 40% of patients with relapsed lymphoma [8].

In 1975, Kohler and Milstein's Nobel Prize winning work made practical the large-scale production of monoclonal antibodies (MAbs) with defined and reproducible specificity. Great excitement surrounding a new era of MAb-directed therapy ensued; however, initial studies with unlabeled MAbs did not meet the expectations of many observers. Over the past decade, two chemically conjugated MAbs, an anti-CD33 MAb chemically conjugated to the cytotoxic drug calicheamicin (gemtuzumab ozogamicin) was approved for the treatment of relapsed acute myeloid leukemia (AML), and more recently an anti-CD30 MAb drug conjugate (brentuximab vedotin) for Hodgkin lymphoma. In addition, several unconjugated MAbs have been approved as cancer therapies in the United States, with two anti-CD20 radiolabeled MAbs, [^{131}I]tositumomab and [^{90}Y]ibritumomab tiuxetan, approved. Each of these unconjugated or chemically conjugated MAbs has shown clinical promise, but most patients treated with these products as single agents subsequently relapse.

As predicted in animal studies, the limitations of unmodified MAb therapy largely result from inadequate host effector mechanisms. In the absence of robust immune effector mechanisms, MAbs bound to antigen on the target cell surface usually do not diminish cell replication and survival sufficiently to eradicate tumors. In addition, antigenic modulation (internalization or shedding) after antibody–antigen complex formation limits the availability of a stable target in many clinical settings. A third impediment to effective cell killing results from variable expression of antigenic targets, including antigen-negative variants in most tumor types. Finally, antigen penetration into large tumors is limited by the vascular supply and the kinetics of intratumoral MAb convection and diffusion.

Throughout the 1980s, interest in radioimmunotherapy (RIT) grew owing to the recognition that radioimmunoconjugates could bypass many of the limitations enumerated above. By providing the MAbs with a cytotoxic mechanism independent of the host immune response, radiolabeled antibodies (RAbs) circumvent the major obstacle to antibody-directed therapy. Furthermore, the use of radionuclides with path lengths that span several cell diameters provides a "crossfire" effect that delivers toxic radiation from the surrounding antigen-bearing cells to tumor cells that are antigen negative or that lack direct access to MAb binding.

In recognition of these advantages, initial preclinical studies with RIT sought to identify both appropriate antigen targets for optimal biodistribution and identification of the factors necessary for tumor cell destruction. These early studies elucidated a number of key principles concerning the biodistribution of RAbs. In addition, these studies documented that 4–10 times more radiation could be delivered to tumors compared with normal organs by using radioimmunoconjugates. Unfortunately, myelotoxicity frequently prevented radiation dose escalation to curative levels in early studies of RIT [9,10]. The understanding that marrow suppression was dose limiting inspired some groups to explore RIT in combination with stem-cell support.

The concept that hematopoietic stem-cell rescue could facilitate dose escalation to curative levels was supported by the work in the early 1970s of Thomas and colleagues, who first defined a role for bone marrow transplantation (BMT) in the treatment of acute leukemia. Clinical trials conducted in Seattle demonstrated the antileukemic effect of total body irradiation (TBI) in combination with high-dose chemotherapy for BMT [11]. Importantly, higher doses of TBI correlated with a reduced-risk of disease recurrence

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after transplant. In two randomized trials, patients with either AML [12] or chronic myeloid leukemia (CML) [13] were given cyclophosphamide (CY) and either 12 or 15.75 Gy of TBI conditioning prior to allogeneic sibling-matched BMT. The rate of post-transplant relapse dropped from 35% to 13% in the AML population and from 30% to 7% in the CML group for those patients receiving the higher dose of TBI. These studies clearly demonstrated the potential for higher doses of radiation to generate more durable responses; however, those in the higher dose group experienced a significant increase in severe or fatal toxicities. Owing to the increased frequency of toxicity (most often radiation-induced pneumonitis, mucositis, or hepatic damage), the higher doses of TBI did not improve overall patient survival. Nonetheless, Thomas' studies demonstrated a steep dose–response curve of the leukemia to radiation and supported the need for improved methods of selectively delivering radiation to malignant cells.

With this background information, researchers in the late 1980s and 1990s finally developed effective clinical applications for Ehrlich's initial concept of "magic bullets" as a form of cancer therapy. Animal models demonstrated that marrow ablation was feasible with RAbs and that subsequent stem-cell infusion could restore marrow function reliably [14]. Non-myeloablative doses of ^{90}Y- and ^{131}I-labeled anti-CD20 MAbs consistently produced remissions in 50–80% of patients with relapsed, refractory, or transformed follicular non-Hodgkin lymphoma (NHL) and led to regulatory approval of both [^{90}Y]ibritumomab tiuxetan (Zevalin™) in 2002 and [^{131}I]tositumomab (Bexxar™) in 2003 by the US Food and Drug Administration (FDA). Despite such high overall response rates, however, most patients treated with standard non-myeloablative doses of radiolabeled anti-CD20 MAbs eventually relapse, indicating the desirability of further dose escalation.

Despite the enormous potential for targeted therapy, problems were identified soon after the early clinical RIT trials were completed. Initially, it was thought that any tumor could be targeted efficiently with MAbs. However, the long circulatory half-life of the blood-borne RAbs causes prolonged high background activity levels, leading to non-specific normal organ exposure to radiation. After the detection of peptide receptor expression by tumors, peptide analogs began to be investigated for specific tumor targeting. Peptides used for tumor targeting show several advantages over antibodies; peptides are small and show rapid diffusion into (target) tissues resulting in rapid pharmacokinetics. Their fast blood clearance could lead to high tumor-to-background ratios shortly after administration of the radiopeptide.

Considerable expansion has been accomplished with use of pretargeted RIT and other methods to enhance tumor-to-normal organ tissue ratios, such as the use of α-emitting radionuclides. This chapter focuses on these clinical therapeutic aspects of targeted radionuclides for diagnosis and therapy of tumors. The current state of clinical use of RAbs and radiopeptides, and the state of development of new compounds and future innovations are reviewed. Discussion of preclinical studies is limited to those that offer insights into future directions for clinical study. The radioisotopes appropriate for diagnosis and therapy and the radiolabeling methods that are currently in use are described.

Components of RIT

Specific antigen targeting with RAbs is designed to produce a biodistribution profile favoring radiation delivery to malignant cells; however, a portion of the radioimmunoconjugate remains unbound in the circulation and contributes to non-specific irradiation of normal tissues. The relative exposure of target and non-target tissues can be non-invasively estimated via imaging. The term biodistribution describes measured values of the relative levels of radionuclide throughout the body. In preclinical research models, biodistributions can be accurately measured by tissue sampling at serial time points after infusion of the radioimmunoconjugate, with quantitative measurements of the radionuclide content per gram of tissue [9]. In large animal models and human subjects, biodistributions are usually determined using radionuclides that possess a γ emission profile suitable for serial quantitative γ camera imaging. Regions of interest including the liver, lung, spleen, kidney, and marrow can be serially imaged and activity curves representing counts per pixel generated. These data are calibrated using direct measurements of radionuclides in accessible tissues such as blood, marrow, and lymph nodes, thus allowing the construction of time–activity curves and calculation of estimated absorbed radiation doses in tumor sites and normal organs (radiation dosimetry). These calculations have been typically based on methods established by the Society of Nuclear Medicine Special Committee on Medical Internal Radiation Dose (MIRD) [15–17]. Factors that influence the biodistribution of RAbs have been delineated through both preclinical animal studies and patient trials (Table 23.1).

Table 23.1 Factors that influence biodistribution of radiolabeled antibodies.

I. *Nature of the targeted antigen*
  a. Tissue distribution
  b. Cell-surface stability

II. *Antibody*
  a. Specificity
  b. Immunoreactivity
  c. Form
  d. Isotype

III. *Antibody pharmacology*
  a. Dose
  b. Infusion schedule
  c. Route

IV. *Labeling procedure*
  a. Choice of radionuclide
  b. Labeling chemistry

Target antigen selection

Appropriate target antigen selection is crucial if a favorable biodistribution of RAbs is to be achieved. Ideally, the target antigen will be expressed homogeneously on the cell surface of all target tumor cells at a high surface density and will not be expressed on any normal cells. Unfortunately, such tumor-specific cell-surface antigens are rare, and in practice the selection of lineage-specific hematopoietic antigens (e.g., CD20, CD45) has proven to be highly successful in the setting of hematopoietic stem-cell transplantation (HCT) where marrow rescue and hematopoietic reconstitution are anticipated. In fact, employing these more widely distributed lineage-specific antigenic targets offers an increased likelihood of eliminating both antigen-negative tumor cell variants and rare diseased cells within a remission marrow due to radioactive crossfire effects from adjacent antigen-positive hematopoietic cells [18].

Antigen density correlates directly with accumulation of MAbs at tumor sites [19]. When the number of antigenic sites is less than 10,000, target saturation occurs at a relatively low MAb dose. This is a significant limitation, as each MAb molecule can be labeled with only a limited amount of radionuclide before immunoreactivity is impaired. Low antigen density theoretically poses less of a problem for studies involving α particles, which require only a single DNA "hit" to kill a cell. However, for the more commonly used β-emitting radionuclides, multiple hits are required to induce irreparable DNA strand breaks resulting in cell death. A second factor in antigen selection is the extent to which the receptor is internalized following binding. Whereas some antigens remain stably on the cell surface after antibody binding, others are rapidly endocytosed and are transported to lysosomes for proteolytic degradation [20,21]. After this process of internalization, the receptor density may remain low for several days. One of the best studied radionuclides in RIT, ^{131}I, is not optimal for targeting internalizing receptors because rapid lysosomal metabolism of the conjugate leads to release of free [^{131}I]- and [^{131}I]monoiodotyrosine from cells [22,23]. This degradation significantly reduces the residence time of the radionuclide in tumor sites and therefore decreases the dose of cytotoxic radiation delivered to the malignant cells. When internalized antibodies are labeled with ^{111}In or ^{90}Y, however, low molecular weight cationic metabolites remain trapped within the lysosomes and their impact is not significantly diminished [24]. Surface antigens that are not internalized following antigen–immunoconjugate complex formation exhibit minimal variation in cellular retention of ^{131}I, ^{111}In, and ^{90}Y [24,25].

CD20 is one of the best studied and most frequently targeted receptors for B-cell neoplasms and possesses many of the favorable attributes noted above. It has a high density of expression on more than 90% of B-cell tumors and most investigators have demonstrated it to be minimally internalized and minimally shed from the cell surface of most B-cell malignancies. Potential limitations of anti-CD20 RIT have emerged, however, including inferior response rates in patients with B-cell depletion and higher serum levels of the unconjugated anti-CD20 MAb rituximab [26]. where preclinical data indicate that CD20 blocking may impair the ability to target CD20 with RIT [27]. One alternative is to circumvent the potential of CD20 blocking and the exclusion of CD20-negative NHL histologies by targeting the pan-hematopoietic antigen CD45 [27]. CD45, also known as leukocyte common antigen, is a cell-surface glycoprotein (size, 180–220 kDa) with tyrosine phosphatase activity [28]. Absent on non-hematopoietic cells, almost all hematopoietic cell lineages express CD45, except mature thrombocytes, mature erythrocytes, and some of their progenitors. Most hematologic malignancies, including 85–90% of acute lymphoid and myeloid leukemias, express CD45 [29–31]. This antigen is expressed in a relatively high copy number (200,000 binding sites per cell) and is not appreciably internalized or shed after ligand binding [24,32]. CD45 exists in several isoforms; however, although isoform-specific MAbs have been developed and investigated for immunotherapy, the CD45-targeting MAbs used so far for RIT are pan-specific.

Similarly to CD45, CD66 antigens are neither internalized nor shed [33]. They are expressed on members of the carcinoembryonic-antigen-related cell-adhesion molecule (CEACAM) family of proteins that play a role in various intercellular-adhesion and intracellular signaling-mediated effects involved in cellular growth and differentiation [34]. Some of these glycoproteins, namely CD66a (CEACAM1, biliary glycoprotein), CD66b (CEACAM8, CGM6), CD66c (CEACAM6, non-specific cross-reacting antigen), and CD66d (CEACAM3, CGM1), are expressed on hematopoietic cells but can also be found on epithelial or endothelial cells. CD66 antigens are found in myeloid cells from the late myeloblast or early promyelocyte stage and reach the highest levels in myelocytes and metamyelocytes, whereas early myeloid progenitors or multipotent progenitors do not express CD66 antigens [35–37]. CD66 antigens are only occasionally found on AML cells, but many B-cell acute lymphoblastic leukemias and some cases of lymphoid blast crisis CML express these antigens [36,38]. Studies with a CD66c-specific MAb suggested restriction primarily to CD10-positive early B-cell acute lymphoblastic leukemia (ALL) [39]. However, many anti-CD66 MAbs recognize several CD66 antigens; for example, BW 250/183, the most widely used MAb for CD66-targeted RIT, recognizes CD66a, -b, and -c [40]. Therefore, although the exact expression patterns may differ between the individual CD66 antigens, these patterns may be currently of limited significance for radioimmunotherapeutic approaches [34,39].

Since CD33 and CD45 are expressed by both normal and malignant cells, MAbs targeting these antigens can deliver radiation to marrow, spleen, and lymph nodes in patients with measurable disease and also in patients in remission. Malignant cells that do not express these antigens may be killed if they are surrounded predominantly by non-malignant hematopoietic cells because of the bystander effect. This bystander effect is particularly important for RIT of myeloid leukemia using CD66, as these antigens are commonly not expressed by the malignant clone.

In addition to CD33, CD45, and CD66, a number of other antigens have been used in clinical studies of RIT of leukemia, including HLA-DR, CD5, CD20, CD22, CD25, and CD37. For example, CD5 is a surface antigen present on T cells, a subset of B cells, and subsets of chronic lymphoid leukemia (CLL) cells [41]. CD25 (interleukin-2 receptor, α subunit) is felt to be an attractive target for RIT as it is only weakly expressed in some normal tissues, including epithelial tissues and lymphocyte subsets, but is found on malignant cells in several diseases, including adult T-cell leukemia, cutaneous T-cell lymphoma, anaplastic large-cell lymphoma, hairy cell B-cell leukemia, and the Reed–Sternberg and associated polyclonal T cells in Hodgkin disease, in addition to some AML [42].

Antibody selection

MAb specificity and binding kinetics also play an important role in optimizing the biodistribution of RAbs. MAbs with a low immunoreactivity do not accumulate at sufficient levels in target tissues and result in a relatively non-specific pattern of distribution. In the setting of HCT, accumulation at high levels in the marrow is anticipated and reversible, hence non-specific cross-reactivity in radiosensitive organs such as lung and liver are frequently dose limiting. In contrast, cross-reactivity with marrow elements is of great significance in the non-HCT setting where myelosuppression frequently limits dose escalation. Another factor in MAb selection relates to stability, since MAbs susceptible to damage or agglutination during the labeling process often accumulate in the liver. In early studies, MAbs with high avidity were presumed to be optimal for RIT [43]; however, subsequent findings have suggested that very high avidity may promote heterogeneity of MAb deposition through enhanced uptake at the most peripheral perivascular target sites. This so-called binding site barrier [44] was subsequently demonstrated in a non-human primate study employing two different CD45 MAbs in which the MAb with slightly higher avidity preferentially localized to the most easily accessible antigen-positive cells and was rapidly cleared from the circulation (~2 hours) [45]. Meanwhile the MAb with slightly lower avidity stayed in the circulation longer and demonstrated superior uptake in the lymph nodes, a less accessible target site.

The use of antibody fragments to improve the rate of penetration and overall distribution of RAbs has been investigated. These smaller fragments produced by enzyme digestion of intact MAbs (Fab, 50 kDa; $F[ab']_2$ 100 kDa) or through cloning and expression of the Fv portions of the immunoglobulin light and heavy chains in *Escherichia coli* (scFv, 25 kDa) permit more rapid diffusion into tumor sites [46,47]; however, their small size results in rapid clearance and short retention times compared with intact immunoglobulin G (IgG) antibody [48–52].

Owing to the murine origin of most MAbs used for RIT, patients may develop a humoral immune response with formation of a neutralizing human antimouse antibody (HAMA). The presence of HAMAs can result in an altered RAb biodistribution due to rapid circulatory clearance and increased hepatic uptake of the resultant immune complexes. HAMAs may also compromise diagnostic in vitro immunoassays employing analytes containing murine immunoglobulins (e.g., hormone assays) [53]. HAMAs usually develop 4–8 weeks after first exposure. HAMAs have posed less of a problem for patients with hematolymphoid malignancies in whom the capacity to mount a robust immune response to neo-antigens is usually impaired. Moreover, HAMA-related complications are generally relevant only for patients treated with repeated doses of murine MAbs, hence HCT patients are at significantly lower risk because they complete RIT conditioning over a short time period. The HAMA response can be limited by the use of immunosuppressive agents (e.g., cyclosporine), development of humanized or chimeric molecules, single infusions, and the use of less immunogenic MAb fragments.

Antibody pharmacology

Studies in murine models, non-human primates, and human subjects have documented the importance of appropriate antibody dosing to achieve optimal RAb biodistributions [9,10,21,54]. Each MAb possesses a specific dose range sufficient for achieving antigen saturation. Low doses result in relatively poor accretion of RAbs in lymph nodes and tumor masses, resulting in unfavorable tumor-to-normal organ ratios. Excessive doses beyond target saturation simply increase the fraction of injected radionuclide that persists in the circulation and non-specifically irradiates normal tissues. The precise MAb dose that delivers the optimal radiation exposure to tumor sites and limits exposure to normal organs in a given clinical scenario also depends on the burden of antigen-expressing cells (tumor load) and the presence of persistent prior antibody (e.g., rituximab) that may compete for target antigens and Fc receptors [27].

In the HCT setting, RAb infusions usually involve a trace labeled test dose (or "dosimetric infusion") to assess the individualized biodistribution, followed by a therapeutic infusion. The rate of infusion is determined by patient tolerance, with fever, dyspnea, and hypotension limiting rapid infusion rates of some MAbs. Based on the presence of a large number of antigen-expressing cells in the circulation of leukemia patients, alternative infusion schedules have been investigated. Animal studies have suggested that a small dose of unlabeled antibody to clear circulating cells expressing the target antigen may lead to an improved biodistribution of the subsequently administered labeled dose [55]. Preliminary patient trials have been inconsistent in this regard, with one group reporting no benefit of this approach in an anti-CD45 MAb (BC8; murine IgG1) model [56] and another demonstrating a significant enhancement in bone marrow uptake when a low-dose of unlabeled anti-CD45 MAb (YAML568; rat IgG2a) is injected prior to therapy [57].

To allow for re-expression of an internalizing antigenic target (CD33), a model in which three divided doses delivered at 48–72 hour intervals has been studied in patients with AML [58]. Fractionation has also been evaluated for B-cell NHL with a non-internalizing target (CD20) to allow a delivery of a higher total absorbed dose [59].

Table 23.2 Radionuclides for radioimmunotherapy

Radionuclide	Particles	$T_{1/2}$	Median energy (MeV)	Comments
Beta-emitters				
Iodine-131	β, γ	8.1 days	0.66 β	Well-defined chemistry, inexpensive, dehalogenation a problem, long $T_{1/2}$
Yttrium-90	β	2.7 days	2.3 β	High energy, no γ, accumulates in liver and bone
Lutetium-177	β, γ	6.7 days	0.50 β	Long $T_{1/2}$, low energy, stable when internalized, lower γ than ^{131}I
Rhenium-188	β, γ	17 h	4.4 β	Limited access
Rhenium-186	β, γ	89 h	1.8 β	
Copper-67	β, γ	2.6 days	0.57 β	Lower γ than ^{131}I, can image with PET, limited access
Alpha-emitters				
Bismuth-213	1α, 2β	46 min	5.84 α	High energy, short path length, very short $T_{1/2}$
Astatine-211	1α	7.2 h	5.87 α	Requires cyclotron for production
Actinium-225	4α, 2β	10 days	5.75–8.7 α	Daughters a potential problem

Labeling procedures

Physical properties that are important in the selection of radionuclides for MAb conjugation include types of emitted particles (α, β, γ), emission energies, half-life, availability, cost, ease of conjugation to proteins, and capacity for imaging (Table 23.2). ^{131}I and ^{90}Y are the best studied radionuclides for RIT and both have been approved by the FDA for treatment of patients with relapsed or refractory B-cell NHL in conjunction with murine anti-CD20 MAb ([^{131}I]tositumomab and [^{90}Y]ibritumomab). ^{131}I has a half-life of 8 days and emits both β particles and γ rays. ^{90}Y is a radiometal with a short half-life (2.7 days) that emits β particles that are four times as energetic as those from ^{131}I, but virtually no γ rays, hence it cannot be easily imaged with a gamma camera. Dosimetry with ^{90}Y is therefore reliant on the administration of a surrogate γ-emitting radionuclide, such as ^{111}In.

Although the relative merits of ^{131}I and ^{90}Y have been debated extensively in the literature on theoretical grounds, no randomized controlled trial comparing them has been performed. Although ^{131}I possesses a number of favorable attributes, including a high energy γ emission energy for imaging, low cost, relative abundance, and a well-understood chemistry for protein labeling, it also possesses several limitations, including rapid release of [^{131}I]tyrosine and free ^{131}I from tumor cells after endocytosis and proteolysis of the radioiodinated MAbs, thus reducing the residence time of the radionuclide at its target [23,60]. In addition, the γ rays emitted by ^{131}I are of sufficiently high energy (364 keV) that they pose a potential safety risk to healthcare workers and require radiation isolation for patients receiving the high doses required for HCT. ^{90}Y also has limitations. It is less abundant and more expensive than ^{131}I and has been shown to accumulate non-specifically in liver and bone [61]. In addition, some groups have raised concerns about the use of ^{111}In as a surrogate for ^{90}Y dosimetry. Recently, there has been increased interest in the lanthanide radiometal ^{177}Lu, which possesses a β energy that is slightly less than that of ^{131}I, emits γ rays suitable for gamma camera imaging, and possesses a half-life of 6.7 days. The lower energy γ rays emitted by ^{177}Lu reduce the potential radiation exposure of family members and healthcare workers compared with ^{131}I. Another radionuclide, ^{67}Cu, like ^{90}Y and ^{177}Lu, is retained intracellularly following internalization. It has both γ- and β-particle emissions and a half-life suitable for RIT. In addition ^{67}Cu emits

^{99m}Tc-like photons that are suitable for imaging via positron emission tomography (PET). Expense and availability have been major limitations to the use of this radionuclide in the past; however, there has recently been renewed interest in increasing the scale of production. Rhenium isotopes ^{186}Re and ^{188}Re have also been used in some studies. Both have γ and β emissions and well-described chelation chemistries; however, the use of ^{188}Re has largely been limited by its short half-life.

α-Emitting radionuclides have considerable promise for RIT. Recent advances in the production and availability of α-emitting isotopes have sparked renewed interest in their use. These particles have a mass that is on average 7000 times that of β particles and energies that range from 3 to 13 times greater than those of the β emitters conventionally employed in RIT. This translates into significantly higher linear energy transfer (LET) for α emitters (approximately 100 keV/μm) compared with β emitters (0.2 keV/μm). LET is defined by the number of ionizations per unit distance traveled. Since the number of ionizations directly correlates with the potential to produce irreparable double-stranded DNA beaks, α particles exhibit a high relative biological effectiveness for cell killing. The α emitters possess a number of theoretical advantages compared with β emitters: they deliver higher energies over a much shorter range (path length <0.1 mm) [62], cause extensive cell damage that is independent of the dose rate, and exhibit no functional impairment in hypoxic environments, in contrast to β emitters [63]. In addition, α emitters generally pose a low risk to healthcare workers owing to their short path length. When conjugated to antibodies, the combination of high energy and short path length provides the capacity to kill only the antigen-expressing target cell while sparing surrounding normal cells, potentially reducing myelotoxicity. The absence of a significant bystander effect may not be optimal for larger volume tumors, but is favorable for targeting small tumors, micrometastasis, and individual tumor cells (i.e., minimal residual disease in patients with leukemia) [64–66]. The α-emitting radionuclides best suited for clinical application include ^{211}At ($T_{1/2}$ = 7.2 hours), ^{225}Ac ($T_{1/2}$ = 10 days), and ^{213}Bi ($T_{1/2}$ = 45.6 minutes) [63,67]. Although the short half-lives of α-emitting radionuclides complicate clinical trial design schemes, a number of groups have successfully conducted patient trials with α-emitting radionuclides conjugated to a MAb (reviewed in sections to follow).

Retaining MAb immunoreactivity is essential during the physical process of radiolabeling and great care must be taken to maintain sterility. The process can be exquisitely sensitive to external factors, including fluctuations in temperature and pH. ^{131}I can be covalently conjugated to antibodies on a tyrosine residue by either the chloramine-T or Iodogen™ methods. However, these simple approaches do not protect against rapid metabolism and release of free radioiodine when the antigen–antibody complex is internalized. Alternative processes have been developed to prevent intracellular degradation. Tyramine cellobiose involves the formation of a polysaccharide bridge between the antigen and halogen radionuclide that allows for retention of the radioligand within the cell [68]. The non-halogen radionuclides (^{90}Y, ^{111}In, ^{177}Lu) cannot be readily linked to antibodies by direct labeling and require a more complicated chemistry for attachment. This process requires the use of an intermediary molecule known as a chelate. Currently the most commonly used chelation techniques include isothiocyanotobenzyl-DTPA (diethylenetriaminepentaacetic acid) or 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*′′,*N*′′′-tetraacetic acid (DOTA).

Dosimetry

Optimal RIT treatment planning requires accurate individualized biodistribution data for calculating the predicted relative exposure of normal and target tissues from delivered radiation. In the clinical setting, this is achieved through the injection of a trace-labeled dose of antibody with subsequent serial single photon emission computed tomography or planar imaging. Activity curves for specific regions of interest are generated and compared with curves derived from banked standard tissue biopsy specimens. This approach has been validated in animal models [54]. The accuracy of this approach is compromised for small, deep-seated tumors or tissues not amenable to biopsy. Other factors limiting accuracy result from signal averaging that occurs when radionuclide content is assessed for whole organs. Current imaging techniques are incapable of identifying the heterogeneous distribution of antibody that occurs at a cellular level. This heterogeneity is most pronounced when evaluating large tumor volumes, areas with poor vascular supply, and in cases when high-affinity MAbs accumulate at the periphery of tumors and in intravascular locations due to the binding site barrier [44]. Radionuclides with longer path lengths (e.g., ^{90}Y) theoretically reduce the impact of cell–to-cell variation at the expense of irradiation of adjacent non-target tissues. In addition, the use of positron emission tomography in conjunction with photon-emitting radionuclides may improve the capacity to identify variable uptake within specific tissues [69].

Based on the measured biodistribution of the RAbs, tables have been developed by the MIRD that allow the estimation of the mean absorbed dose over a specific organ volume for any radionuclide administered [17]. This calculated dosimetry is based on formulas that weigh the contribution of the circulating radionuclide, radiation from neighboring organs to the organ of interest, and the direct contribution from the radionuclide delivered to the target site. Its accuracy can be further enhanced by radiographically quantified individualized organ volumes [70]. This system for calculating dosimetry is not optimized for α-emitting particles, because they deliver high energy over a very short range. Alternative methods involving microdosimetry have been developed for the α emitters [71].

Radiation effects

Conventional fractionated external-beam therapy is capable of delivering high doses of radiation over a short period of time. Classically fractionated doses of 1–2 Gy at 7–25 cGy/min up to a total dose of 10–15 Gy have been used in conditioning regimens for HCT. RAbs, on the other hand, deliver continuous, variable, exponentially decreasing low dose-rate radiation. Although the peak rate of radiation delivery with RIT is substantially lower than with external-beam TBI, there is continuous exposure of tumor cells to radiation, rather than intermittent exposure as with fractionated TBI. The exponential decline of radiation exposure seen with RIT is dependent on the chosen particle's half-life of decay. There has been no systematic evaluation of the biologic impact of these different delivery mechanisms. However, based on strong evidence that the cytotoxic activity of radiation is proportional to its capacity to induce DNA strand breaks, some have suggested a potential advantage arising from continuous radiation exposure that prevents intervals during which cellular DNA repair can occur [72]. Consistent with this hypothesis, a number of human solid tumor xenograft experiments have demonstrated RAbs to have equivalent or even enhanced antitumor activity compared with external-beam radiation [73,74]. Another potential explanation for this finding relates to what has been described as an "inverse dose-rate effect".

At some dose-rate ranges, a lower dose is assumed to be more cytotoxic than a larger dose. It is theorized that this seemingly paradoxical finding is related to induction of cell cycle arrest in cells exposed to a continuous low dose-rate of radiation in the G2 phase of the cell cycle. Since the G2/M interface is the most radiosensitive phase of the cell cycle, the presence of a larger proportion of cells in this phase is believed to increase cytotoxicity. Likewise, there is preclinical evidence to suggest that low dose-rate radiation may best synergize with chemotherapeutics that are involved with interference with single-stranded DNA repair such as the nucleoside analogs [75].

Leukemia

The first studies integrating RIT into HCT targeted the CD33 myeloid-associated glycoprotein in patients with AML. These studies employed [^{131}I]anti-CD33 (p67) followed by a standard CY/TBI (12 Gy) preparative regimen. This regimen was selected because it provided adequate immunosuppression to ensure engraftment, while allowing the possibility for dose escalation of the RAbs. CD33 was selected for initial targeting studies since this antigen is expressed on the leukemic cells in over 90% of AML cases (and also on normal myeloblasts, myelocytes, and metamyelocytes, but not on any non-hematopoietic cells).

As noted previously, identifying a favorable biodistribution of radioactivity as defined by a trace-labeled test dose of MAbs that distributed radiation preferentially to bone marrow and spleen was considered essential prior to delivering a therapeutic RAb dose in these initial studies. The first small trial in Seattle enrolled nine patients, and only four met the biodistribution criterion required by the protocol for proceeding to the therapeutic [^{131}I]anti-CD33 infusion (estimated radiation doses to marrow and spleen that were greater than to any normal organ) [76]. Although all four patients tolerated both the therapeutic infusion of [^{131}I]anti-CD33 MAbs and subsequent HCT without unexpected toxicities, three subsequently relapsed. Eradication of AML cells in this study was likely compromised by the relatively brief residence time of [^{131}I]anti-CD33 MAbs in target tissues (9–41 hours). The rapid clearance of radioactivity from the marrow and spleen was thought to result from rapid internalization of the antibody–antigen complex followed by cellular metabolism of the radioimmunoconjugate resulting in the release of ^{131}I and [^{131}I]tyrosine from the marrow space. In addition, the relatively low density of CD33 per cell resulted in antigenic saturation at doses greater than 0.5 mg/kg. These considerations prevented dose escalation and the study was halted. Despite these limitations, this initial study documented the feasibility of integrating RIT into HCT preparative regimens.

In parallel with the studies in Seattle, investigators at the Memorial Sloan Kettering Cancer Center (MSKCC) in New York also conducted trials targeting CD33 for myeloid leukemias. These studies employed a ^{131}I-labeled murine MAb called M195. Whereas initial biodistribution studies revealed findings similar to the Seattle p67 experience with low concentrations of MAb administered prior to saturation, the reported half-life of ^{131}I in the marrow was somewhat longer. Their first trial of 24 patients with myeloid malignancy involved a dose escalation design. The labeled M195 was administered in divided doses, to allow for CD33 re-expression. About 90% of enrolled patients demonstrated a dramatic decrease in peripheral and marrow blasts. With ^{131}I doses of 5 GBq (135 mCi/m^2) or greater, profound pancytopenia ensued and, while HCT was not required by the study design, eight patients who received <5.9 GBq (160 mCi/m^2) received autologous or allogeneic HCT rescue. In a subsequent study integrating radiolabeled M195 into a busulfan (BU)–CY allogeneic HCT preparative regimen for patients with relapsed or refractory AML, the MSKCC group reported 100% engraftment, with 18 of 19 study subjects achieving a remission.

As a refinement, the MSKCC group developed a humanized version of M195 (HuM195) with pharmacokinetic properties similar to murine M195, but without significant immunogenicity [77]. Cumulative long-term follow-up data from three phase I clinical trials investigating [^{131}I]anti-CD33 antibodies in combination with BU–CY as a conditioning regimen for relapsed or refractory AML ($n = 16$), accelerated/myeloblastic CML ($n = 14$), or advanced myelodysplastic syndrome (MDS) ($n = 1$) revealed a median survival for all patients of 4.9 months (range, 0.3–90+ months) after HCT. The intensified conditioning with [^{131}I]anti-CD33 antibodies resulted in few toxicities beyond those seen with standard BU–CY preparative regimens. A number of limitations were associated with the M195 and HuM195 constructs, however. Radioiodination of these anti-CD33 MAbs at high specific activity decreased the CD33 binding affinity and, as a result, patients required multiple MAb infusions to deliver myeloablative doses to the marrow. In addition, the high doses of ^{131}I required patient isolation to prevent high-energy (γ) emission exposure to hospital staff [58]. To avoid some of these limitations, the MSKCC group subsequently investigated the role of ^{90}Y as the therapeutic radionuclide and demonstrated the feasibility of this approach [78].

In the non-HCT setting, HuM195 conjugated to the α emitter ^{213}Bi has been investigated [79]. A feasibility trial involving 18 patients with either relapsed/refractory AML or chronic myelomonocytic leukemia demonstrated the safety and feasibility of α-particle therapy [80]. All patients developed myelosuppression and the absorbed dose ratios between sites of leukemic involvement (spleen, marrow, and liver) and whole body were reported to be 1000-fold greater than would be seen with β-emitting constructs. However, although 93% of patients experienced a decrease in peripheral blast counts and 78% experienced reductions in marrow blast counts, no complete remissions (CRs) were achieved. There were several limitations associated with this study. First, no maximum tolerated dose was identified because escalation beyond 37 MBq/kg was restricted by production capacity and cost. Second, the patient population studied carried a heavy tumor burden (average 10^{12} cells) that may not be well suited to the short range and high LET properties of α particles. Nevertheless, incorporation of this approach into HCT conditioning regimens for patients in CR or near CR remains a promising prospect, especially with alternative α emitters such as ^{225}Ac [65,81].

Owing to the limitations associated with CD33 targeting identified in their early studies, investigators at the Fred Hutchinson Cancer Research Center (FHCRC) in Seattle selected an alternative target, the protein tyrosine phosphatase CD45, for subsequent investigations. Termed the common leukocyte antigen, CD45 is expressed on the surface of virtually all cells of hematopoietic origin except mature erythrocytes and platelets. CD45 possesses a number of potentially advantageous characteristics for RIT targeting. While the overall percentage of AML and ALL patients whose leukemic cells express CD45 is virtually equivalent to CD33 (85–95% versus 90%), the receptor density is 10-fold greater (100,000–200,000 versus 10,000–20,000 antigenic sites per leukemic cell). In contrast to CD33, which is rapidly internalized after antibody–receptor complex formation, the CD45 antigen remains stably fixed on the cell surface. After a series of murine and non-human primate studies revealing favorable biodistributions with the BC8 anti-CD45

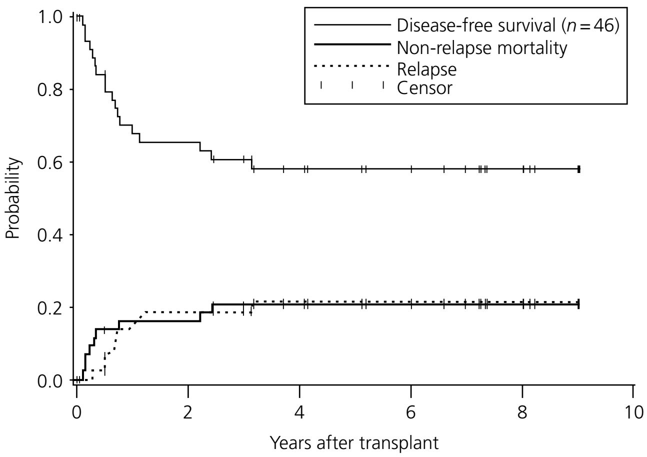

Figure 23.1 Estimates of the probability of disease-free survival, non-relapse mortality, and cumulative risk of relapse among 46 patients with AML in first complete remission who received a therapeutic dose of [^{131}I] BC8 (102–298 mCi) followed by busulfan–cyclophosphamide and allogeneic HCT. Source: Pagel et al. 2006 [86]. Reproduced with permission of the American Society of Hematology.

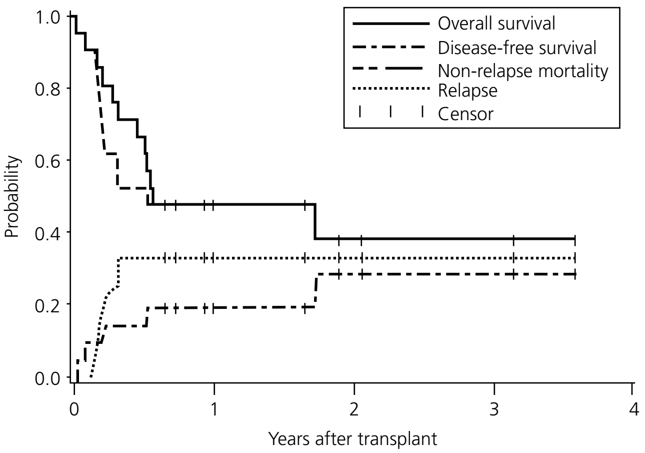

Figure 23.2 Estimates of the probability of overall survival, disease-free survival, non-relapse mortality, and cumulative risk of relapse among patients treated at the MTD of 24 Gy of radiation delivered to the liver by [^{131}I]BC8 MAbs followed by TBI–fludarabine. Source: Pagel J.M. et al. 2009. Allogeneic hematopoietic cell transplantation after conditioning with ^{131}I-anti-CD45 antibody plus fludarabine and low-dose total body irradiation for elderly patients with advanced acute myeloid leukemia or high-risk myelodysplastic syndrome. *Blood* 2009; **114**: 5444–5453. Reproduced with permission of the American Society of Hematology.

MAb, a phase I trial for patients with AML or ALL was initiated, employing an design identical with that of the FHCRC p67 trial [56,82]. The theoretical advantages of CD45 targeting were validated in this trial that exhibited an improved biodistribution profile compared with the prior CD33 targeting experience. The improved biodistribution was likely a function of improved hematopoietic tissue targeting due to a larger number of antigenic sites and a period of red marrow retention that was more than double that seen in the p67 study ($T_{½}$ = 44.2 versus 21.4 hours). Of the 44 patients initially enrolled, 37 had favorable biodistributions (84%) with an average 2–3-fold greater radiation delivered to the marrow than to the liver, which was the normal organ with the highest radiation exposure.

These findings led to a subsequent study for patients with AML in CR1 employing [^{131}I]BC8 MAb combined with a standard preparative regimen of targeted BU 600–900 ng/mL and CY 120 mg/kg. The MAb biodistribution was favorable in 88% of patients. Forty-six patients were treated with 102–298 mCi (3744–11026 MBq) of [^{131}I] BC8 MAb combined with BU–CY, with all but three receiving estimated radiation doses of 5.25 Gy to the liver and average estimated radiation absorbed doses of 11.3 Gy to the bone marrow and 29.7 Gy to the spleen. Overall, 61% of patients were surviving disease free at a median of 3 years after HCT, with eight transplant-related deaths and eight relapses (Figure 23.1). Based on an intention-to-treat analysis, the study patients were compared with an International Bone Marrow Transplant Registry cohort of 509 patients treated with BU–CY alone. Following adjustment for age and cytogenetic risk, the hazard of mortality among the [^{131}I]BC8 RIT treated patients was 0.65 (95% CI, 0.39–1.08; p = 0.09). Subgroup analysis revealed the overall 3-year survival to be 75% (95% CI, 58–92%) among patients with intermediate-risk cytogenetics and 36% (95% CI, 11–62%) for those with unfavorable cytogenetics [83].

These results suggested that this approach might well be applied to older patients. A phase 1 dose escalation trial using [^{131}I]BC8 combined a reduced-intensity conditioning (RIC) approach followed by HCT was conducted for a particularly poor-risk population of patients with advanced AML and high-risk MDS who were older than 50 years of age [84]. This study demonstrated that [^{131}I] BC8 could deliver 5.2–45.9 Gy (mean, 27.5 Gy) to bone marrow, 17.3–155 Gy (mean, 81.2 Gy) to spleen, and 12–24 Gy to the liver (dose-limiting organ) in addition to a standard RIC HCT regimen without a marked increase in day 100 mortality (Figure 23.2) [84]. A remission was achieved in all patients and all had 100% donor CD3$^+$ and CD33$^+$ cell engraftment by day 28 post-transplant. Whether the use of a RAb combined with an RIC transplant will reduce post-transplant relapse for these older patients with high-risk AML/MDS remains to be determined, however.

So far, the majority of clinical experience in targeting CD45 with radioimmunoconjugates has been accumulated with ^{131}I. However, limited data obtained with YAML568, a rat IgG2a MAb that recognizes all CD45 isoforms, suggest that other anti-CD45 MAbs can selectively deliver radiation to hematopoietic tissues when labeled with ^{90}Y, provided that patients are preloaded with unlabeled antibody [57]. In Germany, a number of groups have explored CD66 (a, b, c, e) as an alternative target for patients with MDS or AML. CD66 is expressed at high levels on the surface of normal myeloid cells beyond the promyelocyte stage, but not on leukemic blasts. It is neither internalized nor shed. Targeting this receptor requires that the delivered radionuclide kill tumor cells entirely via by-stander radiation delivered from adjacent non-leukemic cells in the marrow space. Fifty-five patients with AML or MDS who were in CR or partial remission (PR) but judged to be at high risk for relapse after standard HCT were enrolled in this phase I/II trial [40]. All patients enrolled had favorable initial biodistributions and received a therapeutic dose of [^{188}Re]anti-CD66 MAb delivering a mean of 15.6 Gy of additional radiation to the marrow. Patients were then treated with a variety of full-dose conditioning regimens followed by allogeneic HCT. T-cell depletion for graft-versus-host disease (GVHD) prophylaxis was achieved by either CD34$^+$ selection or administering Campath-1H in the donor cell infusion bag. The kidney was the organ receiving the highest dose of supplemental radiation (mean, 7.2 Gy) and late renal toxicity was a complication in 11% of patients with an incidence correlating with the renal dose exposure. There was no increase in treatment-related mortality associated with the addition of RIT. Patients transplanted in CR or good PR (<15% blasts in the marrow) had the best outcomes, with 59% disease-free at median follow-up. In contrast, only 8% of patients who had more than 15% blasts in the marrow were alive at this time point [85]. Less promising results were reported in another study in which [^{188}Re]anti-CD66 was combined with a conventional preparative regimen. A significantly higher rate of transplant-related toxicity and acute GVHD was reported with a transplant-related mortality of almost 50% (nine of the 19 patients enrolled), likely due, at least in part, to the advanced AML or ALL patients treated on this study [86].

Non-Hodgkin lymphoma

NHL cells are ideal targets for RIT because of their exquisite radiosensitivity and their stable expression of well-characterized lineage-specific surface antigens. The direct cytotoxic effect of targeted radiation obviates the need for reliance on the host's immune effector mechanisms, which are frequently impaired in NHL patients. The approval of two CD20 targeted radioimmunotherapeutic agents by the FDA for the treatment of relapsed or refractory follicular and transformed follicular NHL {[^{131}I]tositumomab (Bexxar™) and [^{90}Y]ibritumomab-tiuxetan (Zevalin™)} generated excitement about the promising results seen with both standard and high-dose myelosuppressive treatment regimens.

Some of the earliest NHL patient studies using MAbs employed patient-specific anti-idiotypic antibodies raised against the tumor-specific clonal idiotype of the immunoglobulin (Ig) molecule expressed on B-cell malignancies [87,88]. These trials produced promising results with durable (>5 years) objective remissions in 60–70% of patients studied; however, the technical and financial limitations inherent in patient specific production of anti-idiotypic antibodies rendered the widespread use of this approach infeasible. Subsequently, most investigators focused on targeting lymphocyte differentiation antigens expressed on both normal and malignant lymphoid cell surfaces. The CD20 antigen was a particularly attractive target because of its high density (~150,000/cell) and the presence on more than 90% of B-cell tumors [89]. Further, most investigators believe that it is minimally shed or internalized following antigen binding and, while present on almost all B-lymphocytes, the temporary elimination of normal B cells by anti-CD20 antibodies has not proven a significant limitation.

Initial studies with unmodified murine anti-CD20 MAb were of limited success owing to the restricted capacity of murine Ig to fix human complement or mediate antibody-dependent cell-mediated cytotoxicity (ADCC) with human effector cells. The first patient studies, conducted by Press et al. in Seattle, demonstrated that high doses of the murine anti-CD20 MAb 1F5 could be administered safely to patients, and in individuals receiving the highest doses, dramatic albeit short-lived remissions could be achieved [90]. These initial studies helped to pave the way for the development of a human–mouse chimeric anti-CD20 MAb, rituximab, which ameliorated the murine MAb-associated limitations [91]. Early studies by Maloney et al. reported objective remissions in 46% of patients treated with rituximab in a multicenter phase II trial [92]. These results were confirmed in a subsequent study in which 48% of patients with indolent NHL achieved objective responses and 6% achieved CR. The median duration of response was approximately 1 year [93].

Although the initial studies with rituximab were encouraging, almost half of all patients with relapsed indolent NHL and 60–70% of patients with relapsed aggressive lymphomas failed to respond to single-agent rituximab. Furthermore, only 5–10% of patients with indolent NHL achieved CR with rituximab and the median response duration was less than 1 year. In light of these factors, a number of investigators continued to pursue mechanisms for amplifying the therapeutic promise of MAbs by conjugating them to radionuclides.

The first studies to evaluate the potential efficacy of RAbs in NHL were conducted by DeNardo et al. in the mid-1980s [94]. In these studies, an antibody recognizing a DR variant antigen (Lym-1) was conjugated to ^{131}I. DeNardo's group demonstrated both the feasibility and safety of RIT in a population of advanced-stage patients with relapsed B-cell malignancy; moreover, they documented an objective remission rate of approximately 50% in a population of patients with disease that had been resistant to standard therapy. Subsequently, a robust literature developed reporting clinical trial data that demonstrated the safety and efficacy of non-myeloablative RIT in B-cell NHL. While most centers focused on CD20-targeted RIT at standard non-myeloablative doses without the need for hematopoietic progenitor support, two basic approaches have emerged for incorporating RIT as part of a transplant conditioning regimen for NHL. The first approach maximizes the therapeutic potential of RIT by escalating the dose to the non-hematopoietic toxicity threshold either with or without high-dose chemotherapy much akin to the use of high-dose TBI. A second tactic adds standard non-myeloablative doses of RIT to a maximized myeloablative chemotherapy conditioning regimen.

Myeloablative RIT-based autologous conditioning regimens

In the mid-1980s at the FHCRC in Seattle, a series of studies were initiated to investigate the possibility of replacing external-beam TBI with targeted RIT as a conditioning regimen for HCT [95]. Integration of RIT into HCT conditioning regimens was tested to determine if it could maximize the tumor-to-normal organ ratios of delivered radiation, thereby improving cure rates while minimizing additional toxic effects. The first such study was designed to compare the biodistribution profiles for a number of different B-cell antibodies, to determine the impact of MAb dose on biodistribution, and to assess the toxicity and potential efficacy of increasing doses of ^{131}I conjugated to the B-cell antibodies under study [21,96]. This phase I dose-escalation trial studied the biodistribution of anti-CD37 and anti-CD20 MAbs in 43 patients with relapsed B-cell NHL. In the patients who received therapeutic infusions, maximal doses to normal organs were calculated according to dose-escalation parameters allowing delivery of radiation between 10 and 31 Gy to critical normal organs with therapeutic ^{131}I doses ranging from 234 to 777 mCi. Pulmonary doses of 27 Gy defined the maximum tolerated dose (MTD) with two of four patients developing reversible cardiopulmonary toxicity at this level. Objective responses were seen in 95% of patients receiving therapeutic infusions of ^{131}I-MAb with 85% achieving a CR. Eight patients remained in CR 46–95 months following therapy.

Patients assessed with anti-CD20 MAbs (tositumomab or 1F5) exhibited biodistributions demonstrating superior targeting compared with the other MAbs evaluated (anti-CD37 and anti-idiotype). Based on this finding, a phase II study with high-dose [^{131}I-]tositumomab (formerly known as anti-B1 MAb) was performed. The regimen was well tolerated with minimal toxicity compared with standard TBI based conditioning regimens. Furthermore, in a series of 125 consecutive patients with indolent B-cell NHL undergoing high-dose conditioning and autologous HCT, the outcomes in the RIT patients compared favorably in a multivariate model with the control population treated with conventional autologous HCT conditioning regimens [48% 5-year progression-free survival (PFS) in the RIT group versus 29% in the conventional transplant group, $p = 0.03$] [97].

Although the rates of CR and prolonged remissions in this population with multiply relapsed B-cell NHL were encouraging, maximal doses of RIT with stem-cell support still averted eventual relapse in only about 50% of the patients studied. To improve further the fraction achieving durable CR, Press et al. conducted a phase I/II study combining [^{131}I]tositumomab with high-dose etoposide, CY, and autologous HCT [72]. Fifty-two patients under the age of 60 years were treated on this protocol and the MTD was determined as 25 Gy of ^{131}I-tositumomab (1.7 mg/kg), 60 mg/kg etoposide and 100 mg/kg CY. At 2 years, the estimated overall survival for all 52 patients enrolled was 83% and PFS was 68%. When compared with a non-randomized control group of 105 NHL patients who were treated with TBI–CY–etoposide and autologous HCT in a multivariate analysis that incorporated disease grade (indolent versus aggressive), stage, and chemosensitivity, the patients in the RIT-based conditioning regimen group demonstrated superior PFS and overall survival (OS) [Figure 23.3; 38% ($p = 0.006$) and 50% ($p = 0.01$)].

Patients with mantle cell lymphoma (MCL) comprise 4–10% of all NHL and represent a special challenge as they exhibit the poorest long-term survival of any lymphoma subtype. There is no consensus regarding optimal first-line treatment for MCL patients and the disease is generally considered incurable with chemotherapy regardless of the regimen employed (survival ~2–3 years) [98]. Furthermore, conventional HCT performed after relapse has generally been unsuccessful [99,100]. The Seattle group performed a pilot study evaluating outcomes in 16 patients with relapsed MCL with the above autologous HCT conditioning regimen employing [^{131}I]tositumomab followed by CY and etoposide [101]. As a result, the overall response rate for the small number of evaluable patients was 100% with 91% achieving a CR. A large ($n > 100$) phase II trial by the Seattle group evaluating this combined high-dose chemo-RIT approach in patients with aggressive, indolent, and MCL has completed accrual and is expected to be reported soon.

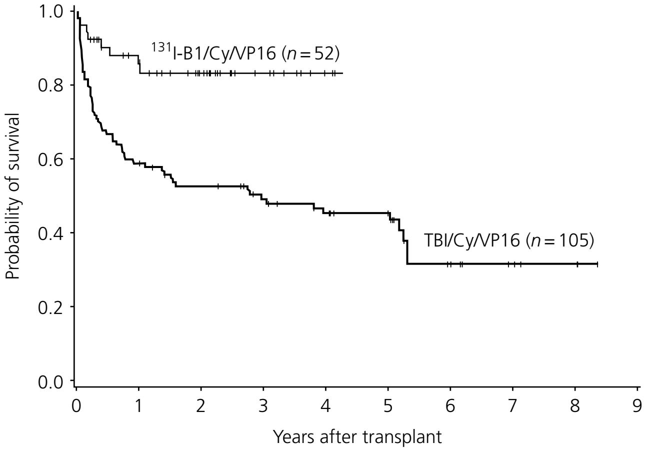

Figure 23.3 Overall survival of patients with relapsed B-cell lymphomas who underwent high-dose chemoradiotherapy including either [^{131}I] tositumomab at myeloablative doses (52 patients; top line) or a standard total body irradiation (TBI)-based preparative regimen (105 control patients; bottom line) followed by autologous HCT. Patients in the RIT group received up to 25 Gy to normal organs; the TBI group received 1.5 Gy twice per day for 4 days. Both groups were treated with identical doses of etoposide and cyclophosphamide. Source: Press et al. 2000 [72]. Reproduced with permission of the American Society of Hematology.

A similar strategy was also employed by Nademanee et al. at the City of Hope Cancer Center, where [^{90}Y]ibritumomab tiuxetan was escalated in place of TBI and combined with 60 mg/kg etoposide and 100 mg/kg CY prior to autologous HCT [102]. In this phase I/II trial, patients were given escalating doses of radiation based on dosimetry during phase I, and the MTD to critical organs was calculated to be 10 Gy. Subsequently, 31 patients with follicular ($n = 12$), diffuse large B-cell ($n = 14$), and MCL ($n = 5$), including seven in first CR or PR, were treated with 10 Gy of [^{90}Y]ibritumomab tiuxetan (36.6–105 mCi) in combination with either 40 mg/kg (cohort 1; six patients) or 60 mg/kg (cohort 2; 25 patients) of etoposide and a fixed-dose CY (100 mg/kg). There were five relapses and 24 of the 31 patients were alive and in remission at a median follow-up of 22 months after therapy. The 2-year RFS and OS were 78% and 92%, respectively. These findings provide further data on the promise of integrating CD20-targeted RIT into HCT conditioning regimens. Although it is enticing to compare the results from this ^{90}Y-based trial with ^{131}I findings reported by Press et al., it should be reiterated that the patient profiles in these two studies were very different in terms of inclusion criteria, differences in radiation dose, and number of prior therapies. A similar study of relapsed and refractory B-cell NHL patients was conducted by Winter et al. at Northwestern University [26]. In this phase I trial, 44 patients received dose-escalated [^{90}Y]ibritumomab followed by high-dose carmustine, etoposide, cytarabine, and melphalan (BEAM) and autologous HCT. One patient developed transient veno-occlusive disease at the 700 cGy level and two dose-limiting toxicities occurred at the 1700 cGy dose level; one patient developed grade IV stomatitis on day 10 and a second patient developed septic pulmonary emboli on day 13. In addition, one heavily pretreated patient developed MDS on day 291. With a 21-month median follow-up, the 3-year OS and PFS were 52% and 37%, respectively.

Patients aged over 60 years comprise over half of all new diagnoses of NHL in the United States and are at particularly high risk for poor outcomes when diagnosed with NHL [103], yet individuals in this age group have been excluded from most HCT trials owing to concerns about the toxicity of the conditioning regimens. Gopal et al. sought to exploit the reduced non-specific normal organ toxicity seen with RIT-based conditioning regimens in a phase II safety and efficacy trial employing single-agent high-dose [^{131}I]tositumomab conditioning prior to autologous HCT for this group of older adults [104]. Twenty-four heavily pretreated NHL patients ≥60 years of age (median age 64 years) received 328–1154 mCi of [^{131}I]anti-CD20 RAb to deliver a maximum of 25–27 Gy to critical organs. The estimated 3-year OS and PFS rates were 59% and 51%, respectively. Based on these promising safety and efficacy data, the desire to improve outcomes further, and the preclinical data suggesting a potent supra-additive effect for RIT combined with nucleoside analogs [75,105], a phase I trial of combined fludarabine–[^{131}I]tositumomab trial for patients ≥60 years has been completed [106]. This study of 36 B-cell NHL patients with a median age of 65 years (range, 60–76 years) demonstrated that up to 210 mg/m^2 of fludarabine could be delivered concurrently with [^{131}I] tositumomab delivering ≤27 Gy to critical normal organs. Toxicity was again moderate compared with conventional high-dose regimens with no early treatment-related deaths, 69% of patients remained outpatients following discharge from radiation isolation, and only two developed grade 4 non-hematologic toxicity (hypokalemia and depression) with a 3-year PFS of 52%.

More recently, high-dose RIT approaches have also been explored by other groups. Ferrucci et al. dose escalated [^{90}Y]ibritumomab tiuxetan based on weight, using three predefined dose levels (0.8, 1.2, and 1.5 mCi/kg) [107]. Patient-specific dosimetry was performed in all cases to evaluate the distribution of activity in critical organs. Absorbed doses to target organs were highly variable for any of the cohorts. At the most recent update, 25 patients were reported including those at the highest dose level of 1.5 mCi/kg [108]. The median activity of ^{90}Y was 100 mCi and all patients engrafted promptly. Similarly, Devizzi et al. in Milan reported on 29 patients with B-cell NHL who received either 0.8 or 1.2 mCi/kg followed by tandem reinfusions of mobilized peripheral blood progenitor cells on days +7 and +14 [109]. No significant non-hematologic toxicities were reported and engraftment was rapid, although it cannot be concluded that the tandem infusions were of benefit over the conventional approach. Flinn et al. likewise dose escalated [^{90}Y] ibritumomab tiuxetan with mobilized peripheral blood progenitor cell support in patients with relapsed or refractory B-cell NHL [110]. As suggested by these three series, dose escalation of single-agent ^{90}Y-ibritumomab tiuxetan is possible, but whether dose escalation should be performed using dosimetry to define a specific absorbed dose to the critical organ or by weight remains to be established. If dosed according to weight, 1.2–1.5 mCi/kg would appear to be the preferred dose range.

Other groups have also investigated high doses of single-agent RIT, including a small trial conducted in Germany. Behr et al. reported on seven patients treated with potentially myeloablative doses of either [^{131}I]rituximab (five patients) or a humanized]^{131}I] anti-CD22 MAbs (two patients). Complete responses were reported in all five patients who received stem-cell support [111].

Other RIT targets have also been explored in a more limited fashion in this setting. The CD22 surface receptor has been targeted by a number of groups. Goldenberg's group reported on three patients treated with potentially myeloablative doses (90 mCi/m^2) of [^{131}I]anti-CD22 (LL2 IgG) followed by autologous bone marrow support. The two evaluable patients had PR lasting 2 and 8 months [112]. Vose et al. treated 21 patients in a phase I/II trial using multiple doses of [^{131}I]LL2 MAb with an overall CR rate of 24% [113]. Five patients in this study required autologous HCT due to prolonged cytopenias. Based on preclinical data, the Seattle group is currently evaluating targeting the pan-hematopoietic antigen CD45 as a method to treat both T- and B-cell NHL and circumvent the potential risk of CD20 blocking by rituximab [27,114].

One of the limitations associated with high-dose ^{131}I RIT conditioning regimens is the requirement that patients remain in inpatient isolation until their body radiation levels return to safe levels. Likewise, the use in RIT of doses of ^{90}Y above what is commercially available can pose technical and financial challenges. To study the efficacy of a straightforward outpatient-based approach, a phase I trial was conducted combining conventional, non-myeloablative doses of [^{131}I]tositumomab with the BEAM high-dose chemotherapy regimen followed by autologous HCT in 23 patients with aggressive chemo-refractory NHL. The total body dose of radioimmunoconjugate was escalated up to the standard 75 cGy dose approved for this radiopharmaceutical. There was no significant difference in toxicity for patients receiving 75 cGy of ^{131}I compared with patients receiving high-dose BEAM alone. The CR rate after HCT was 57% and the overall response rate was 65%. At a median follow-up of 38 months, event-free survival and OS were 39% and 55%, respectively [115,116].

A subsequent phase II trial by the City of Hope Cancer center employed a similar strategy with encouraging results by adding [^{90}Y]ibritumomab tiuxetan to high-dose BEAM chemotherapy conditioning prior to autologous HCT. Forty-one patients with B-cell NHL whose age or prior radiotherapy deemed them ineligible for TBI or high-dose [^{90}Y]ibritumomab tiuxetan were studied. All patients received a standard non-myeloablative dose of [^{90}Y]ibritumomab (0.4 mCi/kg), with no dose adjustment for neutropenia or thrombocytopenia, followed by high-dose BEAM therapy 1 week later. After a median follow-up of 18.4 months, the estimated 2-year OS was 88.9% and PFS was 69.8% [117]. Overall, the rates of treatment-related toxicities were not different from those reported for patients receiving BEAM therapy alone. A subsequent multivariate comparison of the study patients with those treated with TBI-based conditioning favored the RIT plus BEAM approach [118].

Shimoni et al. in Israel have also reported on the use of combined standard-dose [^{90}Y]ibritumomab tiuxetan with high-dose BEAM chemotherapy for aggressive NHL [119]. Their findings were similar to those in the previous studies with an overall CR rate of 76% in the 21 evaluable patients (of the 16 patients in CR, five were initially in PR but reached CR after additional radiation). The 2-year PFS and OS were 52% and 67%, respectively. These data led to at least two randomized phase III trials evaluating the potential benefit of adding standard-dose anti-CD20 RIT to BEAM versus BEAM or rituximab–BEAM. The largest study to date, led by Vose et al., compared [^{131}I]tositumomab plus BEAM with rituximab plus BEAM in patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) [120]. Disappointingly, OS, PFS, and relapse rates were similar in both groups, with the best predictor of outcome in either arm being pre-transplant CR. A second randomized trial was reported by Shimoni et al. This study compared standard [^{90}Y]ibritumomab tiuxetan (0.4 mCi/kg, 32 mCi maximum) plus BEAM with BEAM alone, again with autologous stem-cell support [121]. In contrast to the study by Vose et al., patients receiving [^{90}Y]ibritumomab tiuxetan plus BEAM demonstrated an improved 2-year OS compared with those receiving BEAM alone (91% versus 62%; p = 0.05). A multicenter expansion of this trial is under way to confirm or refute these results.

RIT-based allogeneic transplant conditioning regimens for NHL

Until recently, limited data were available on the use of RIT in conditioning regimens for allogeneic HCT, primarily in the form of case reports and small case series [122]. Within the last few years, there have been at least four series evaluating the use of RIT as part of an allogeneic transplant conditioning regimen for NHL. Shimoni et al. described a series of 12 B-cell NHL patients who received standard [^{90}Y]ibritumomab tiuxetan (0.4 mCi/kg, capped at 32 mCi) on day −14 prior to a regimen on fludarabine either BU (n = 6) or melphalan (n = 6) [123]. The 2-year PFS and non-relapse mortality (NRM) were 33% and 42%, with the best outcomes observed in the three patients with follicular NHL. These data suggested that this approach was feasible, but this regimen yielded a high NRM rate due to acute GVHD in most patients, attributed to an abbreviated duration of GVHD prophylaxis. Bethge et al. conducted a prospective multicenter trial of standard [^{90}Y]ibritumomab tiuxetan (0.4 mCi/kg, capped at 32 mCi) prior to a conditioning regimen of fludarabine and 2 Gy TBI followed by allogeneic HCT from matched donors [124]. This study included 40 patients with relapsed/refractory indolent B-cell NHL or MCL without pre-transplant progressive disease. The 2-year OS and EFS of these patients were 51% and 43%, respectively, with a 45% 2-year cumulative incidence of NRM. The best outcomes were observed in those with follicular NHL, chronic GVHD, and acute GVHD less than grade 3.

Gopal and colleagues published a similar trial of 40 patients with relapsed/refractory B-NHL using standard [^{90}Y]ibritumomab tiuxetan (0.4 mCi/kg, capped at 32 mCi) prior to a fludarabine and 2 Gy TBI conditioning regimen with matched related or unrelated allogeneic HCT [125]. Notable differences between this trial and that of Bethge et al. were the inclusion of patients with aggressive B-cell NHL, the exclusion of patients who were in CR prior to HCT, and earlier administration of fludarabine in the conditioning regimens to maximize the potential antitumor synergy between RIT and the nucleoside analog. The estimated 30-month survival, PFS, and NRM of this approach were 54.1%, 31.1%, and 15.9%, respectively. Again, the best outcomes were seen in those patients with indolent histology and those with baseline platelet counts over 25,000/μL and related donors. Notably, disease control and outcomes were excellent in those who achieved a CR or PR by 3 months post-HCT, suggesting that if early disease control can be achieved, long-term remissions can be maintained by the allogeneic graft. Khouri et al. also published data evaluating standard [^{90}Y]ibritumomab tiuxetan (0.4 mCi/kg, capped at 32 mCi) prior to a fludarabine–CY-based conditioning and allogeneic transplant in 26 patients with relapsed follicular NHL [126]. Patients in this trial also experienced an excellent outcome, with 80% alive and progression free at 3 years.

A second study by Bethge's group evaluated the ability to improve outcomes further in patients with aggressive B-cell NHL by further escalating the dose of ^{90}Y [127]. This phase I trial of 20 patients demonstrated that ^{90}Y up to 0.8 mCi/kg could be safely administered prior to a fludarabine, melphalan, and alemtuzumab conditioning regimen, although the long-term survival in this extremely high-risk group of patients was only 20%. Building on this approach and the data using high-dose [^{90}Y]ibritumomab tiuxetan prior to autologous transplantation, the Seattle group is currently conducting a phase II trial of 1.5 mCi/kg prior to fludarabine, 2 Gy TBI, and allogeneic HCT for patients with aggressive B-cell NHL. Overall, data collected to date indicate that standard or moderately escalated [^{90}Y]ibritumomab tiuxetan can be safely added to a variety of allogeneic RIC regimens and may yield improved outcomes in patients with refractory or bulky indolent B-cell NHL with little additional toxicity, although comparative trials would be required to verify this hypothesis.

Hodgkin lymphoma

The protein antigen ferritin is found at elevated concentrations in the Reed–Sternberg cells of Hodgkin lymphoma. Polyclonal antiferritin antibodies labeled with ^{131}I were initially studied at nonmyeloablative [128,129] and myeloablative [130] doses, documenting the safety and efficacy of this approach. Schnell et al. reported a trial with [^{131}I]anti-CD30 MAbs in patients with Hodgkin disease [131]. Although the patient population in this study admittedly had highly resistant disease with a majority having failed prior autologous HCT, the selection of CD30 as a target antigen may also have been a limitation to the success of this approach. The presence of CD30 is limited to Reed–Sternberg cells that have a relatively sparse distribution, hence only a small amount of radioactivity can be delivered to target sites using anti-CD30 MAbs. More recently, Dancey et al. reported the use of ^{131}I-labeled anti-CD25 MAbs in patients with Hodgkin lymphoma [132]. Given the limited antigenic targets on the Reed–Sternberg cells, an alternative strategy is to target bystander cells, for example via the CD45 antigen that is present on most all hematolymphoid cells, and rely on a principle of cross-fire to deliver the therapeutic radionuclide.

Solid tumors

Solid tumors are generally less sensitive to the effects of radiation than hematologic malignancies and therefore less attractive for studies of RIT. Nevertheless, selected tumor types are sufficiently radiosensitive to merit evaluation with this approach. DeNardo et al. at the University of California (Davis) have explored ^{131}I-labeled chimeric MAbs reactive with the L6 cell-surface antigen, highly expressed on lung, breast, colon, and ovarian carcinomas [133]. In a small study, three patients were given ^{131}I-labeled MAbs followed by autologous stem-cell rescue, with one patient demonstrating a minimal response.

Anti-carcinoembryonic antigen (CEA) MAbs have been generated against a variety of CEA epitopes and used for therapy in a variety of tumors of epithelial origin. In breast cancer patients, a murine IgG1 MAb, T84.66, was studied in six patients treated with [^{90}Y]DTPA-cT84.66 followed by autologous HCT [134]. Patients received between 15 and 22.5 mCi/m^2. All showed temporary but measurable therapeutic responses and the MTD was not reached. Another group reported phase I dose-escalation data using high-dose ^{90}Y conjugated to a MAb reactive with a human milk fat-associated antigen (BrE-3) [135]. In this study, nine patients with advanced breast cancer were treated with 15–20 mCi/m^2 of [^{90}Y]BrE-3. Four of eight patients had partial responses. Anti-MUC-1 MAb 170H.82 is directed against an asialo-GM1 antigen that is highly expressed in adenocarcinoma of the breast. This anti-MUC-1 MAb was labeled with ^{90}Y and given to three patients with metastatic breast cancer with subsequent reinfusion of previously collected peripheral blood stem cells (PBSCs). A PR was reported in one patient [136]. A second study by the same group reported on three additional patients who received PBSC infusion to hasten hematopoietic recovery after ^{90}Y-labeled anti-MUC-1 MAb therapy. Two patients experienced a minor reduction in soft tissue disease lasting less than 1 month [137]. Autologous stem-cell support has also been used to enable dose escalation with [^{131}I]anti-CEA MAbs in patients with metastatic medullary thyroid cancer [138] and in children with neuroblastoma receiving ^{131}I-labeled 3F8 anti-GD2 MAbs [139]. Most studies of RIT for solid tumors since those described above have focused on its use without stem-cell support.

Toxicity

Toxicity, in the context of RAbs in HCT, falls into three general categories: acute infusional reactions, short-term toxicities associated with radiation delivery, and long-term sequelae. Acute infusion-associated toxicities are MAb specific. The anti-CD20 MAbs (rituximab, ibritumomab tiuxetan, and tositumomab) are usually associated with only mild reactions and, in the case of rituximab, tolerance improves with repeated infusions. In contrast, anti-CD45 BC8 MAbs can lead to fever, chills, and mild hypotension (when administered rapidly). The severity of these reactions can usually be reduced by premedication with diphenhydramine, meperidine hydrochloride, or hydrocortisone. The short-term toxicities seen after high-dose RIT for HCT are usually related to myelosuppression. Pancytopenia is an expected consequence and relatively prolonged periods of thrombocytopenia are not atypical. Residual radionuclide in the marrow can potentially pose a toxic risk to newly transplanted marrow cells. To prevent such damage, transplants are usually delayed until the residual radiation in the marrow space is of minimal risk. Press et al. performed the only study to date that delineates the dose-limiting non-hematopoietic toxicities from single-agent RAb therapy [96]. In this study, 27 Gy to the lung was dose limiting, which represents a significantly higher dose than has been tolerated with external-beam TBI (maximum dose 16 Gy). Other short-term toxicities seen with RIT are similar to those associated with external-beam TBI. These include mucositis, nausea, and vomiting. Alopecia is uncommonly seen with high-dose RIT, in contrast to external-beam TBI. Longer term sequelae include hypothyroidism in almost 70% of patients receiving ^{131}I therapy (even when prophylactic potassium iodide is given). Nephropathy was reported in 6 of 93 patients treated with [^{188}Re]anti-CD66 RAb as part of an allogeneic HCT conditioning regimen [140]. In a multivariate comparison of 125 patients who received either high-dose RIT or conventional high-dose therapy with TBI followed by autologous HCT, the long-term risk of therapy-related MDS/AML was between 6 and 7% for both groups, suggesting that no increased risk was associated with the RIT-based regimen [97].

At standard (reduced-intensity) doses, RIT in NHL has a CR rate of ~30% with 20–25% of patients experiencing long-term DFS; therefore, a majority of non-myeloablative RIT patients will ultimately require additional treatment. The impact of RIT on subsequent autologous stem-cell collection has been a subject of interest and concern. In a study by Ansell et al. designed to assess subsequent therapies after [^{90}Y]ibritumomab tiuxetan, a cohort of eight patients were described who underwent PBSC after RIT. One patient in this group required an additional bone marrow harvest to achieve adequate cell counts, but all eight patients engrafted normally [141].

Future directions

Preclinical studies in experimental rodents and primates were instrumental in demonstrating the feasibility and potential efficacy of RIT as a treatment modality [142]. Undoubtedly, preclinical studies remain critical for the development of radioimmunotherapeutic approaches. This research will focus its efforts in several different directions, including the identification of alternative target antigens that demonstrate enhanced efficacy for RIT and also the exploration of alternative radionuclides that offer improved specificity of tumor targeting and lower non-specific toxicities. Other attempts will focus on improving the specificity of radiation delivery and enhancing the tumor penetration with targeted radionuclides.

The number of antigens targeted with RIT may expand significantly over the coming years. Many are under active investigation, including CD22, CD25, CD30, and CD52. For example, even though CD25 has so far not gained major importance for RIT of leukemias [143], it remains an attractive target owing to its relatively selected expression on a high proportion of malignant cells in certain forms of lymphoid neoplasms, including adult T-cell leukemia. Given this expression pattern, α-particle therapy may be particularly appealing in an attempt to spare normal hematopoietic tissue. To this end, a ^{211}At-labeled murine anti-CD25 IgG2a MAb (7G7/B6) has been developed and tested with promising results in immunodeficient mice bearing CD25-positive leukemia and lymphoma cells [144]. Using a similar model, the same group also achieved promising results using a ^{211}At-labeled murine anti-CD30 IgG1 MAb (HeFi-1) in prolonging survival of immunodeficient mice bearing tumors that express CD30, an antigen that is found on some lymphocyte subsets but is also expressed on some lymphoid neoplasms [145]. Together, these findings may set the foundation for progression to clinical studies using ^{211}At-labeled MAbs for the treatment of patients with either CD25- or CD30-expressing leukemias. CD52 is normally expressed on B and T-lymphocytes, monocytes, macrophages, eosinophils, natural killer cells, dendritic cells, and cells of the male reproductive tract [146]. However, CD52 is also expressed by a variety of lymphoid neoplasms and has therefore been exploited as a target for MAb-based therapies, in particular for CLL. Alemtuzumab (Campath-1H), a humanized rat IgG1 MAb directed against CD52, has shown activity alone and in combination with other therapeutics for untreated and previously treated patients with CLL [146–148]. More recently, this MAb has been explored for RIT using ^{188}Re [149]. Preclinical studies found [^{188}Re] alemtuzumab to have good in vitro stability and showed high uptake in blood with biexponential clearance and some increased uptake observed in kidneys and heart [149]. Among the targets explored for treatment of CLL is also CD23. An unconjugated anti-CD23 MAb, lumiliximab (IDEC-152), has shown promising activity in clinical studies [148,150,151] and it is conceivable that this, or other MAbs under development may be used to deliver radiation to tumor sites.

Parallel efforts are ongoing to identify novel radionuclides and radionuclide–antibody conjugates and define their antitumor properties. For example, a ^{213}Bi-labeled anti-CD45 MAb (YAML568) is under preclinical development that shows interesting properties regarding induction of apoptosis and breakage of chemoresistance and radioresistance by overcoming DNA repair mechanisms in leukemia cells [152]. The high LET of ^{213}Bi RIT may also provide synergistic cytotoxic activity when combined with antileukemic chemotherapeutics, for example, for the treatment of CLL [153]. Other examples of radioimmunoconjugates that potentially are exploited in the clinical setting include [^{90}Y]B4, [^{90}Y]BU12 and [^{90}Y] HD37 (mouse IgG1 anti-CD19 MAb), [^{90}Y]RFB4 (mouse IgG1 anti-CD22 MAb), [^{211}At]hTac (humanized anti-CD25 MAb), [^{213}Bi] rituximab (humanized anti-CD20 MAb), and ^{177}Lu-labeled anti-CD20, anti-CD22, and anti-HLA-DR MAbs [154–160].

Clinical data have demonstrated that RAb therapy is capable of delivering 2–4-fold more radiation to target sites than surrounding normal tissues. When patients with relapsed lymphoma receive myeloablative doses of anti-CD20 RIT followed by stem-cell rescue, rates of response have improved dramatically. Objective remissions are seen in 85–90% of such patients, with 75–80% experiencing durable CR, with many lasting 5–18 years [72,96,161,162]. Optimizing the therapeutic index of RIT further by improving biodistribution ratios in favor of target cells may improve outcomes in both hematologic malignancies and solid tumors. A number of pharmaceutical and academic research centers are focusing on the identification of new antigenic targets that are highly expressed on malignant cells and minimally expressed on normal tissues. The increased availability of β-emitting radionuclides with favorable physical characteristics, such as ^{177}Lu and ^{67}Cu, may provide another avenue for future investigations. The α emitters, including those with longer half-lives such as ^{225}Ac, also show great promise [163]. Investigators in Seattle and at the University of Tübingen (Germany) have demonstrated in a canine model that TBI can be entirely replaced with [^{213}Bi]anti-CD45 MAbs in allogeneic reduced-intensity HCT [164].

A number of novel approaches are also being explored to improve the ratio of target to non-target exposure. As noted previously, dose escalation in high-dose RIT for HCT is limited by non-specific irradiation of normal tissues. The cause is both the slow distribution of the RAbs to target cells and the presence of unbound labeled

MAbs persisting in the circulation. One approach, pretargeted RIT, is a process in which patients are initially treated with tumor-reactive MAbs that are not bound to therapeutic radionuclides. This allows localization to tumor sites without subjecting the rest of the body to non-specific irradiation. After maximal accumulation of MAbs in the tumor, a low molecular weight radioactive moiety with high affinity for the tumor-reactive MAbs is administered. Because of its small size, this second reagent rapidly penetrates tumors, where it binds to the pretargeted MAbs. The unbound molecules of the second (radioactive) reagent are small enough to be rapidly cleared from the blood and excreted in the urine within minutes. To improve further the targeted delivery of the radioimmunoconjugate, a clearing agent can be injected shortly before the radiolabeled small molecule to remove unbound MAbs from the bloodstream. The high-affinity binding that occurs between the pretargeted MAbs and subsequently administered radiolabeled small molecule can be achieved through a number of proposed mechanisms. One of the most promising exploits the exceptionally high affinity of avidin for biotin, and another approach employs bispecific MAbs. In animal models, pretargeted RIT has generated therapeutic tumor-to-normal organ ratios of greater than 10:1 (Figure 23.4) and cures have been achieved in 100% of animals with lymphoma and solid tumor xenografts [165,166]. Early patient trials have exhibited similarly encouraging findings in both solid tumors and lymphoma [167–169]. A pretargeted RIT trial for patients with relapsed/refractory AML is ongoing in Seattle.

Nanomaterials also have enormous potential in a wide range of applications associated with HCT. Recently, increased attention has been placed on nanomaterials for this application since the nanostructured surfaces offer a large surface area and greater reactivity, making nanovectors potentially ideal for targeted RIT [170,171]. Nanoparticle materials can be synthetically modified for RIT using well-established techniques after radionuclide loading. Multiple studies over the past decade have been performed to determine the efficacy of nanoparticle carriers for drug delivery (i.e., radiopharmaceutical and chemical). While initial studies determined that blood circulation time and hepatic clearance were primarily dependent on nanoparticle size [170,172,173], a number of more recent studies have shown that there are many factors that determine circulation time and clearance, for example, surface coating and surface charge [171,174–177]. presence of a high immunological protein concentration on the particle surface [178–180], and particle size [171,173,174,181,182]. In fact, the term “stealth particle” has been coined to describe the characteristics necessary in a successful carrier nanoparticle; it should be targeted for delivery of its payload, have a sufficient contact time with the target via an antibody or small molecule, and not be removed by the reticuloendothelial system (RES) so that toxic levels of drug, radiation, or particles are not accumulated in the liver and spleen [170]. To target blood-borne cancer cells, a radiolabeled nanoparticle must have an appropriately long circulation time and have its accumulation in the RES (liver and spleen) and uptake on the bone surfaces slowed sufficiently to maximize therapeutic efficacy and minimize the dose to healthy tissues.

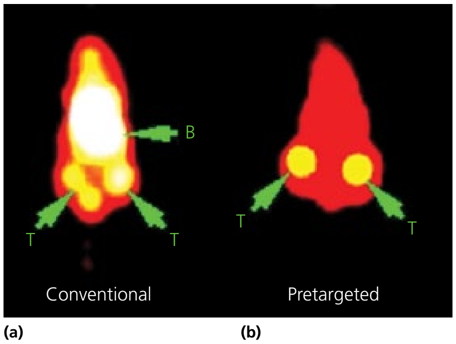

Figure 23.4 Efficacy of pretargeting as demonstrated by planar gamma camera images of Burkitt lymphoma xenograft tumors in athymic mice. Animals were injected with either directly labeled [^{111}In]anti-CD20 Ab (a) or pretargeted with anti-CD20–streptavidin conjugate followed 24 hours later by clearing agent and then by [^{111}In]DOTA–biotin (b). Arrows indicate radioactivity in tumors (T), and blood pool (B) 24 hours after injection of ^{111}In-labeled reagents. Bladder activity is also seen in the conventional mouse. Source: Subbiah K. et al. 2003. Comparison of immunoscintigraphy, efficacy, and toxicity of conventional and pretargeted radioimmunotherapy in CD20-expressing human lymphoma xenografts. *J. Nucl. Med.* 2003; **44**: 437–445. Reproduced with permission of the Society of Nuclear Medicine. (For a color version of this figure, please see the color plate section.)

Extracorporeal adsorption therapy (ECAT) offers another promising means for optimizing biodistribution. In this process, RAbs are injected and allowed 24–48 hours to circulate and distribute to antigen-bearing cells. Then patients undergo a hemodialysis-like procedure during which their blood volume is circulated across an avidin–agarose column. In this way, the biotin-conjugated RAbs that remain in the circulation are removed, thereby minimizing the non-specific radiation to normal organs typically caused by perfusion with blood containing RAbs. In a small safety and efficacy trial conducted in Sweden [183], seven patients with relapsed or refractory B-cell NHL received biotin-conjugated [^{90}Y]rituximab, followed 24–48 hours later by ECAT filtration. The procedure was demonstrated to be safe and effective and ~96% of the unbound [^{90}Y]rituximab-biotin was cleared from the blood.

An exciting new avenue to improve the efficacy of radioimmunoconjugates and potentially reduce non-specific toxicities entails MAb modifications that could allow the clinical use of the Auger electron-emitting radionuclide ^{111}In in combination with anti-CD33 MAb. Specifically, M195 and HuM195 were modified with a 13-mer peptide (CGYGPKKKRKVGG) harboring the nuclear localization sequence of SV-40 large T antigen [184]. This resulted in efficient internalization and improved routing of the radioimmunoconjugate to the nucleus of leukemia cells compared with MAb constructs lacking the nuclear localization sequence. This effect was associated with enhanced killing of drug-sensitive and -resistant AML cell lines and primary AML cells by the radioimmunoconjugates containing nuclear localization sequences [184,185]. An equally exciting avenue to explore is the development of small synthetic multidentate ligands that mimic the properties of MAbs and bind to cell-surface antigens with high affinity and specificity, as shown, for example, for the HLA-DR antigen, and due to the smaller size may provide better tumor penetration [186,187]. Such small engineered molecules may therefore provide an alternative means of targeted delivery of radiation to sites of disease involvement. Finally, an alternative approach to increase accessibility of tumors to radioimmunoconjugates lies in the manipulation of tumor blood flow, and early studies with interleukin-2 suggest that increasing vascular permeability to achieve this goal might be a valid strategy worth exploring in the future [188].

Conclusion

An increasing number of proof-of-principle clinical studies demonstrate that RIT and HCT are safe and feasible and have acceptable toxicities, even in older adults. Research over the last two decades has established important principles of directing RAbs to malignancies, and has provided encouraging results. Nevertheless, RIT is a complex, multidisciplinary effort that still faces many challenges on the road to success; significant obstacles remain that must be overcome to achieve optimal targeting and elimination of target cells by radioimmunoconjugates. For example, the optimal therapeutic radionuclide–antibody–antigen combination for RIT remains unclear, and better controlled clinical trials will ultimately be required to address this question and to determine whether addition of radioimmunoconjugates truly improves patient outcomes over standard treatment. Undoubtedly, future studies will explore alternative antigenic targets and novel radionuclides to improve imaging, dosimetry, and therapy. Furthermore, innovative strategies such as pretargeted RIT and extracorporeal immunoabsorption may optimize the administration of radionuclide therapy and allow more widespread use of very short half-life materials such as the α emitters for RIT. With continued research, RIT holds the potential to establish itself as a radiation boost in combination with other treatments, such as stem-cell rescue, or as adjuvant therapy. Thereby, RIT-based approaches may become an important cornerstone to improve the outcome of patients and will remain an exciting avenue due to constant methodological refinements. It is anticipated that innovations such as those enumerated above will further enhance the feasibility, safety, and efficacy of RIT, with and without HCT.

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CHAPTER 24

Documentation of Engraftment and Characterization of Chimerism After Hematopoietic Cell Transplantation

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Genetic markers in hematopoietic cell transplantation

Marrow cells, growth factor-mobilized apheresis products, and cord blood cells used for hematopoietic cell transplantation (HCT) contain a wide variety of cell types that differ in function and life span. Hematopoietic stem cells (HSCs) in the graft are of central importance for the recovery of marrow function after HCT. With the use of genetic markers, the survival, distribution, and differentiation of engrafted donor cells can be traced throughout the entire life of the recipient. When HSCs are obtained from a syngeneic donor, the only markers that might distinguish donor and recipient cells after HCT are those related to the underlying disease in the recipient. When autologous HSCs are used, genetic markers have sometimes been introduced into the graft in order to demonstrate that the graft contributes to long-term marrow function and to determine whether recurrent malignancy after HCT originated from neoplastic cells that survived the preparative regimen before HCT or from neoplastic cells infused with the graft (see Chapter 8). In allogeneic HCT, donor and recipient cells can be distinguished by testing informative genetic markers.

Informative genetic markers in allogeneic HCT are those that distinguish cells of the donor from those of the recipient, and those that distinguish cells of the recipient from those of the donor. In many cases, the donor and recipient alleles at a single genetic locus will be informative in both directions. For a heterozygous donor with alleles "*a*" and "*b*" and a heterozygous recipient with alleles "*a*" and "*c*," the "*b*" allele is informative for donor-derived cells and the "*c*" allele is informative for recipient-derived cells. In some cases, the alleles at a single locus will not be informative in both directions. For a homozygous donor with the "*a*" allele and a heterozygous recipient with alleles "*a*" and "*c*," the "*c*" allele is informative for recipient cells, but this locus is not informative for donor cells. Evaluation of genetic markers in the donor and recipient before HCT allows loci that are informative to be identified for testing after transplantation.

The probability of finding informative markers depends on the number of loci tested, the number of alleles at each locus, the allele distribution, and the relationship between the donor and recipient. Within members of a family, no more than four alleles can be defined at any single locus, whereas the number of alleles at any given locus in the population can be much larger. For this reason, it is easier to find informative genetic markers with unrelated donors and recipients than with related pairs, but with current molecular biological techniques, markers that distinguish between the donor and recipient can be identified in all patients other than those who have syngeneic donors.

Methods for evaluating chimerism

Historical perspective

In ancient Greek mythology, Chimera was a fearsome three-headed creature – lion at the front, goat in the middle, and serpent at the rear. In today's medical parlance, the term "chimerism" has been used to describe the presence of allogeneic hematopoietic or lymphoid cells in an individual. HCT recipients are sometimes described as having "full chimerism" when all hematopoietic cells and lymphoid cells are derived from an allogeneic donor. The terms "partial chimerism" and "mixed chimerism" are sometimes used when recipient hematopoietic or lymphoid cells persist together with donor cells after HCT. The term "split chimerism" is sometimes used when donor cells are present within some hematopoietic or lymphoid lineages but not in others. The claim that full chimerism has been established must always be qualified by understanding the limits of detection of the assay used to measure chimerism and by defining the hematopoietic and lymphoid lineages encompassed in the test. During the first few weeks after HCT, recipient-derived cells can be found in the blood or marrow of virtually all patients when sensitive tests are used [1,2].

The genetic markers and laboratory methods used for testing chimerism have evolved considerably during the history of HCT. Erythrocyte antigens were among the first markers to be widely used for confirmation of donor-cell engraftment in HCT recipients. Antigens tested for the analysis of chimerism after HCT have included those of the ABO, MN, and Rhesus systems and those of the Kell, Kidd, Duffy, Lutheran, Ss, and P systems. With assays for a wide variety of erythrocyte antigens, informative donor and recipient markers can be identified in more than 80% of sibling pairs. Mixed agglutination and flow cytometry assays can detect admixtures with a sensitivity of 0.1–0.5% [3]. Because erythrocytes have a relatively long life span in circulation, assay results can be confounded by transfusions before or after HCT unless precautions are taken to ensure that the HCT donor has a marker distinctive from those of the recipient and all transfusion donors. Although the long life span of erythrocytes makes it difficult to assess the function of the graft in an ongoing manner, this shortcoming can be circumvented by

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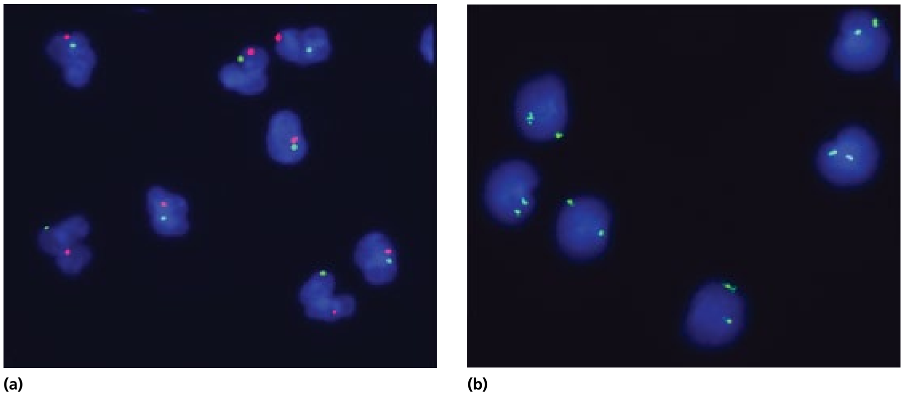

Figure 24.1 Assessment of chimerism in sorted granulocytes (a) and leukemic blasts (b) by dual-color fluorescence in situ hybridization (FISH). Hybridization was carried out with a biotin-labeled Y chromosome-specific DNA probe and a digoxigenin-labeled X chromosome-specific DNA probe. Hybridization was detected with Texas Red-conjugated avidin and fluorescein-conjugated antibody against digoxigenin. Cells were counterstained with 4′,6-diamidino-2-phenylindole (API). Male cells contain a single red fluorescent spot and a single green fluorescent spot, whereas female cells contain two green fluorescent spots and no red fluorescent spots. In this case, granulocytes were derived from a male donor and leukemic blasts were derived from the female recipient. (For a color version of this figure, please see the color plate section.)

assays that define antigens on reticulocytes [3]. Finally, hemolysis caused by ABO incompatibility can delay the appearance of donor erythrocytes in patients who are well engrafted with donor myeloid cells, and results of chimerism studies limited to the erythroid lineage could be misleading.

Conventional cytogenetic analysis of metaphase chromosomes was historically well established as a method for evaluating chimerism in the blood and marrow after allogeneic HCT. Gender disparity between the donor and recipient allows sex chromosomes to be used as convenient genetic markers [4]. In some cases, a constitutional structural rearrangement or heteromorphism can be used as a genetic marker in same-sex pairs. Four major problems limit the applicability of cytogenetic assays for the assessment of chimerism after HCT. First, informative markers are generally not available for same-sex pairs except in cases where a disease-specific cytogenetic abnormality is present in the recipient. Second, techniques for cytogenetic analysis are highly labor intensive and cumbersome. Third, results are limited to cells in metaphase at the time of harvest. Finally, the number of metaphase cells that can be evaluated in any single assay is limited.

During the late 1980s, techniques of molecular biology revolutionized the use of genetic markers in HCT. The two tests most widely used today are in situ hybridization with sex chromosome-specific probes and typing of variable number of tandem repeat (VNTR) or short tandem repeat (STR) polymorphisms by DNA amplification. In situ hybridization permits examination at the level of single cells, but this method is applicable only when the donor and recipient are of opposite sex or when an informative autosomal marker can be identified [5,6]. Informative VNTR/STR polymorphisms can be identified in virtually all allogeneic donor–recipient pairs, but testing at the level of single cells is not possible. Both methods have high sensitivity and specificity, and both methods can provide quantitative results. Both methods can be adapted easily for use in clinical laboratories, and both can be used for evaluating archival specimens.

Molecular cytogenetics

Molecular cytogenetic techniques have many advantages over conventional cytogenetic analysis of metaphase chromosomes. Techniques for in situ hybridization are simple and highly time efficient, and the analysis can be applied equivalently for interphase and metaphase cells [7,8]. Techniques are sufficiently flexible to allow the simultaneous determination of genotype and morphology or surface-marker expression in single cells [7–9]. Large numbers of cells can be analyzed, allowing high precision and sensitivity.

Initial reports of molecular cytogenetic techniques for chimerism testing described the use of a human Y chromosome-specific repetitive DNA sequence probe for fluorescence in situ hybridization [1,10,11]. The upper limit of false-negative results in males was 5.6% and the upper limit of false-positives in females was 2.7% [1]. Age-associated loss of the Y chromosome in cells from older males [12], Y chromosome loss associated with the tumor cell karyotype, and constitutional variation in the Y chromosome DNA probe target sequences are potential limitations of the assay. These limitations make it essential to test specimens either by conventional cytogenetics or by in situ hybridization before HCT to ensure Y chromosome integrity. Cells that show no signal with the use of a Y chromosome-specific probe are inferred to be of female origin. The absence of signal with a Y chromosome-specific probe could result from technical errors that prevent hybridization. This potential problem can be avoided by the use of a mixture of X and Y chromosome-specific probes each labeled with a different chromophore to allow dual-color detection (Figures 24.1 and 24.2). With these reagents, the upper limit of false-positive XX cells in males was 0.63% and the upper limit of false-positive XY cells in females was 0.30% [13]. Chimerism in sex-matched donor–recipient pairs can be analyzed by in situ hybridization with the use of probes for informative deletion polymorphisms [5,6].

VNTR and STR polymorphisms

Certain core DNA sequences are repeated in tandem in the genome, and the number of such tandem repeats (TRs) at any given locus varies among different individuals (Figures 24.3 and 24.4 [14,15]). VNTR sequences are comprised of “minisatellite” cores 8–50 base pairs in length [16], whereas STR sequences are comprised of “microsatellite” cores 2–8 base pairs in length (e.g. CACACA and complementary GTGTGT) [17,18]. Polymorphisms in the number of tandem repeated core sequences within a locus are inherited as codominant Mendelian traits. A large number of loci are available

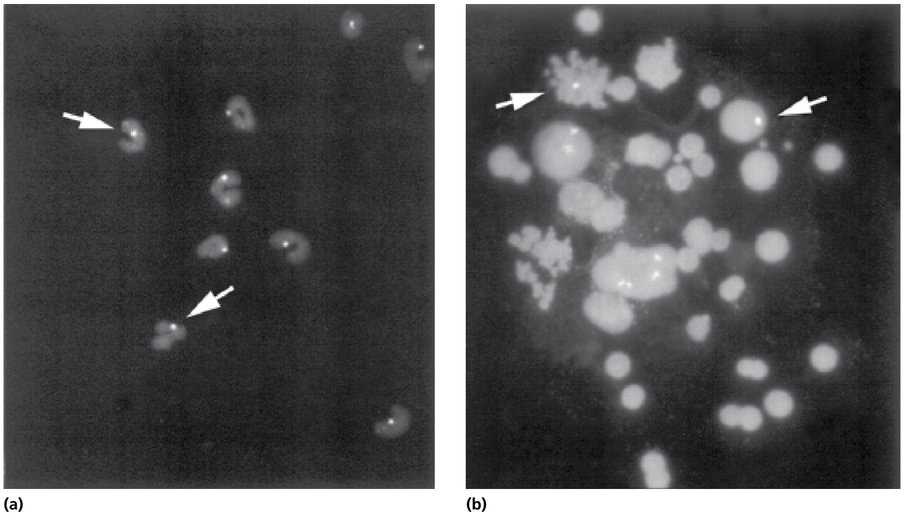

Figure 24.2 Assessment of chimerism by fluorescence in situ hybridization (FISH). A biotinylated Y chromosome-specific DNA probe was hybridized to interphase and metaphase cells obtained from peripheral blood. Hybridization was detected with fluorescein-conjugated avidin, and cells were counterstained with propidium iodide. A fluorescent spot (arrows) is seen in male interphase cells and on the Y chromosome in metaphase cells. Female cells show no hybridization. Granulocytes (a) and phytohemagglutinin (PHA)-stimulated mononuclear cells (b) are shown from a female patient after T-cell-depleted marrow transplantation from a male donor. Granulocytes originated entirely from the donor, whereas mononuclear cells and PHA blasts originated from both the donor and the recipient.

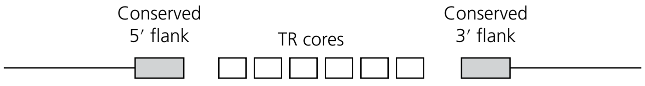

Figure 24.3 Variable number of tandem repeat (TR)/short TR structure. Alleles reflecting differences in the number of TRs can be distinguished by the length of fragments generated after DNA amplification with 5′ and 3′ primers specific for conserved sequences flanking the TR core region.

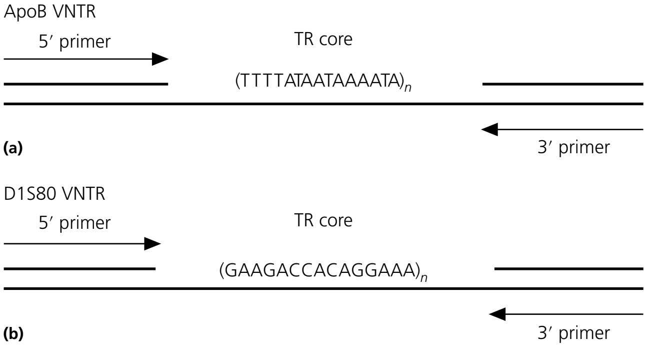

Figure 24.4 Representative TR core sequences for (a) the apoB locus [15] and (b) the D1S80 locus [14]. Arrows show the position of primers complementary to conserved sequences flanking the TR regions. Core sequences at each locus show some heterogeneity resulting from base substitutions, insertions, or deletions.

for testing, and certain loci have more than 25 alleles [7,8,19]. Informative donor and recipient markers can be identified in virtually all allogeneic sibling pairs with a panel of as few as six loci.

VNTR/STR polymorphisms can be identified most conveniently by using the polymerase chain reaction (PCR) to amplify defined segments of DNA [15,20]. In these assays, oligonucleotides complementary to non-polymorphic 5′ and 3′ sequences on opposite strands flanking the polymorphic VNTR/STR core (Figure 24.3) are used to prime DNA synthesis through repeated cycles of denaturing, primer annealing, and heat-stable polymerase-mediated extension. When the two primer binding sites are spaced within a short distance of each other, each cycle of extension creates a primer-binding site for initiation of opposite strand synthesis in the next cycle. The 10^6–10^9-fold amplification of donor- and recipient-derived DNA during the reaction allows fragment length to be analyzed directly by staining with ethidium bromide or silver. With the use of fluorescent primers, reaction products can be visualized by a DNA sequencer or by capillary gel electrophoresis [7,8,21–24] (Figures 24.5 and 24.6 [25]). Efficiency can be improved by simultaneous amplification of different loci in a single "multiplex" reaction [7,8,22,23]. The ability of a multiplex reaction to amplify several loci simultaneously circumvents the need to identify informative loci before testing is carried out, but donor samples and recipient samples obtained before HCT are still needed for the interpretation of results. The multiplex reaction should be designed to amplify selected loci that are most likely to be heterozygous and therefore, informative in a given population [26].

The use of DNA amplification allows chimerism testing with extremely small amounts of starting material, a major advantage when attempting to analyze samples from patients with graft failure and severe leukopenia. Depending on fragment length and the efficiency of amplification, the sensitivity for detecting admixtures is 1–5% and can approach 0.1% in certain cases. Methods involving DNA amplification can be confounded by contamination, which can be recognized when the DNA has originated from an individual other than the donor or recipient. Concurrent amplification of control samples obtained from the donor and recipient before HCT together with the test sample after HCT generally allows unambiguous identification of donor-specific and recipient-specific reaction products and avoids confusion that could be caused by background bands. With the inclusion of standards, assays can be adapted for quantitative determination. Assay results can be affected when alleles have large differences in base-pair length, since shorter alleles may be preferentially amplified [27]. Assay results can also be affected by technical artifacts such as stutter peaks that reflect

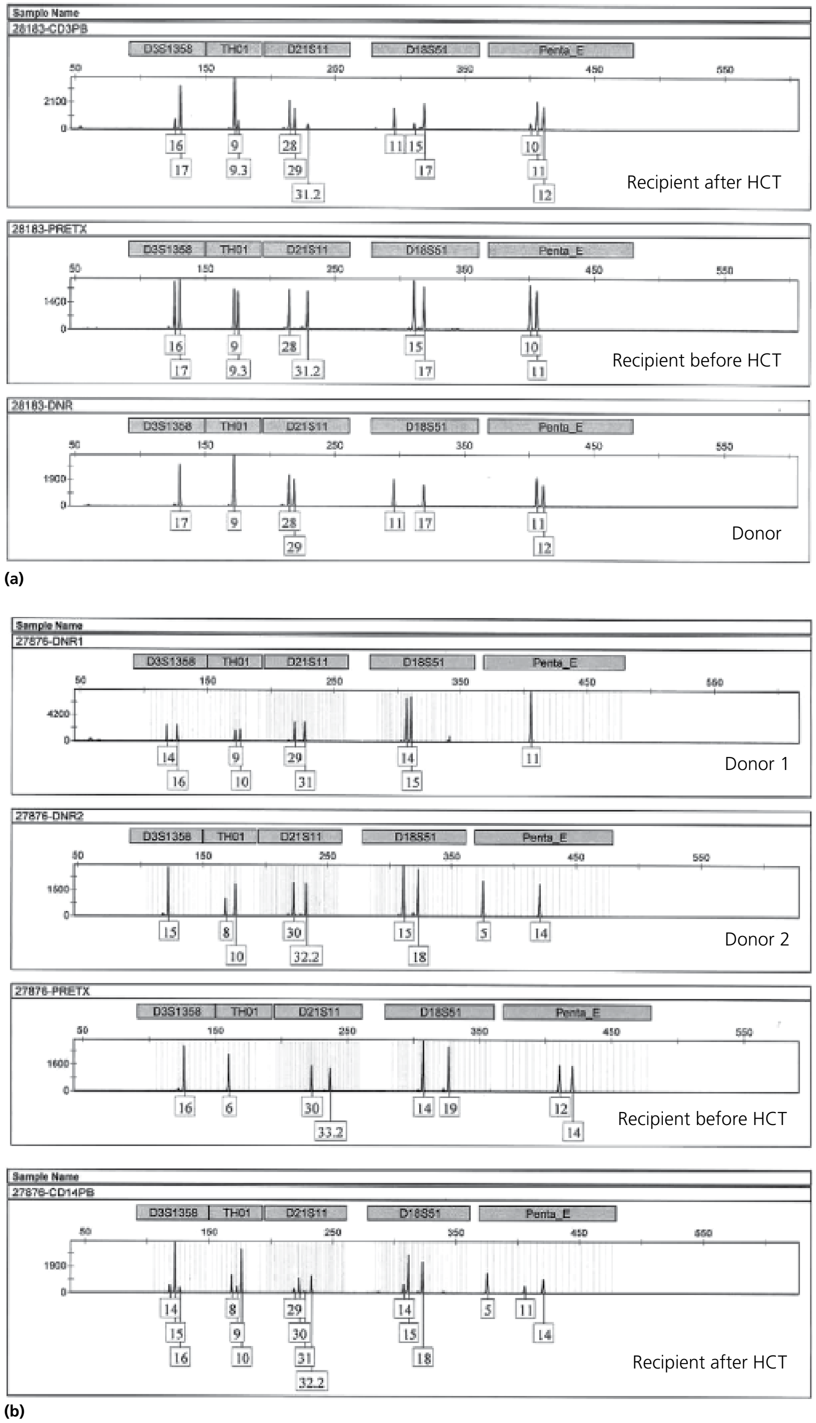

Figure 24.5 Assessment of chimerism by testing short tandem repeat (STR) polymorphisms. (a) Mixed chimerism in CD3$^+$ blood cells tested at 1 year after HCT with a reduced-intensity conditioning regimen for treatment of acute myeloid leukemia. The figure shows capillary gel electrophoresis chromatograms of products after multiplex amplification of five loci (listed in the gray bars) with samples from the donor (bottom), recipient before HCT (middle), and recipient after HCT (top). Allele designations are numbered in boxes below each chromatogram. In this case, informative donor markers include D21S11 allele 29, D18S51 allele 11, and Penta E allele 12. Informative recipient markers include D3S1358 allele 16, TH01 allele 9.3, D21S11 allele 31.2, D18S51 allele 15, and Penta E allele 10. The percentage of donor DNA was calculated according to the height of informative donor peaks divided by the sum of the heights of all informative donor and recipient peaks at each locus. The percentage of donor DNA can also be calculated according to the areas under the respective peaks. The median percentage of donor-derived DNA among values for all informative loci in this specimen was 75%.
(b) Engraftment with two different cord blood units. The sample obtained from the recipient after HCT represented CD14$^+$ monocytes isolated by immunofluorescent staining and flow cytometry. The figure shows chromatograms from each of the two donors (top two profiles), the recipient before HCT (third from top), and the recipient after HCT (bottom). Informative recipient alleles (TH01 allele 6, D18S51 allele 19, and Penta E allele 12) were not detected in the sample from the recipient after HCT. The bottom profile shows that 20% of the sample was derived from one cord blood unit and 80% was derived from the other cord blood unit.

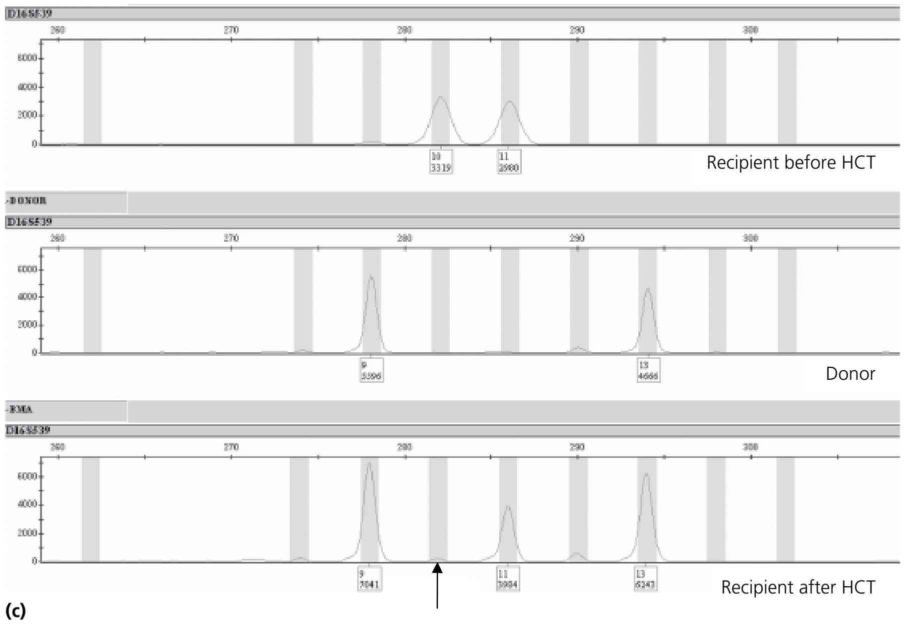

Figure 24.5 (*continued*) (c) Missing allele caused by chromosomal deletion in a patient with multiple myeloma. The figure shows chromatograms of products after amplification of the D16S539 locus from the donor (middle), recipient before HCT (top), and recipient after HCT (bottom). The sample from the recipient after HCT was a marrow aspirate obtained on day 497 after HCT. Both donor peaks (alleles 9 and 13) and both recipient peaks (alleles 10 and 11) are informative. Vertical gray bars across each profile indicate the expected positions of D16S539 alleles. The small peak corresponding to allele 9 in the recipient profile before HCT and the small peaks corresponding to alleles 12 and 8 in the donor profile are artifacts termed "stutter fragments" that contain one less repeat than the normal allele. The marrow sample from the recipient after HCT shows a reduced amount of allele 10 (arrow) compared with allele 11. This imbalance resulted from loss of one chromosome 16 in myeloma cells that persisted after HCT.

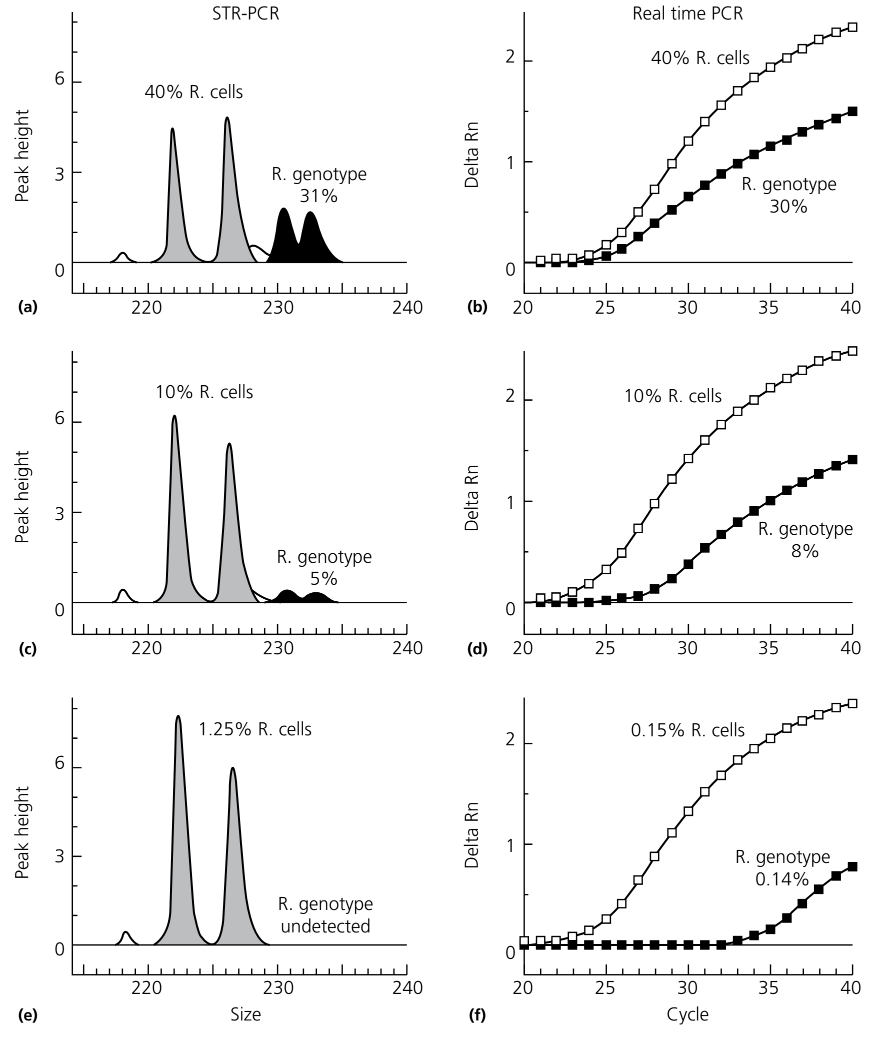

Figure 24.6 Comparison of short tandem repeat polymerase chain reaction (STR-PCR) and real-time PCR chimerism assays. Mixtures contained 40% (a and b), 10% (c and d), 1.25% (e) or 0.15% (f) "recipient" cells. In this case, the D21S11 STR alleles of the recipient (black peaks) were longer than those of the recipient (gray peaks) (a and c). This STR-PCR assay was able to detect the presence of 40% or 10% recipient cells (a and c), but 1.25% recipient cells could not be detected (e). Preferential amplification of shorter alleles is characteristic of STR-PCR assays, which explains the under-representation of recipient alleles (a and c). Donor and recipient alleles were tested individually in separate real-time assays (b, d, and f). The increase in fluorescence indicates the accumulation of amplification products as the reaction progresses from one cycle to the next. With decreasing amounts of the recipient S09a allele, fluorescence exceeds the threshold of detection (horizontal line) at progressively later cycles, shifting the curve to the right. With increasing amounts of the donor S 09b allele, fluorescence exceeds the threshold of detection earlier, shifting the curve slightly to the left. The real-time assay was easily able to detect 0.15% recipient DNA (f). Source: Alizadeh et al. 2002 [25]. Reproduced with permission of the American Society of Hematology.

amplification products containing one less repeat than the normal allele (Figure 24.5) [27]. Accuracy may be improved by averaging results from all informative loci in a multiplex reaction [27]. Nomenclature for describing assay results has been proposed [28], and computer software is available for analyzing and summarizing the results [27].

DNA amplification of other loci for assessment of chimerism

With amplification of certain Y chromosome-specific sequences, sensitivity for detection of male cells in female recipients can approach 0.01% [29,30]. Loci with biallelic polymorphism not involving VNTR/STRs have also been used for quantitative and highly sensitive measurements of chimerism. Two different approaches have been used for these assays. The first involves the use of oligonucleotide primers specific for single nucleotide polymorphisms or short two-base-pair insertions or deletions that differ between the donor and recipient [25,31,32]. Assay conditions are carefully controlled so that the primer binds to only one of the alleles, thereby allowing selective amplification of an informative donor or recipient allele. The second involves the use of oligonucleotide primers that are specific for longer insertion or deletion polymorphisms or null alleles that differ between the donor and recipient [33]. Informative single nucleotide markers or short insertion or deletion markers were identified for more than 90% of related donor–recipient pairs by testing 7–11 biallelic loci [25], and at least one recipient-informative marker was identified in approximately 80% of related donor–recipient pairs by testing 10 loci with longer insertions or deletions or null alleles [33].

Unlike assays with VNTR/STR loci in which amplification products from both donor and recipient DNA are measured simultaneously at the end of the reaction, assays with these loci measure amplification products from DNA of either the donor or the recipient in real time as they accumulate during the reaction in comparison with known standards. Real-time quantitative DNA amplification assays had 0.01–0.1% sensitivity (Figure 24.6).

Clinical application of chimerism tests

Samples used for chimerism testing

Alleles that are informative for the donor and recipient must be identified before samples from the recipient can be tested for chimerism after HCT. This identification can be easily accomplished by testing blood cells from the donor and recipient before HCT. When a recipient sample obtained before HCT is not available, material from a buccal scraping, hair follicle or skin biopsy can be used to identify recipient alleles [34]. Buccal scrapings and mouthwash specimens contain large numbers of cells that originate from the blood and cannot be used reliably to identify recipient alleles after HCT [35].

Cells from blood or marrow are most frequently used for clinical chimerism testing. In most circumstances, results from testing blood cells can provide the necessary clinical information. One exception is the detection of minimal residual disease when malignant cells infiltrate the marrow but are not found in the circulation. Selected lineage-specific populations of interest can be isolated by flow cytometry in order to improve diagnostic accuracy and sensitivity for detecting small numbers of recipient- or donor-derived cells [8,36–38]. For example, separation of blood samples into T-lymphocyte and granulocyte fractions can be very helpful in interpreting results in patients who have received a reduced-intensity conditioning regimen [36,37,39]. Likewise, the diagnosis of recurrent malignancy can be confirmed by demonstrating that a small population of cells with an aberrant cell surface phenotype originated from the recipient [40,41]. Chimerism testing with isolated cell populations is particularly helpful in confirming the diagnosis of recurrent malignancy when the aberrant population represents less than 1% of the sample and when the aberrant phenotype detected after HCT resembles a normal pattern of hematopoietic regeneration or differs from the original malignant phenotype.

General principles

Chimerism tests have been used for a variety of purposes in human HCT (Table 24.1). With sufficiently sensitive assays, recipient cells have been shown to persist for longer than 2 years in most patients after treatment with a myeloablative conditioning regimen [2]. A complex interplay between prior intensive chemotherapy, the intensity of the conditioning regimen before HCT, the number of HSCs in the graft, the immunosuppressive regimen after HCT, and the effects of donor T cells in the graft governs the ability of recipient-derived lymphoid and hematopoietic cells to survive after HCT (see Chapter 9). In general, prior intensive chemotherapy and more intensive conditioning regimens would be expected to decrease the persistence of recipient cells after HCT, whereas higher numbers of HSCs in the graft and the occurrence of acute graft-versus-host disease (GVHD) would be expected to accelerate engraftment [11,42–45]. Given the critical role of donor T cells in helping to eliminate recipient lymphoid and hematopoietic cells that survive the conditioning regimen, depletion of donor T cells would generally be expected to increase the incidence of mixed chimerism after HCT [1,19,44,46]. In some studies, disappearance of recipient cells within the first 100 days after HCT has been associated with an increased incidence of acute and chronic GVHD [47], whereas persistence of recipient cells at 2–6 months after HCT with myeloablative conditioning has been associated with an increased risk of recurrent malignancy [48] or decreased risks of chronic GVHD and overall mortality [49].

General recommendations for chimerism testing after allogeneic HSC have been published [37]. Before individual applications of chimerism testing are discussed in detail, several points should be emphasized:

1 The clinical context weighs heavily in the interpretation of chimerism test results. For example, the absence of donor cells in patients with marrow aplasia could indicate rejection, but a similar absence of detectable donor cells in the non-fractionated marrow

Table 24.1 Clinical applications of chimerism tests in hematopoietic cell transplantation

1. Routine documentation of donor-cell engraftment
2. Evaluate the persistence of donor cells in:
   a. Patients with inadequate marrow function
   b. Patients who are candidates for donor lymphocyte infusion
   c. Patients who are candidates for a second allogeneic transplant
   d. Long-term follow-up patients who are at increased risk of occult rejection
3. Assess the prognostic risks of graft-versus-host disease (GVHD), rejection, or recurrent malignancy
4. Define whether recurrent malignancy or lymphoproliferative syndrome has originated from donor or recipient cells
5. Identify maternal cells in pretransplant patients with severe combined immunodeficiency
6. Correlate immune reconstitution with engraftment after transplantation for treatment of severe combined immunodeficiency
7. Determine whether cells from a transfusion donor can be implicated in causing GVHD
8. Verify the genetic identity of twins
9. Identify the origins of engraftment after HCT with multiple cord blood units

or blood of a patient with florid leukemia relapse could be caused by an overwhelming preponderance of recipient cells.

2 Test results are reported as a ratio in which changes in the numbers of donor and recipient cells can occur independently. Thus, an increase in the proportion of donor cells can be caused both by an increase in the number of donor cells and by a decrease in the number of recipient cells.

3 The interpretation of results depends on an awareness of the types of cell tested in the assay. For example, blood mononuclear cells can include T- and B-lymphocytes, monocytes, immature myeloid precursors, and leukemic blasts. More informative results can be obtained by analyzing purified cell populations.

4 The presence of a particular cell population does not allow direct inferences concerning its functional capabilities or effects. For example, the detection of recipient T cells after HCT does not necessarily indicate that rejection is likely to occur.

5 The inability to detect a particular cell population does not necessarily indicate its absence. The interpretation of test results must always be made with an understanding of the assay sensitivity.

6 Time trends are often more informative than test results at a single time point. For example, the presence of 5% recipient cells at day 28 after HCT might have no clinical significance in itself, but a progressive increase in the proportion of recipient cells could herald rejection or recurrent leukemia.

7 Abnormalities within a single patient can have multiple causes. Thus, the inability to detect donor cells after HCT could indicate the occurrence of both rejection and recurrent malignancy.

8 The inability to detect recipient markers after HCT could result from the absence of recipient cells or from chromosome loss or deletion in malignant cells (Figure 24.5).

9 In the absence of a clinical trial, routine chimerism testing is not needed after HCT with myeloablative conditioning regimens. Chimerism testing may be indicated if patients have evidence of graft failure or recurrent malignancy.

Chimerism testing in patients at risk for graft failure

Although chimerism tests can be carried out for routine monitoring of donor-cell engraftment after HCT [50], the results are of greatest importance in patients who have inadequate marrow function and in patients who might be candidates for donor lymphocyte infusion or a second HCT from the same donor. Marrow graft rejection is usually defined by the absence of donor cells in a patient with pancytopenia and reduced marrow cellularity, but "occult" rejection and complete reconstitution with recipient cells can occur, especially in patients who have received a reduced-intensity conditioning regimen and in patients with chronic myelogenous leukemia (CML) [51]. As noted, the presence of donor cells can be obscured by a florid leukemia relapse or by a malignant population with myelosuppressive effects. Testing the origin of mature T cells can be helpful in excluding rejection in this situation since these cells are not usually affected by recurrent malignancy.

Diagnostic assessments of chimerism test results are especially difficult in patients with poor marrow function. The presence of recipient cells in the blood or marrow does not necessarily indicate that rejection is the cause of poor marrow function since recipient cells are frequently identified early after HCT in patients with normal marrow function [1,2]. Thus, a patient with "benign" mixed chimerism [30] could have poor marrow function because of drug toxicity, viral infection, or a possible marrow stromal defect. Likewise, the inability to detect recipient cells in any single test does not eliminate rejection or occult recurrent malignancy as a possible cause of poor marrow function. In these situations, serial testing can be highly informative.

In patients who are candidates for donor lymphocyte infusions as treatment for recurrent malignancy (see Chapter 70), the detection of persisting donor cells by chimerism tests offers reassurance that rejection has not occurred. In addition, the risk of transient or irreversible aplasia after donor lymphocyte infusion may be much higher in patients with a low proportion of donor cells in the blood or marrow compared with those with a high proportion of donor cells in the blood or marrow [52]. In patients who are candidates for a second HCT from the original donor, the persistence of donor cells indicates that the recipient is still tolerant of donor cells. In this situation, the risk of rejection after a second HCT is negligible, and the preparative regimen can be designed for the sole purpose of eliminating malignant cells.

Serial monitoring of chimerism can be used to monitor initial engraftment after HCT for the treatment of severe aplastic anemia or thalassemia. Mixed chimerism has been associated with an increased risk of rejection in patients with thalassemia [53] and other non-malignant diseases [54]. The presence of less than 5% donor cells in the blood was associated with irreversible graft loss after cord blood transplantation [55]. In some cases, infusion of donor lymphocytes might prevent rejection in patients with decreasing chimerism after HCT [56]. Late "occult" rejection and complete hematopoietic reconstitution with recipient cells have been observed in long-term follow-up after HCT for the treatment of aplastic anemia [57]. This distinctly unusual outcome is of biologic interest but probably has no medical significance except for theoretical concerns that aplastic anemia might recur or that myelodysplasia might develop in the future.

Chimerism testing in patients at risk for recurrent malignancy

In certain circumstances, chimerism tests can be useful in predicting recurrent malignancy after HCT. Findings that demonstrate an increasing proportion of recipient-derived cells in the blood or marrow after HCT can unquestionably herald relapse [19,38,58–62]. By itself, stable mixed chimerism in non-fractionated blood or marrow cells does not predict recurrent malignancy [63,64], although persistence of recipient cells within the CD19$^+$ population has been correlated with an increased risk of relapse in patients with pre-B lineage acute lymphoblastic leukemia [65]. Chimerism testing to demonstrate the recipient origin of isolated cell populations with an aberrant expression of cell-surface antigens can facilitate the early detection of recurrent leukemia [40,41] (Table 24.2). The presence of malignant cells can also be inferred when quantitative STR assays show loss or gain mutations associated with the original disease [66].

In patients with malignant diseases that have aberrant expression of cell-surface antigens, a more direct and unambiguous measure of minimal residual disease can come from flow cytometry or molecular diagnostics, as opposed to chimerism testing (see Chapter 25). Highly sensitive tests and very frequent monitoring would likely be necessary to gain early warning of impending relapse in patients with rapidly progressive diseases such as acute leukemia [50,67]. In several studies, selected patients with increasing levels of mixed chimerism have had immunosuppressive medications withdrawn or have received donor lymphocyte infusions in an effort to prevent recurrent malignancy after HCT [60,61,67–70]. In some cases, patients who had such interventions survived longer than those

Table 24.2 Testing of isolated cell populations by single- or dual-color fluorescence in situ hybridization (FISH) to detect recurrent leukemia

Sample	Percent in original sample	Genotype (%)			
		Y	XY	XX	X
Unsorted marrow		98.5			
Granulocytes	89.0		100.0	0.0	
Lymphocytes	1.6		97.7	0.3	
Blasts	0.2		2.0	86.9	11.1

Single-color FISH with a Y chromosome-specific probe on day 45 after the transplant did not detect an appreciable population of female recipient cells in unsorted marrow from this patient. Granulocytes were isolated by flow sorting of cells with high side scatter characteristics and low expression of CD45, and lymphocytes were isolated by sorting cells with low side scatter characteristics and high expression of CD45. A small population of blasts was isolated by sorting cells with high expression of CD34 and low expression of CD45. Two-color FISH demonstrated that the granulocyte and lymphocyte populations were derived from the male donor, whereas the blasts with aberrant antigen expression were derived from the female recipient. Some of the malignant cells in this patient had a single X chromosome.

who did not have interventions, but bias in the selection of patients for intervention makes these results difficult to interpret. Serial monitoring of chimerism after HCT could offer greater benefit if interventions for treatment of recurrent acute leukemia were more effective [60].

Serial chimerism testing has been useful for early detection of recurrent malignancy in patients with less rapidly progressive diseases such as CML [71] or myeloma [72]. A correlation between persistence of recipient T cells after HCT and an increased risk of recurrent malignancy has been found in patients who received T-cell-depleted marrow for the treatment of CML [73]. The absence of donor T cells in the graft presumably allowed both T cells and malignant HSCs of the recipient to survive after HCT. With the use of a more intensive conditioning regimen before HCT, persistence of recipient lymphocytes after T-cell-depleted HCT was not associated with an increased risk of recurrent CML [74]. The persistence of recipient cells in the blood or marrow during the first 3 months after HCT has not been associated with an increased risk of relapse in patients who received unmodified (T-cell-replete) marrow for the treatment of CML [57]. This result is consistent with earlier findings that the detection of *BCR–ABL* rearrangements in the blood or marrow during the first 3 months after HCT is not associated with an increased risk of relapse [75].

Chimerism testing after reduced-intensity conditioning regimens

A two-stage strategy for HCT involves the initial establishment of mixed chimerism with the use of a reduced-intensity conditioning regimen, combined with potent immunosuppression after HCT to prevent rejection and GVHD. After engraftment has been established, immunosuppression is withdrawn, allowing donor T cells to eliminate residual hematopoietic cells or malignant cells in the recipient through immunological mechanisms (see Chapter 21). In some situations, donor lymphocytes may be infused if withdrawal of immunosuppression does not have the desired clinical effect.

Genetic marker studies and state-of-the-art chimerism tests play an essential part in clinical management with this approach to HCT and in the evaluation of results from these clinical trials. High levels of donor chimerism early after HCT have been associated with improved overall survival and relapse-free survival. Serial testing is likely to be more informative than any single test. In one study, for example, declining proportions of donor cells in the blood or marrow were associated with a high risk of relapse, whereas rising proportions of donor cells were associated with a low risk of relapse [76]. Results of serial monitoring have suggested that full donor T-cell engraftment precedes the development of full donor myeloid engraftment, GVHD, and antitumor effects [37,39,77]. Chimerism tests after reduced-intensity conditioning showed that low levels of donor T-cell and natural killer-cell engraftment at early time points after HCT were associated with an increased risk of rejection and the absence of antitumor effects, whereas high levels of donor T-cell engraftment were associated with an increased risk of GVHD, and earlier establishment of natural killer-cell chimerism was associated with improved progression-free survival [78,79]. In some cases, a progressive decline in donor-cell engraftment has been reversed by the infusion of donor lymphocytes [37,80]. These results suggest that serial monitoring of donor-cell engraftment can help to determine whether donor lymphocytes should be given pre-emptively in order to prevent rejection (see Chapter 77). In another study, recipient T cells persisted for a longer time after HCT in patients prepared with fludarabine and melphalan as compared with fludarabine and busulfan, but no correlation was found between the onset of complete donor chimerism and the occurrence of acute GVHD or the risk of disease progression after HCT [81]. The contrasting results among these studies emphasize that correlations between chimerism and clinical outcomes after HCT may vary according to the intensity of the conditioning regimen.

Other applications for genetic marker studies

Genetic marker studies have found a variety of other useful applications in HCT. In rare cases, for example, it has been possible to demonstrate that hematologic malignancy after HCT originated in donor cells rather than recipient cells. Chimerism tests allow these unusual cases to be identified for further intensive study, although the mechanisms involved in malignant transformation of donor cells remain obscure. With chimerism tests, it has likewise been possible to assess the involvement of donor- or recipient-derived B-lymphocytes in lymphoproliferative disorders caused by Epstein–Barr virus infection (see Chapter 90).

In rare cases, genetic marker studies have demonstrated that GVHD was caused by the cells from a blood product transfusion or from a solid organ allograft. In HCT recipients, evidence implicating cells of a blood transfusion donor came from tests demonstrating the presence of genetic markers different from those of either the donor or the recipient. In some cases, it has been possible to trace the donor through further testing. The universal practice of irradiating all blood product transfusions should eliminate any risk of transfusion-induced GVHD in HCT recipients.

Chimerism tests can be used to identify the origins of engraftment after HCT with multiple cord blood units (see Chapter 39). In this situation, testing must employ markers that are informative for both the recipient and each of the donors. STR markers are very useful for this purpose, since these loci typically have more than two alleles (Figure 24.5).

An extremely useful clinical application of genetic marker studies in HCT is the assessment of monozygosity in twins. With an identical twin donor, there is no risk of rejection or GVHD and hence no need for immunosuppressive treatment before or after HCT. In this situation, the conditioning regimen before HCT is designed solely to eliminate malignant cells. The alleles of a genetic locus are fully informative in a family study when they allow unambiguous assessment of inheritance, which requires parental heterozygosity and the absence of allele matching between the parents. With human leukocyte antigen-identical twins of the same sex and with identity at three additional unlinked fully informative genetic loci, the probability that the twins are not genetically identical is less than 1:100. With identity at four unlinked fully informative loci in addition to HLA, the probability is less than 1:500, and with identity at five unlinked fully informative loci in addition to HLA, the probability is less than 1:1000 [82].

Biologic insights from genetic marker studies

Beyond the clinical applications described above, genetic marker studies have contributed valuable information towards understanding the biology of HCT. Genetic marker studies have recently emerged as powerful tools in studies of HCT for the treatment of congenital immunodeficiency. Infants with severe combined immunodeficiency syndrome (SCID) often have occult maternal T cells in the circulation. The presence of these cells might require immunosuppression to avoid rejection (see Chapter 73). Important information for understanding immune reconstitution after HCT for the treatment of SCID has been obtained by analyzing the lineage-specific patterns of donor-cell engraftment [83,84].

Results of genetic marker studies have made it clear that clinical resolution of non-malignant diseases can often be achieved without complete donor replacement of the defective recipient lymphohematopoietic system [84]. The need for red cell transfusions in patients with thalassemia can be averted by establishing mixed hematopoietic chimerism with only 25% normal donor cells in the marrow, and stable mixed chimerism can ameliorate the effects of sickle disease [85].

Genetic marker studies after HCT have been used to characterize the diversity of marrow-derived cells throughout the body. From these studies, it is now recognized that marrow-derived cells include not only hematopoietic and lymphoid populations, but also tissue macrophages, Kupffer cells in the liver, Langerhans cells in the skin, dendritic cells in the blood, and microglial cells in the brain [86–90]. The donor origin of tissue macrophages and microglial cells has given impetus to the application of HCT for treatment of certain congenital enzyme deficiency diseases (see Chapter 74). In some studies, small proportions of donor-derived stromal cells and mesenchymal cells have been detected in the marrow [91]. Some investigators have reported that marrow-derived cells can differentiate into mature hepatocytes [92,93], epithelial cells of the gastrointestinal tract [93,94], epithelial and endothelial cells of skin affected by GVHD [95], and hair follicles [96]. Rigorous experiments in mice reconstituted with single HSCs, however, did not show an appreciable contribution of donor cells in the brain, kidney, gut, liver, or muscle, suggesting that transdifferentiation of HSCs is a rare event [97]. The small numbers of donor-derived, non-hematopoietic cells observed after HCT in human recipients could reflect cell fusion events or the progeny of other types of stem cells [98–100].

Conclusion

The origin of marrow and blood cells after HCT can be identified by testing informative genetic markers that distinguish the donor and recipient. Molecular methods for in situ hybridization and DNA amplification have made chimerism tests widely accessible for a variety of clinical applications after HCT. Biologic insights gained from testing genetic markers will continue to guide future progress as new approaches are developed in using HCT for the treatment of malignant and non-malignant diseases.

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CHAPTER 25

The Detection and Significance of Minimal Residual Disease

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Introduction

Relapse is the major obstacle to cure in leukemia, and this problem persists despite new chemotherapy regimens, "targeted" molecular therapy, the study of mechanisms of drug resistance, and attempts to leverage immunotherapy against the leukemia clone. Unfortunately, once hematologic relapse occurs, the prospects for achieving a subsequent durable remission (and cure) are slim. One strategy to combat relapse is to detect and treat minimal residual disease (MRD) prior to overt relapse. Over the last two decades, the technology to detect minuscule quantities of leukemia has developed and matured, and there are now ample examples demonstrating the strong association of MRD detection and relapse.

The problem of defining remission and relapse is illustrated in Figure 25.1. At diagnosis, patients may have a leukemia burden of up to 10^{10}–10^{12} cells. Thus, potentially millions of leukemia cells may persist after therapy despite the morphologic appearance of remission. MRD detection may have a number of potential roles in leukemia treatment: (i) as an early measure of disease response, (ii) as a post-therapy prognostic factor to assign patients for optimal consolidation therapy, (iii) for early diagnosis of relapse for initiation of pre-emptive therapy prior to overt relapse, and (iv) as a surrogate end-point in clinical trials to evaluate efficacy of novel therapies.

Methods of MRD detection

The standard approach to evaluate residual leukemia by routine pathologic examination of bone marrow (BM) is limited by the subtle morphologic differences between malignant and normal cells. Fortunately, several techniques can be used to find the "needle in the haystack"; these are summarized in Table 25.1.

Chromosomal assays

Conventional metaphase cytogenetics can detect approximately one leukemia cell in 100 normal cells (denoted here as "10^{-2}" sensitivity) if enough metaphases are analyzed (generally 20 metaphases) [1,2]. Although cytogenetics is the method of choice for screening leukemic samples for risk stratification at diagnosis, these studies are also useful to define early response to induction therapy (cytogenetic remission) or to look for new, unanticipated chromosomal changes during therapy. Of note is that cytogenetics samples cells that divide in culture.

Molecular cytogenetics is the analysis of genomic alterations using fluorescence in situ hybridization (FISH)-based technology. FISH uses chromosome-specific or locus-specific probes to target tumor-specific genetic aberrations in metaphase or interphase cells. FISH is useful for rapidly screening 200–10,000 cells for numerical chromosomal aberrations and has the ability to identify minor abnormal clones that may be unrelated to or associated with early clonal evolution of disease undetected by the conventional karyotypic study. Compared with conventional cytogenetics, the sensitivity of FISH ranges from 10^{-1} to 10^{-3} (depending on the number of probes and number of nuclei scored). The sensitivity of FISH increases on combining it with other special techniques, such as in combination with morphologic or immunohistochemical staining (Figure 25.2) [3–5], on specific flow-sorted cell populations, or by increasing the number of mitotic cells analyzed by hyper-metaphase FISH [6–8].

Flow cytometry assays

Cell surface antigen expression can distinguish malignant from normal cells, as malignant cells often express surface antigens in subtly different patterns compared with normal cells [9]. By using combinations of multiple antibodies, "multiparametric" flow cytometric (MFC) assays uses aberrant antigen expression patterns to "fingerprint" the malignant clone (Figure 25.3). If combinations of several antibodies are used to define the aberrant antigen expression, the sensitivity can reach as low as 10^{-3} or less in experienced hands. This technique has been very useful in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), where leukemia blasts frequently have abnormal antigen expression compared with normal hematopoietic cells. The current practice of three- or four-color flow cytometry is quickly evolving to methods that allow seven colors or more.

Polymerase chain reaction (PCR)

The most sensitive approach to detect MRD involves nucleic acid amplification using PCR (Figure 25.4) [10]. In the PCR, a specific genetic lesion target becomes the "fingerprint" of the malignancy in order for PCR-driven reactions to have the desired sensitivity and specificity. Two different techniques are currently used: (i) PCR using genomic DNA for detection of clonal rearrangements of immunoglobulin (Ig) and T-cell receptor (TCR) genes in ALL (sensitivity 10^{-3}–10^{-5}), and (ii) reverse-transcriptase PCR (RT-PCR) for detection of gene rearrangements, mainly fusion gene, transcripts

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Stephen J. Forman, Robert S. Negrin, Joseph H. Antin and Frederick R. Appelbaum.
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resulting from chromosomal translocations (sensitivity 10^{-3}–10^{-6}). RT-PCR is usually combined with quantitative PCR methods [real-time quantitative PCR (RQ-PCR)] that allow quantitative detection of gene expression. Overexpressed genes in leukemic cells may be used as a target for MRD detection by RQ-PCR. Gene translocations, such as t(9;22) in chronic myelogenous leukemia (CML), and t(15:17), t(8;21), and inv(16)/t(16;16) found in AML, are straightforward leukemia-specific markers for the detection of MRD [11–17]. In ALL, the most common translocation involves the Philadelphia (Ph^{+}) chromosome; however, around 40–50% of cases of ALL (and AML) have a translocation that can be detected by PCR assays [18]. In ALL, the most used PCR markers involve the leukemia-specific "fingerprints" caused by the immunoglobulin heavy chain (IgH) and TCR rearrangement [19,20]. The sensitivity of PCR ranges from 10^{-4} to 10^{-6}, depending on the specific genetic target.

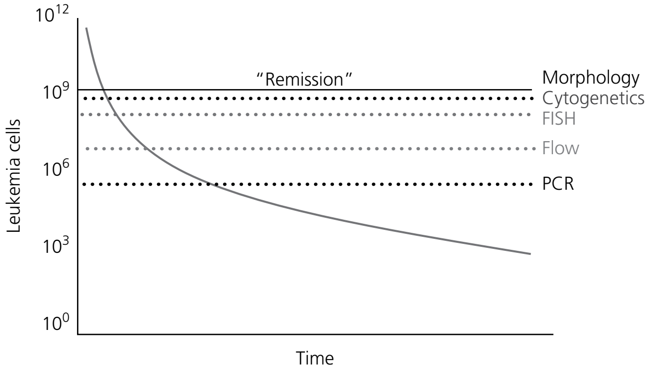


Figure 25.1 The measurement of minimal residual disease (MRD). At diagnosis, there may be as many as 1012 malignant cells present (curved line). After an effective induction therapy that yields a "3-log kill," a huge number of leukemia cells still remain despite morphologic remission. Various techniques can be used to test for MRD as the leukemia burden further lessens with continued therapy. The relative depth of remission plumbed by these techniques is illustrated. FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction.

Since much of the sensitivity is derived from the difference of the genetic target from the background "wild type" genome, it stands to reason that mutations subtly different to normal are more difficult to assay and detect. Thus, point mutations in oncogenes (e.g. N-*ras*) are difficult to assess with great sensitivity with PCR methods. FLT3-ITD mutations are common in AML, but also difficult to adapt to PCR methods, since the composition and length of the insert vary from patient to patient, hence each patient must have custom, "patient-specific" probes, much like Ig VDJ rearrangements in ALL.

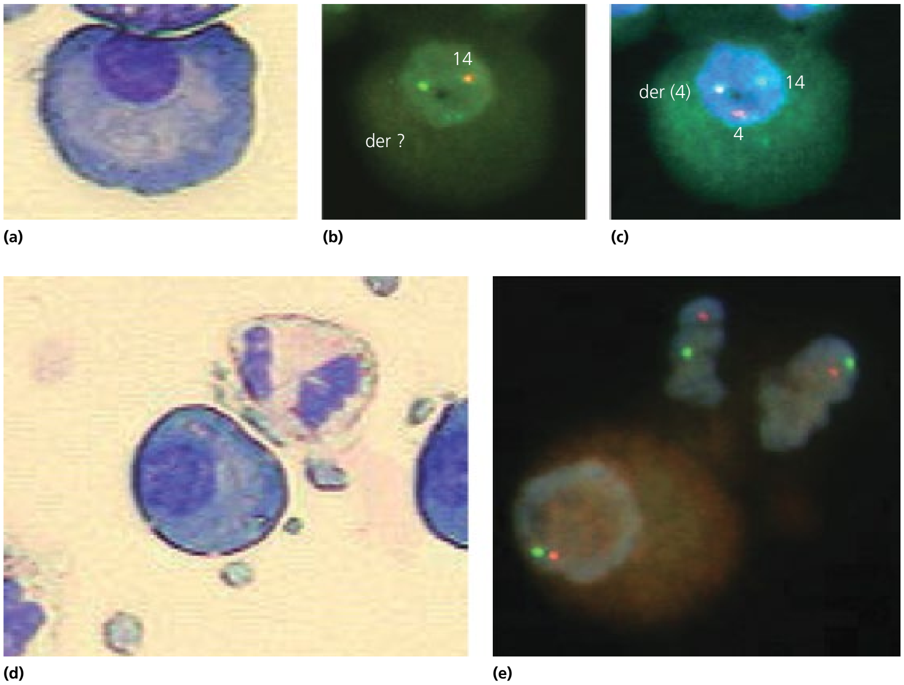
High-throughput sequencing

During the last decade, there has been rapid development of methods to sequence and analyze complete genomes. There are many platforms for high-throughput sequencing (HTS), but most share common steps. First the DNA is fragmented into short single stands, and the DNA is isolated on a bead or in a water–oil emulsion. Each isolated DNA fragment is amplified, and these copies provide the template for a sequencing reaction. Depending on the technology, millions of ~30–250 nucleotide "reads" of sequence are produced. The DNA sequence in total is pieced together by combining the millions of overlapping sequences [21]. Although not yet in routine clinical use, new DNA sequencing technology is bearing down on the clinical laboratory, and is likely to have a major impact on MRD studies in the near future. The technology has the major advantage of being able to uncover all types of genetic lesions – insertions, deletions, translocations, and mutations. In addition, theoretically the sensitivity is limited only by the number of copies of genomes sequenced and by the intrinsic error rate of the DNA polymerases. HTS has been used to detect TCR rearrangements in T-cell ALL after HCT, achieving the sensitivity of detecting a single specific T-cell rearrangement in a background of 100,000 cells [22]. Indeed, in a head-to-head comparison with MFC, it appeared that HTS detected MRD in cases negative by flow cytometry. A similar study of 106 pediatric B-cell ALL demonstrated a sensitivity of 10^{-6}, with high correlation with flow cytometry and gene-specific PCR methods [23]. HTS has been used for MRD monitoring in AML patients with FLT3-ITD or NPM1 mutations [24].

Table 25.1 Common assays used for detection of minimal residual disease (MRD)

Detection method	Target	Sensitivity (%)	Applicability	Comments
Pathologic examination	Cellular morphology	5	Defines CR	Standard clinical practice
Cytogenetics	Chromosome structure	1–5	Upfront risk stratification Response indicator Defines clonal evolution	Assays dividing cells Limited sensitivity
FISH	Specific genetic marker(s)	0.1–5	Leukemia-specific target Rapid assay for known marker	Evaluates single cells Assays all nucleated cells
Combined morphology–FISH or combined IHC–FISH	Specific cell type and specific genetic marker	~0.01–1	Targeting rare event tumor cells Screening stem-cell harvests	Time consuming Requires automation
Flow cytometry	Surface antigen (protein) expression	0.001–0.1	Rapid, quantitative measure of response	Measures single cells Immunophenotypes may shift over time Sensitivity based on number of "colors" and expertise
PCR for chromosome aberrations	mRNA sequence	0.0001–0.1	Diagnosis to detect translocations MRD assessment	Limited to patients with known fusion gene transcripts
PCR for Ig/T-cell receptor genes	DNA sequence (junctional region)	0.001–0.1	MRD assessment	Patient specificity means time consuming at diagnosis but rapid assay at follow-up False negatives may result with clonal evolution or phenotype shifts
High-throughput sequencing	Sequence of large genome fragments	0.0001–0.001	MRD assessment	Not yet in routine clinical use, but transition of new sequencing methods to the clinical laboratory is under way

CR, complete remission; FISH, fluorescence in situ hybridization; IHC-FISH, sequential immunohistochemistry-FISH; PCR, polymerase chain reaction.



Targeted scanning with CD19 monoclonal antibody

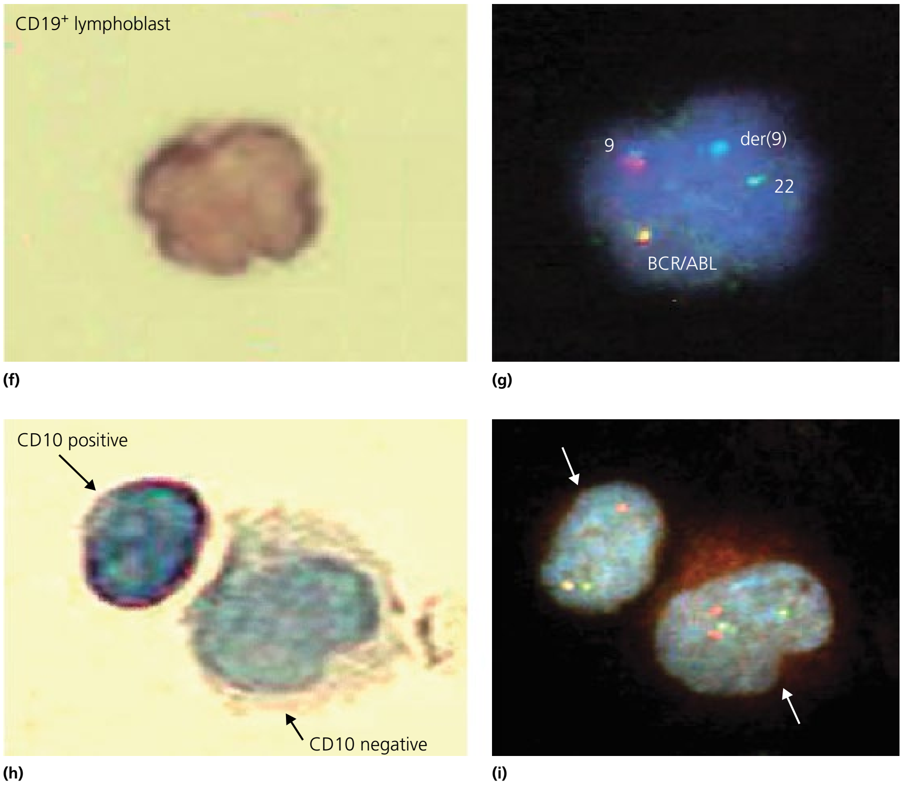


Figure 25.2 Plasma cell-specific fluorescence in situ hybridization (FISH) analysis. (a–e) Cytospin slides are made from the bone marrow aliquot and stained with May–Grünwald stain. After the cells have been mapped to the slide, the slide is destained and hybridized with the selected DNA probe. For details, see [5]. (a) A plasma cell that has been mapped. This same cell is seen in (b), showing a variant immunoglobulin H (*IGH*) pattern using a dual-color *5′IGH/3′IGH* break-apart probe. Once an *IGH* gene rearrangement is found in a patient with multiple myeloma, the same slide is rehybridized to determine its partner chromosome in the IGH translocation. (c) A fusion signal is detected with the *FGFR3* (fibroblast growth factor receptor-3)/*IGH* probe indicating the derivative chromosome 4 of the t(4;14) or der(4)t(4;14). The second fusion that would have indicated the der(14)t(4;14) is not present in this patient, the result of loss of the derivative 14 chromosome. In another slide from this patient [(d) and (e)], monosomy 13 is shown in the neoplastic plasma cells, but the segmented neutrophil adjacent to the plasma cells shows both chromosome 13 signals (one each in each of the segments of the nucleus). The chromosome 13 probe set used was composed of D13S319 probe (red), which maps to 13q14.3, and the *LAMP1* (lysosomal-associated membrane protein-1)/13q34 reference probe labeled in green. Sequential immunohistochemistry (IHC)/FISH is shown in (f)–(i). (f) A CD19^{+} lymphoblast that is *BCR/ABL1* fusion gene positive (g), indicating residual disease and not a hematogone. Similarly, (h) and (i) show the cell that is CD10^{+} is positive for the *BCR/ABL1* fusion gene, and the CD^{-} cell is negative for the *BCR/ABL1* fusion gene. The *BCR/ABL1* probe used for the sequential IHC/FISH studies was the single fusion probe spiked with 9q34/argininosuccinate synthetase gene probe labeled in Spectrum aqua. All probes were obtained and reproduced with permission from Abbott Molecular Inc. (Des Plaines, IL, USA). (For a color version of this figure, please see the color plate section.)

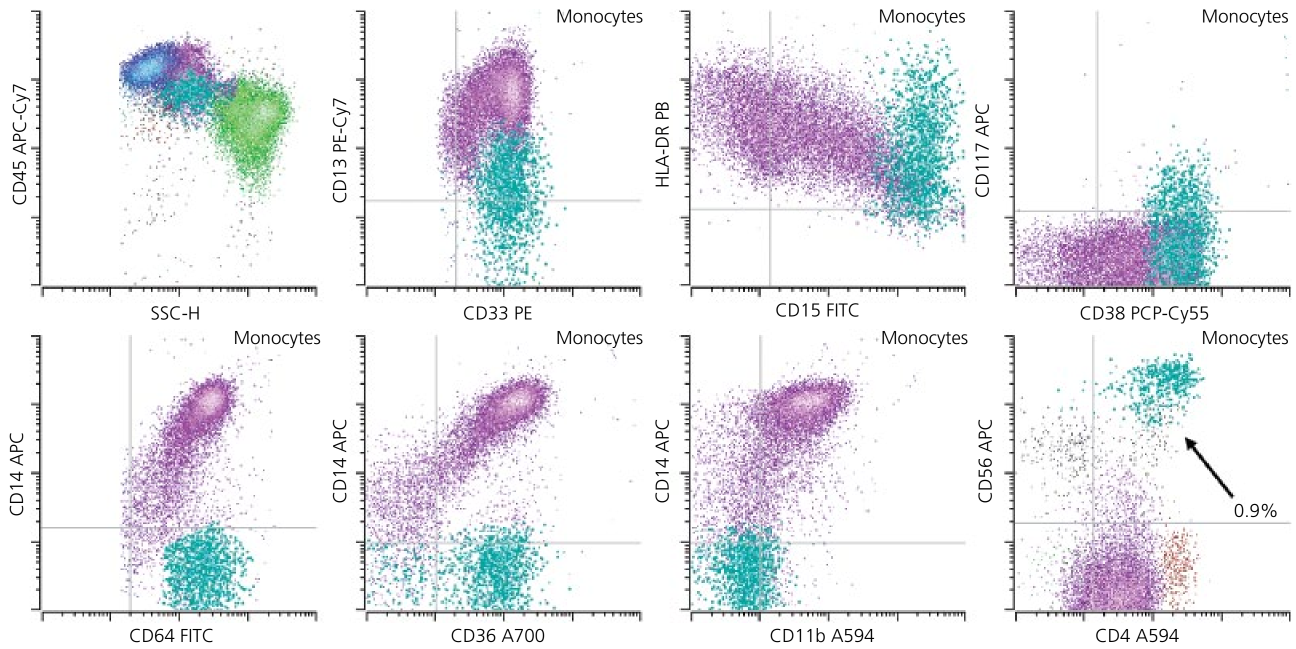


Figure 25.3 Flow cytometry assays for detection of minimal residual disease. Leukemia samples can be labeled with multiple fluorescent antibodies, and cells can be distinguished by the size of the cell and the types of antibody that bind the cell surface. Normal hematopoiesis has very stereotypical cell antigen expression linked to differentiation; leukemia cells are distinguished by inappropriate expression patterns of these antibodies. In the example, a residual acute monocytic leukemia is analyzed with multiple antigens. In the bottom left panel, the sample shows an inappropriate expression of CD56 and CD4 consistent with residual disease. In this example, these cells made up 0.9% of the total nucleated cells. Source: courtesy of Dr. Brent Wood, University of Washington. (For a color version of this figure, please see the color plate section.)

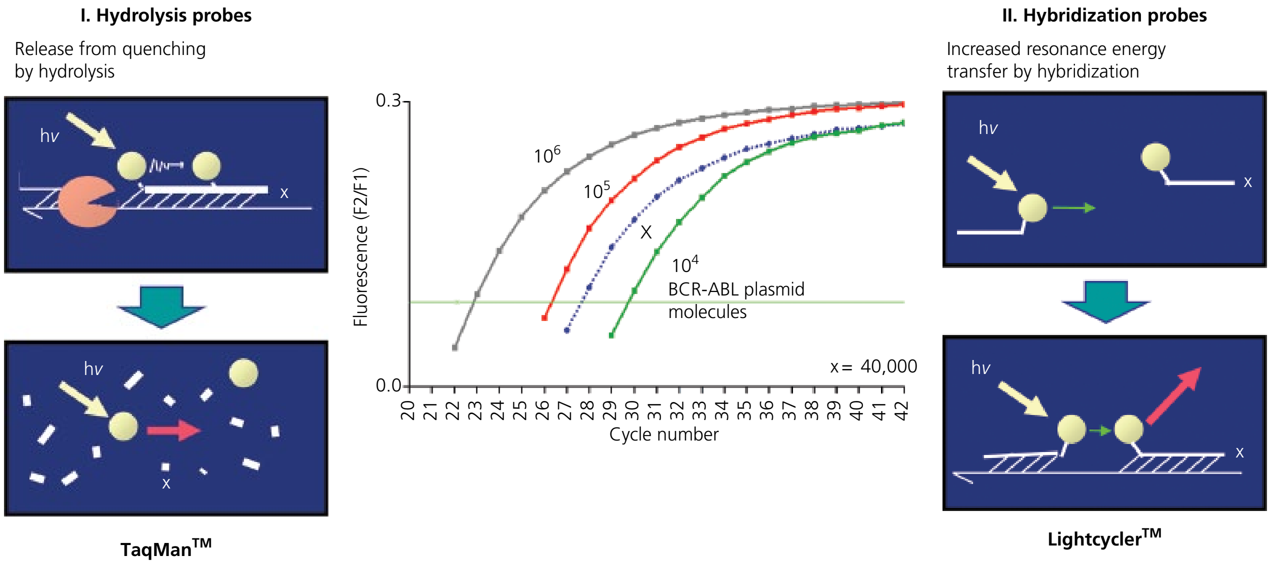

I. Hydrolysis probes
Release from quenching by hydrolysis
hv
x
hv
x
TaqMan™
0.3
0.0
Fluorescence (F2/F1)
10⁶
10⁵
X
10⁴
BCR-ABL plasmid molecules
x = 40,000
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42
Cycle number
II. Hybridization probes
Increased resonance energy transfer by hybridization
hv
x
hv
x
Lightcycler™

Figure 25.4 Quantitative polymerase chain reaction (PCR). The cartoon shows the principle behind the two most popular quantitative approaches: the Taqman (ABI) and the LightCycler (Roche). In the Taqman reaction (I), the probe is a 5′ reporter dye and a 3′ quencher molecule. When the probe is intact, the quencher blocks laser excitation of the reporter. During PCR, the 5′ exonuclease activity of Taq polymerase releases the reporter, which, free from the quencher, is excited by the laser detection system. In the LightCycler system (II), annealing of juxtaposition probes during PCR synthesis allows for the transfer of resonance energy that can be stimulated by the laser and detected. In both systems, a standard curve is generated that plots dilutions of the standards versus the PCR cycle number at which a threshold signal is obtained (middle panel). The starting amount of target in unknown samples is thus detected by a back-calculation of cycle threshold in the unknown (Ct) against the standard curve. Source: Reproduced with permission of Dr. John Goldman. (For a color version of this figure, please see the color plate section.)

The clinical significance of MRD

Many studies have demonstrated the effect of MRD on relapse and outcome in the setting of conventional chemotherapy and HCT. Detection of MRD after conventional therapy can bring patients to transplant in a timely manner, and detection of MRD after transplant may be used in order to start preemptive antileukemic therapy prior to overt relapse.

MRD detection in acute lymphoblastic leukemia (ALL) patients receiving chemotherapy

In ALL, MRD detection is accomplished by either MFC, PCR, or, more recently, HTS. Flow cytometry assays rely on the unique and aberrant constellation of surface antigens in the leukemia clone. PCR-based assays rely on the clonal IgH (V-D-J) or TCR gene rearrangements that can be used to follow MRD in approximately 70% of ALL cases. This PCR method takes advantage of the unique gene rearrangements that occur in these genes as cells develop into functional B and T cells. While all individual normal B and T cells should have unique gene rearrangements, a clonal outgrowth of malignant cells should all have identical gene rearrangements, and this "fingerprint" can later be used to distinguish malignant versus normal cells in "remission" samples. Although both methods have excellent sensitivity, both are susceptible to clonal "shifts" that occur with disease progression in 10–20% of cases, whereby the relapsing clone appears with a different gene rearrangement or surface antigen pattern than the presenting leukemia [25–28]. The combined use of these two methods should permit MRD monitoring in almost all patients and prevent false-negative results due to clonal evolution and phenotypic shift [29,30]. Based on several prospective clinical trials, detection of MRD has emerged as part of standard evaluation and treatment guidance of both pediatric and adult ALL [31].

The clinical impact of MRD in ALL depends on the time point at which it is assessed. Detection of MRD can be used to evaluate early treatment response, and thereby allows the identification of low- and high-risk patients who may profit from therapy reduction or therapy intensification, respectively. Early clearance of leukemia cells is a favorable prognostic indicator in childhood and adult ALL [19,32–34]. Low or absent MRD in the BM after the completion of induction therapy predicts a favorable outcome, as MRD-negative patients have an overall relapse rate of only 2–10% (Table 25.2) [19,35–38]. On the other hand, high MRD levels at the end of induction treatment have been associated with high relapse rates of 70–100% (Table 25.3) [19,35–37,39]; these patients at risk for relapse may benefit from considering alternative treatment approaches such as transplantation [19,40–42]. MRD status after induction therapy may be the most significant prognostic factor in ALL, independent of other clinically relevant risk factors [19,32,37,43]. In pediatric ALL cases who relapse after achieving remission, MRD status after re-induction treatment is similarly important [41,44]. For example, Coustan-Smith et al. [44] studied

Table 25.2 Minimal residual disease (MRD) studies in childhood acute lymphoblastic leukemia

Study	*N*, diagnosis	MRD method	MRD time point	Impact of MRD on clinical outcome
Cave et al. 1998 [46]	178 (B and T ALL)	Ig/TCR-PCR	After induction therapy and during the first 6 months after complete remission	8% of patients with negative MRD after induction relapsed vs. 38% of patients with positive MRD. The risk of relapse was 7.3 times higher in patients with detectable disease than in those with undetectable disease after consolidation
van Dongen et al. 1998 [47]	240 (B and T ALL)	Ig/TCR-PCR and *TAL1* deletion	Post-induction therapy	MRD-positive was associated with 39–86% relapse rate at 3 years, compared with 3–15% relapse in MRD-negative. Risk of relapse in patients with high-degree MRD early after induction was three times higher than in patients with low-degree MRD. During further follow-up even a low degree of MRD was associated with high relapse rates
Coustan-Smith et al. 2000 [48]	195 (B and T ALL)	Flow cytometry	End of induction and during weeks 14, 32, and 56 of continuation therapy	Detection of MRD was significantly associated with a greater likelihood of relapse. At the end of induction therapy, the 5-year cumulative incidence of relapse was 43% for MRD-positive patients compared with 10% for MRD-negative patients
Dworzak et al. 2002 [49]	108 (B and T ALL)	Flow cytometry	Four time points during the first 6 months of treatment	Presence of MRD at day 33, week 12, and weeks 22–24 was associated with a greater likelihood of relapse, but not at day 15. The MRD level that was associated with increased relapse risk decreased with time under treatment (day 15, ≥1.0%; day 33, ≥0.1%; week 12, ≥0.01%; weeks 22–24, any MRD positivity)
Nyvold et al. 2002 [50]	104 (B and T ALL)	Ig/TCR-PCR	Day15 and day 29 after start of induction therapy	Patients who relapsed had higher day 15 and day 29 MRD levels than patients who stayed in remission (median levels: day 15, 1% versus 0.1%, $p = 0.03$; day 29, 0.4% versus 0.01%, $p = 0.0001$)
Marshall et al. 2003 [51]	85 (B and T ALL; Ph negative)	Ig/TCR-PCR	1, 12, and 24 months from diagnosis	Patients with $\leq 10^{-3}$ residual blasts at 1 month after diagnosis had 81% 5-year RFS compared with 23% for patients with $>10^{-3}$ residual blasts. 5-year RFS for MRD-negative patients at 12 months after diagnosis was 71% vs. 36% for MRD-positive patients; 5-year RFS for MRD-negative patients at 24 months after diagnosis was 74% vs. 36% for MRD-positive patients.
Paganin et al. 2008 [52]	60 (relapsed B and T ALL)	Ig/TCR-PCR	3–5 weeks after initiation of treatment, 6–8 weeks after initiation of treatment, and 9–16 weeks	3-year EFS was 79% for patients with negative MRD at first time point, 43% for patients with low positive MRD and 18% for patients with high positive MRD. The relative risk of relapse for positive MRD patients at the first time point available vs. with negative-MRD patients was 4.4
Van der Velden et al. 2009 [53]	99 (infants with B ALL)	Ig/TCR-PCR, MLL rearrangement	During and after induction, consolidation, and maintenance	51% of patients with high MRD at day 15 relapsed vs. 11% of patients with low MRD. All patients with MRD $\geq 10^{-4}$ during consolidation relapsed. After consolidation, 71% of MRD-positive patients relapsed vs. 17% of MRD-negative patients
Scrideli CA et al. [54]	229	Ig/TCR-PCR	Day 14 and day 28 of induction therapy	5-year EFS was 85% for patients with negative MRD on both day 14 and day 28, 76% for patients with positive MRD on day 14 and negative on day 28, and 28% for patients with positive MRD on both days

Ig/TCR-PCR, immunoglobulin/T-cell receptor polymerase chain reaction.

Table 25.3 Studies examining the impact of minimal residual disease (MRD) on outcome of adult acute leukemia (ALL and AML) in the non-transplant setting

Study	*N*, diagnosis	MRD method	MRD time point	Impact of MRD on clinical outcome
Vidriales et al. 2003 [55]	102 (B and T ALL)	Flow cytometry	Day 35 of induction	Patients with low MRD levels (<0.05%) had a significantly longer RFS than patients with high MRD levels (median RFS: 42 months vs. 16 months)
Brüggemann et al. 2006 [56]	196 (B and T ALL)	Ig/TCR-PCR	Up to 9 time points in the first year after induction	Patients with a rapid MRD decline to lower than 10^{-4} or below detection limit at day 11 and day 24 had a 3-year relapse rate (RR) of 0%. Patients with MRD of 10^{-4} or higher until week 16 had a 3-year RR of 94%. The remaining patients had an RR of 47%
Holowiecki et al. 2008 [57]	116 (B and T ALL)	Flow cytometry	After induction therapy	The incidence of relapse at 3 years was 26% for patients with MRD <0.1% after induction vs. 81% for patients with MRD ≥0.1%
Bassan et al. 2009 [58]	112 (B and T ALL)	RQ-PCR (Ig/TCR and/or fusion genes)	3 time points after initiation of induction	5 year OS/DFS were 75%/72% in the MRD-negative group compared with 33%/0.14% in MRD-positive group. MRD was the most significant risk factor for relapse (HR 5.22)
Patel et al. 2010 [59]	159 (B-ALL)	RQ-PCR (Ig/TCR)	3 time points after initiation of induction	At completion of phase I induction, 5-year RFS was 69% in MRD-negative patients and 42% in MRD-positive patients. At completion of phase II induction, 5-year RFS was 71% in MRD-negative patients and 15% in MRD-positive patients
Gökbuget et al. 2012 [60]	580 (B and T ALL)	RQ-PCR (Ig/TCR)	Day 71 and week 16 after induction	Patients with molecular CR after consolidation had a significantly higher probability of continuous complete remission (74% vs. 35%) and of overall survival (80% vs. 42%) compared with patients with no molecular CR
Pane et al. 2005 [61]	42 B-ALL; Ph+	RT-PCR (*BCR-ABL*)	End of induction and end of consolidation	In patients with good molecular response (>2 log reduction of residual disease after induction and >3 log reduction after consolidation therapy), the probability of RFS, DFS and OS at 2 years was 38, 27, and 48%, respectively, compared with 0, 0, and 0% in patients with poor molecular response
Ottmann et al. 2007 [62]	49 B-ALL; Ph+	Qualitative PCR (*BCR-ABL*)	After end of induction and each consolidation cycle	Median DFS was significantly longer in patients in molecular CR compared with patients with no molecular CR (18.3 vs. 7.2 months)
Yanada et al. 2008 [63]	100 B-ALL; Ph+	RT-PCR (*BCR-ABL*)	Day 28 and day 63 after induction, end of consolidation	Negative MRD at the end of induction therapy was not associated with longer RFS or lower relapse rate. Molecular MRD elevation during hematologic CR is predictive of subsequent relapse, but allogeneic hematopoietic stem-cell transplantation can override its adverse effect (12/13 patients who had not undergone allogeneic HCT experienced a relapse, whereas 10/16 patients who had undergone allogeneic HCT were alive without relapse at a median of 2.9 years after transplantation)
Lee et al. 2009 [64]	52 B-ALL; Ph+	RT-PCR (*BCR-ABL*)	After induction chemotherapy, 1st course TKI, consolidation, 2nd course TKI	No prognostic impact of MRD after induction chemotherapy before TKI. However, a reduction in *BCR-ABL* transcript levels of at least 3 log after the first 4 weeks of imatinib therapy was the most powerful predictor of lower relapse rate (12.1% vs. 45.1%) and better DFS (82.1% vs. 41.7%).
Venditti et al. 2000 [65]	55 (AML)	Flow cytometry	After induction and after consolidation	The most predictive MRD cutoff value after induction was 4.5×10^{-4} cells: 58% of patients with 4.5×10^{-4} cells or more after induction had relapsed, compared with 40% of those with fewer than 4.5×10^{-4} cells. After consolidation, the most predictive MRD cutoff value was 3.5×10^{-4}cells: 77% of these patients had relapsed, compared with 17% patients with lower MRD levels.
Venditti et al. 2002 [66]	63 (AML)	Flow cytometry	After consolidation	81% of MRD-positive patients after consolidation relapsed vs. 27% of MRD-negative patients. MRD positivity at consolidation was associated with a high probability of relapse ($p < 0.00026$) and short duration of RFS ($p = 0.008$).
Kern et al. 2004 [67]	93 (AML)	Flow cytometry	After induction and after consolidation	The degree of disease reduction between diagnosis and CR was significantly related to RFS after both induction ($p = 0.0001$) and consolidation ($p = 0.00008$).
Buccisano et al. 2006 [68]	100 (AML)	Flow cytometry	Several time points after induction and consolidation	Post-consolidation MRD-negative patients had a superior relapse rate, OS and RFS ($p < 0.001$, for all comparisons), regardless of the MRD status after induction
Maurillo et al. 2008 [69]	142 (AML)	Flow cytometry	After consolidation	5-year RFS rates were 60% and 16% for MRD-negative and -positive patients, respectively. 5-year OS rates were 62% and 23%, respectively
Al-Mawali et al. 2009 [70]	54 (AML)	Flow cytometry	After induction and after consolidation	MRD level post-induction influenced RFS ($p = 0.004$) and OS ($p = 0.003$). Multivariate analysis showed that MRD post-induction was a powerful independent prognostic factor for both RFS ($p = 0.037$) and OS ($p = 0.026$)

the clinical significance of MRD in ALL after first relapse using flow cytometry for MRD detection. Of the 35 children with morphologic remission after induction treatment, 19 (54%) had an MRD of 0.01% or more, and their subsequent relapse rate was 70%, compared with 28% for the 16 MRD-negative patients. The value of continuing MRD monitoring during maintenance therapy and off therapy is less clear, since frequent monitoring has been difficult to employ [33,37,45].

There is increasing evidence that in adult ALL, as in children, an early decrease in MRD level after induction therapy is an important predictor of prognosis and response to chemotherapy [55–64,71–73]. Assessment of MRD kinetics at different follow-up time points defined risk groups based on a specific threshold MRD level in standard-risk adult ALL patients [74]. Patients with a rapid MRD decline to lower than 10^{-4} or under the detection limit at day 11 (during first induction) and day 24 (at the end of induction) were at low risk, with a 3-year disease-free survival (DFS) and overall survival (OS) of 100%. In contrast, patients with a persistent MRD of 10^{-4} until week 16 were at high risk, with a 3-year DFS of 5.8% and a 3-year OS of 45.1%.

MRD measurement in the first year of treatment is also a useful outcome predictor for adult T-cell ALL patients [75]. MRD-positive patients prior to consolidation therapy had a probability of relapse at 2 years of 82%, compared with 39% in MRD-negative patients. In a larger study of 102 adolescent and adult ALL patients, MRD at day 35 was found to be the most powerful independent prognostic parameter by multivariate analysis [76]. Patients in morphologic complete remission (CR) and a low MRD level (<0.05%) had a median relapse-free survival (RFS) of 42 months versus 16 months in the case of higher MRD levels. An excellent prognosis with a projected 5-year RFS of 90% was observed when MRD levels at day 14 were very low (<0.03%). The German Multicenter Study Group for Adult ALL evaluated MRD by real-time PCR directed to TCR and Ig gene rearrangements in 580 patients with B- and T-lineage Ph$^-$ ALL with cytologic complete remission (CCR) after induction and consolidation chemotherapy [12]. MRD+ after consolidation chemotherapy had the worst prognostic effect on CCR after 5 years. The probability of CCR after 5 years was 74 ± 3% for patients with MRD– versus 35 ± 5% for patients with MRD+. OS was significantly inferior in MRD+ than in MRD– patients (42 ± 5% vs. 80 ± 3%).

In adult Ph$^+$ ALL, MRD is followed by measuring *BCR-ABL* transcript levels with RQ-PCR [61–64,77,80]. Using this approach, it has been demonstrated that 42 patients with complete hematologic response following induction therapy were divided into two prognostic groups: good molecular responders (with a >2 log reduction of *BCR-ABL* transcript levels after induction, and a >3 log reduction after consolidation therapy was correlated with significantly better 2-year survival (48%) compared with patients with higher MRD levels (0%) [80]. However, in Ph+ ALL patients treated with combinations of tyrosine kinase inhibition and chemotherapy, rapid molecular response alone is not necessarily associated with a favorable prognosis, in that a single observation of elevated MRD is associated with an increased risk relapse [63].

MRD in ALL in the transplant setting

MRD detection prior to and following transplantation is strongly predictive of outcome. Many reports have shown that the MRD burden prior to conditioning is the most powerful single predictive factor for relapse after HCT (Table 25.4) [78,79,81–89]. In these studies, the event-free survival (EFS) for the MRD-negative groups prior to HCT was often more than 70%, whereas for those in the MRD-positive group the EFS fell to 17–50%. Both PCR and flow cytometry were used to detect MRD in these studies, and various criteria have been used to determine "low" from "high" levels of

Table 25.4 Studies examining the impact of pre- or post-hematopoietic cell transplantation minimal residual disease (MRD) levels in ALL and AML on subsequent relapse and transplant outcome

Study	*N*, diagnosis	MRD method	MRD study time point	Impact of MRD on clinical outcome
Knechtli et al. 1998 [78]	64 (ALL)	PCR of Ig or T-cell receptors	~1–12 weeks pre-transplant	2-year EFS for high-level MRD (>10^{-2}), low-level MRD (10^{-3}–10^{-5}), or MRD negative was 0%, 36%, and 73%, respectively
van der Velden et al. 2001 [79]	17 (ALL)	Ig/TCR gene by Southern blotting, PCR heteroduplex analysis, sequencing, and Ig/TCR-PCR	Pre-HCT (no specific time indicated)	2/11 patients with negative MRD relapsed post-transplant compared with 4/6 patients with positive MRD
Uzunel et al. 2001 [81]	30 (ALL)	Ig/TCR-PCR	0–30 days (median 9 days) before HCT	13/25 patients with MRD positivity pre-transplant relapsed post-transplant vs. 0/25 MRD-negative patients.
Bader et al. 2002 [82]	41 (ALL)	PCR amplification of antigen receptor gene rearrangements and clone-specific oligoprobing	Prior to conditioning (no specific time indicated)	5-year EFS was 23%, 48% and 78% for high-level MRD positivity, low-level MRD positivity and negative MRD, respectively
Krejci et al. 2003 [83]	140 (ALL)	Semiquantitative PCR, RQ-PCR, and oligoprobe blotting-based analysis of TCR and Ig gene)	Prior to conditioning (no specific time indicated)	16% of patients with negative MRD relapsed post-transplant compared to 65% of patients with positive MRD.
Sanchez et al. 2002 [84]	24 (ALL)	Flow cytometry	Prior to conditioning (no specific time indicated)	4/6 MRD-positive patients relapsed post-HCT vs. 3/18 MRD-negative patients. 2-year DFS for MRD-positive patients was 33 % vs. 74% for MRD-negative patients.
Uzunel et al. 2003 [85]	32 (ALL)	Ig/TCR gene rearrangement, QR-PCR	Before and after HCT	11/24 patients MRD positive before HCT relapsed vs. 1/5 for MRD-negative patients
Sramkova et al. 2007 [86]	25 (ALL)	Ig/TCR gene rearrangement, QR-PCR	1 week prior to conditioning	6/8 MRD-positive patients relapsed post-HCT vs. 1/17 MRD-negative patients. Multivariate analysis identified pre-transplant MRD as the only significant risk factor for relapse post-HCT
Spinelli et al. 2007 [87]	37 (ALL)	RQ-PCR for fusion chimeric genes and Ig/TCR rearrengements	Prior to conditioning (no specific time indicated)	3-year overall survival for MRD-negative patients was 80% vs. 49% for MRD-positive patients. The cumulative incidence of relapse was 0% and 46%, respectively.
Bader et al. 2009 [88]	91 (ALL)	Ig/TCR gene rearrangement, QR-PCR	1–39 (median 13) days before conditioning	Probability of EFS and cumulative incidence of relapse in MRD-positive patients was 27% and 57% vs. 60% and 13% in MRD-negative patients.
Leung et al. 2012 [90]	122 (ALL, AML)	Flow cytometry	Prior to conditioning (no specific time indicated)	6% of patients with negative MRD at the time of HCT died of relapse vs. 29% of MRD-positive patients. The 5-year cumulative incidence of relapse after HCT was 40% in patients with high levels of MRD (≥0.1% leukemia cells in ALL and ≥1.0% in AML), 16% among patients with low level of MRD (0.01 to <0.1% leukemia cells in ALL and 0.1 to <1% in AML), and 6% in patients with no MRD
Walter et al. 2011 [91]	99 (AML)	Flow cytometry	Prior to conditioning (no specific time indicated)	2-year estimates of OS were 30.2% and 76.6% for MRD-positive and -negative patients, respectively; 2-year estimates of relapse were 65% and 18%. MRD-positive HCT was associated with increased overall mortality (HR 4.1) and relapse (HR 8.5) relative to MRD-negative HCT
Fang et al. 2012 [92]	424 (AML)	Flow cytometry, cytogenetics	Before and after HCT (~1 month, ~3 months, and ~1 year)	Positive cytogenetics or flow cytometry pre- or post-HCT predicted a significantly worse outcome than negative results. Patients with discordant results had significantly worse outcomes than patients with negative results. The HRs for reduced OS with either positive cytogenetic results or flow results pre-transplantation were 3.1 and 2.5, respectively. The HRs for decreased RFS were 2.7 and 4.1 respectively
Miyazaki et al. 2012 [93]	31 (AML), 10 (ALL)	Flow cytometry and Q-RT-PCR for *WT1* mRNA	Post-HCT on days 28, 56, 100, and 365	The duration of OS was significantly associated with the detection of MRD by flow cytometry ($p = 0.014$), whereas no significant difference of RFS was observed between the MFC-positive and -negative groups ($p = 0.068$)
Zhao et al. 2012 [89]	139 (B and T ALL; Ph+/–)	Flow cytometry and Q-RT-PCR	Different time points after allogeneic HCT	MRD-positive patients had cumulative incidence of relapse of 54% vs. 8% in MRD-negative patients. MRD status after transplantation was an independent prognostic factor for EFS, OS, and relapse ($p < 0.001$, $p = 0.013$, and $p < 0.001$, respectively)

MRD burden. Taken together, however, the data are fairly consistent, showing a very high risk of relapse (~75%) in cases with high levels of MRD prior to HCT, an intermediate risk of relapse in cases with low-level MRD (~50%), and a much smaller risk of relapse in those without MRD (~15%).

MRD detection following transplant is likewise associated with a high risk of subsequent relapse. This is true for Ph^+ ALL and Ph^- ALL cases. In the latter setting, studies have found the relative risk of relapse associated with MRD to be roughly 5–10-fold, with relapse occurring within a few months of MRD detection [94,95]. The situation in Ph^+ ALL is more complicated; PCR must be performed on both the "p210" *BCR-ABL* variant transcript (found in CML and some ALL), and the "p190" *BCR-ABL* transcript (found only rarely in CML but common in ALL). All studies of Ph^+ ALL have demonstrated excess relapses in MRD-positive cases following transplant [96–98]. However, the data suggest that the risk of relapse associated with p190 *BCR-ABL* MRD is stronger than with p210 *BCR-ABL* [98]. For p190 MRD, the relative risk was 8.7, whereas for p210 *BCR-ABL* transcripts it was 2.2. Note that this difference in risk with different *BCR-ABL* transcript variants was also seen in the pre-transplant MRD assessment. The median time from first MRD detection to relapse was 75 days. Spinelli et al. [99] correlated MRD clearance immediately before and 30 and 100 days after HCT with outcome in 43 adult "high-risk" ALL patients by RQ-PCR using one or two molecular probes for *BCR-ABL, MLL/AFF1* or rearrangements of the T-cell receptor or immunoglobulin genes. At 36 months, the OS of the 37 patients who underwent HCT in hematologic remission was 80% for those who were PCR negative before transplantation compared with 49% for PCR-positive patients and the cumulative incidence of relapse was 0% and 46%, respectively. The relapse rate of patients who were PCR negative at day 100 after transplantation was remarkably low (7%) compared with that among patients who were PCR positive (80%).

Achieving a molecular CR *before* HCT is a highly desirable associated subsequent response to the transplant. Zhao et al. studied 139 patients with ALL undergoing allogeneic HCT, and found that those with MRD after transplantation had lower EFS (54%) and a higher cumulative incidence of relapse (54%) compared with an EFS of 80% and a CIR of 8% in MRD-negative patients. Multivariate analysis showed that MRD status after transplantation was an independent prognostic factor. A good correlation was found between the MRD results of flow cytometry and RQ-PCR [89]. Thus, the achievement of a pre-HCT molecular remission is a desirable goal in high-risk ALL, though it is not known if continued, more aggressive, or experimental therapy to treat MRD pre-transplant (1) can change an MRD-positive to MRD-negative status or (2) is associated with an increased toxicity that might actually adversely affect transplant outcome.

Of note is that most MRD studies have used BM samples rather than peripheral blood (PB). In precursor B-cell and Ph^+ ALL, the level of MRD is higher in BM than PB, which makes BM the preferred source for disease monitoring. However, the use of PB to detect MRD has several practical advantages over the use of BM, especially in children, as PB sampling is simple and relatively painless, allowing more frequent monitoring, which may offset the sensitivity advantage of BM [100,101].

MRD detection in acute myeloid leukemia (AML) following chemotherapy

In AML, the use of PCR-based detection of MRD is most commonly used for cases containing unique breakpoint fusion regions, such as t(15;17), t(8;21), and inv(16). In contrast, flow cytometry assays can be used to detect abnormal patterns of lineage and differentiation-specific markers suggestive of leukemia blasts in the majority of AML cases.

Acute promyelocytic leukemia (APL) is characterized by the t(15;17) translocation, which results in the promyelocytic leukemia/retinoic acid receptor-alpha or *PML/RARα* gene juxtaposition. APL patients who have residual disease after induction or consolidation therapy have a significant risk of relapse [102,103]. Serial quantitative MRD monitoring by PCR until completion of maintenance therapy identifies patients at high risk of relapse in APL [104], and the detection of MRD after first consolidation is a powerful predictor of relapse. Patients with an MRD level of 10^{-3} or more had a >10-fold higher relapse rate at 5 years compared with a level of $<10^{-3}$ In APL; there is evidence that the analysis of peripheral blood may be as informative as that of BM [105].

Disruptions of the core binding factor genes, runt-related transcription factor-1 (*RUNX1, AML1*), and core binding factor β subunit (*CBFB*) are involved in the t(8;21) and inv(16)/t(16;16) "favorable" risk translocations, found collectively in 15–20% of adult and pediatric AML cases. However, the mere qualitative detection (yes or no) of the *RUNX1/RUNX1T1* and *CBFB/MYH11* gene transcripts has limited value in monitoring MRD and predicting relapse since these transcripts persist during long-term remission [106–114]. Reciprocal rearrangements of the "mixed lineage leukemia" (MLL) gene on chromosome 11q23 are associated with unfavorable prognosis. The most frequent 11q23/MLL rearrangements detected by RT-PCR are the t(11;19)/*MLL/MLLT1* and the t(9;11)/*MLL/MLLT3* [115]. Intragenic mutations of the *MLL* gene (*MLL-PTD*; partial tandem duplications) have an unfavorable prognostic relevance [116]. *MLL-PTD* positivity detected by RT-PCR or RQ-PCR after standard chemotherapy significantly correlates with leukemia recurrence [117].

Quantification of MRD is essential in these AML variants [14,109]. Several groups have attempted to define the critical level of MRD that accurately segregates good and poor outcome. In general, a post-therapy MRD level of less than 1% as determined by PCR analysis of fusion or mutated genes has been associated with a relatively low risk of relapse [118–123]. MRD investigation at the end of treatment might have the best prognostic value in core binding factor-leukemia [124]. Schnittger et al. created a score for risk stratification of AML by combining the transcription ratios at diagnosis and after 3–4 months during treatment [125].

Immunophenotypic detection of MRD by flow cytometry is useful in AML, although the published experience is less than that in ALL. The clinical significance of positive MRD can vary with the time of assessment during the clinical course, and several studies have identified specific cut-off values of MRD after the induction and consolidation therapy that are associated with outcome [126]. Cut-off levels to distinguish flow cytometry associated with significant survival impact MRD positive from MRD negative vary, ranging from 0.035% to 1%, with most studies using 0.1% as the level of clinically significant MRD [118]. Coustan-Smith et al. [128], using a flow cytometry MRD assay, reported a 2-year OS of approximately 30% in patients with MRD compared with more than 70% in patients without MRD. Sievers et al. [129] found immunophenotypic abnormalities in 16% of 252 children with AML who achieved morphologic CR with initial induction therapy, with MRD highly associated with a poor outcome. Multivariate analysis has shown MRD to be independently associated with relapse and survival [118,130].

The expression of the Wilms tumor gene (*WT1*) is a potential target for MRD detection, as it is overexpressed in approximately

90% of AML cases, and is expressed at low levels in normal cells [131,132]. Several groups have reported that a high *WT1* level at diagnosis is associated with poor prognosis [133]. In some studies, the increase in *WT1* transcripts during therapy was associated with subsequent relapse [131,134] and correlated with other disease markers, such as leukemia-specific fusion transcripts [132]. Polak et al. demonstrated that quantitative monitoring of *WT1* expression in PB of patients with AML before and after HCT is a useful tool for MRD monitoring that correlates with clinical outcome [135].

The most common genetic mutations found in AML involve fms-related tyrosine kinase-3 (*FLT3*) and nucleophosmin (*NPM1*) and occur in approximately 25–35% of cases or more [136]. The most common *FLT3* gene alteration is an internal tandem duplication that causes a 5–100 base-pair addition to the juxtamembrane domain. Primers can be made that allow for leukemia-specific amplification for a specific patient [137–139]. However, the *FLT3* mutation is not very stable, and around 10–20% of the time patients relapse without the *FLT3* mutation [140,141]. Mutation-specific PCR reactions have been constructed for the most common point mutations in *NPM1*, and many potential mutations exist [142], including *CEBPA*, *MLL*, *DNMT3A*, and *IDH1/2,* among others [136]. However, it is unlikely that PCR mutation monitoring will replace the faster and nearly equally sensitive flow cytometry for MRD evaluation.

MRD in AML in the HCT setting

MRD at the time of HCT has a major impact on transplant outcome in AML. Walter et al. [91] reported that pre-HCT MRD is associated with increased risk of relapse and death after myeloablative HCT for AML in first morphologic CR. Among 99 AML patients who underwent myeloablative HCT, 24 had positive MRD pre-HCT as determined by flow cytometry. Two-year estimates of overall survival were 30% and 77% for MRD-positive and MRD-negative patients, respectively; 2-year estimates of relapse were 65% and 18%, respectively. After adjustment for cytogenetic risk, secondary disease, incomplete blood count recovery, and abnormal karyotype pre-HCT, MRD-positive HCT was associated with increased overall mortality (hazard ratio, HR 4.1) and relapse (HR 8.5) relative to MRD-negative HCT. Fang et al. [92] reported that among 424 patients, positive cytogenetic or flow cytometry results at time of transplant, compared with patients who had double-negative test results, had a significant impact on overall survival and relapse-free survival. The HRs with either positive cytogenetic results or positive MFC results pre-transplantation were 3.1 and 2.5, respectively, for reduced overall survival and 2.7 and 4.1, respectively, for decreased recurrence-free survival.

There have been few studies documenting the prevalence and significance of MRD in AML following HCT. This due in part is to the fact that the most common translocations found in AML – for example, t(15;17), t(8;21), and inv(16)/t(16;16) – are associated with reasonable outcomes with conventional chemotherapy, and therefore are underrepresented in the transplant population.

The significance of MRD following transplantation depends largely on the specific genetic subtype of AML, and generally correlates with the "rules" seen in the chemotherapy setting. Thus, in t(15;17) AML, the detection of the *PML/RARα* transcript following HCT is highly correlated with relapse [143,144]. In t(8;21) AML, the detection of *RUNX1/RUNXITI* transcripts remarkably often persists after allogeneic transplant, without subsequent relapse [108–110]. Too few cases of inv(16)/t(16;16) have been studied in the transplant setting to draw a firm conclusion about its association with relapse [107,145].

The tumor suppressor gene *WT1,* which is overexpressed in >90% of patients with AML, has been used as a marker of MRD following HCT, with a number of studies demonstrating correlation between *WT1* expression after transplant and relapse [93,146–148]. Ogawa et al. [147] studied 72 patients (50 AML, 15 ALL, and 7 CML), post HCT, and all 20 patients who relapsed showed elevated *WT1* transcripts at the time of relapse. After relapse, patients were treated with some form of immunomodulation (donor lymphocyte infusions or discontinuation of immunosuppressive therapy). The calculated *WT1* transcript doubling time that occurred with relapse for patients was significantly longer for patients who responded to immunomodulation (a median doubling time of 26 days) compared with non-responders (a median of 6 days). It is unclear, however, if the same information with regard to the kinetics could also be produced by serial flow cytometry studies. *WT1* being investigated as a potential target for immunotherapy and *WT1* transcript levels thus may indicate if particular patients are more susceptible to this therapeutic approach.

MRD in chronic myelogenous leukemia (CML) following tyrosine kinase inhibitor (TKI) therapy

Monitoring the *BCR-ABL* transcript level during therapy with TKIs is essential to evaluate response and guide therapy. Indeed, the guidelines of the European Leukemia Network (ELN) and the National Comprehensive Cancer Network (NCCN) are based on response milestones defined by cytogenetic and molecular response, with the PB PCR for *BCR-ABL* being the primary method of monitoring once a cytogenetic remission is completed. The rate of decline and level of *BCR-ABL* after imatinib therapy predicts the achievement of major molecular response (MMR), achievement and duration of complete cytogenetic response, and progression-free survival [149–152]. In addition, an increase in *BCR-ABL*, particularly the loss of MMR, is associated with a loss of cytogenetic response and the presence of ABL kinase domain mutations [150,153]. More recent studies demonstrated that after 3 months of TKI therapy, the failure to reduce *BCR-ABL* to less than 10% IS (International Scale) are associated with worse outcomes, compared with those patients who have a dramatic reductions to <1% IS, who have a superior outcome [154]. The achievement of an MMR is a "safe haven" associated with rare cases of resistance or progression. ELN and NCCN guidelines recommend PCR *BCR-ABL* testing on PB at 3-monthly intervals, with BM examinations for morphology and cytogenetics, in addition to testing for *ABL* mutations, in cases where the *BCR-ABL* rises significantly (~5–10-fold over two measurements 1 month apart).

MRD detection in CML after transplantation

The clinical utility of MRD detection of the chimeric *BCR-ABL* transcript was first shown in the HCT setting, and a large number of results demonstrate the strong association of MRD and relapse in CML after transplantation. Early studies, prior to the development of quantitative PCR, showed that the mere qualitative detection of *BCR-ABL* was associated with relapse, and that *BCR-ABL* detection was equally informative from PB or BM specimens [94]. The advent of "real-time" quantitative *BCR-ABL* has made the evaluation of disease burden, and thus the risk of relapse, more precise, although the perfection of robust quantitative assays requires a dedication to rigorous laboratory procedures [155,156].

The highest risk of relapse associated with *BCR-ABL* transcript detection appears to be between 3 and 12 months after transplant [157,158,161]. In one study of 346 patients post-transplant, a positive

BCR-ABL transcript assay 6–12 months post-transplant was associated with a 42% risk of relapse compared with a 3% risk of relapse in PCR-negative patients. Tests earlier than 3 months post-HCT were not strongly associated with relapse. MRD can be detected by quantitative PCR for years after HCT. *BCR-ABL* transcripts have been detected in 25–50% of patients 3 or more years post-transplant, with subsequent relapse rates of around 10–20% [160–163]. Occasional patients in CR for longer than 10 years post-HCT have been found to be persistently MRD positive [164].

The type of transplant (allogeneic, T-cell depleted or unrelated) affects the association of MRD and relapse. This is presumably due to the immunologic effect associated with an allograft ["graft-versus-leukemia" (GVL)]. Transplants from unmanipulated unrelated donor grafts had a lower risk of relapse compared with matched related transplants. Patients undergoing T-cell depletion have a very strong association of MRD detection and subsequent relapse [160,165]. Thus, although T-cell depletion decreases graft-versus-host disease, it clearly does so at the cost of the associated GVL effect.

The quantification of *BCR-ABL* transcripts by quantitative PCR further refines the predictive value of MRD detection in CML [163,166–172]. For example, Lin et al. demonstrated that low residual *BCR-ABL* transcript levels after transplant were associated with a very low risk of relapse (1%), compared with a 75% relapse rate in patients with increasing or persistently high *BCR-ABL* transcript levels [173]. Olavarria et al. studied 138 CML patients "early" (3–5 months) after transplant and found that the *BCR-ABL* transcript level was highly correlated with relapse [170]. Patients with undetectable *BCR-ABL* transcripts had a 9% risk of relapse, compared with patients with a "low" (<100 *BCR-ABL* transcripts/μg RNA) or "high" (>100 copies *BCR-ABL*/μg) level of transcripts, who had a cumulative relapse rate of 30% and 74%, respectively. In addition, in a study of 379 CML patients "late" (>18 months) post-HCT [163], 90 (24%) had at least one positive assay and 13 of the 90 (14%) relapsed. Conversely, only three of 289 patients who were persistently *BCR-ABL* transcript negative relapsed (HR of relapse = 19). A rising *BCR-ABL* transcript level was a reliable portent of relapse. The kinetics of *BCR-ABL* can also be used to monitor response in patients receiving donor lymphocyte infusions (DLIs) for relapsed CML [174]. It is common to monitor *BCR*-ABL in PB every three months until 2 years post-transplant, then every 6 months until 5 years and yearly afterwards.

MRD in chronic lymphocytic leukemia (CLL)

In CLL, MRD can be measured by studying IgH V-D-J rearrangements or by flow cytometry. An international standardized approach for flow cytometric four-color analysis has been developed that is applicable to all CLL sample types and treatment regimens [175,176]. In head-to-head comparisons of RQ-IgH-PCR and flow cytometry, PCR has been found to be slightly more sensitive, with approximately 20% of flow cytometry-negative cases being found to be PCR positive for clonal rearrangements. However, there was a close correlation and 95% concordance with RQ-PCR for detection of CLL above 0.01% [175]. The increased sensitivity of PCR must be weighed against the relative ease and speed of flow cytometry compared with the more complicated and costly PCR technique.

Allogeneic HCT plays a role in the treatment of CLL (see Chapter 60), especially in patients with high-risk CLL prognostic factors of unmutated IgV_H, del(11q), and del(17p) [177–180]. In CLL cases treated with either anti-CD52 antibody-based therapy (alemtuzumab) or autologous transplant, the presence of MRD (by either flow cytometry or PCR) is very strongly associated with subsequent relapse [181,182]. By contrast, MRD negativity following allogeneic transplantation is associated with a very low relapse risk, although some patients remain with low-level MRD but do not overtly relapse. Slow clearance of MRD after HCT has been seen in a number of studies where some patients became MRD negative several months after transplant [182,183]. Moreno et al. analyzed MRD by quantitative PCR and flow cytometry in 40 patients with CLL who underwent HCT. Fifteen of 25 patients receiving autografts suffered a relapse, with increasing levels of MRD being observed before relapse in all of them. In contrast, in allografted patients, MRD did not correlate with outcome [184]. Itala et al. evaluated MRD after allogeneic HCT in 11 patients with poor-risk CLL by flow cytometry or allele-specific oligonucleotide-PCR (ASO-PCR). Flow cytometry did not detect MRD in any of the samples that were PCR negative. In contrast, ASO-PCR detected MRD in samples that were negative for MRD by flow cytometry in 22% of the analyses. All patients treated with allogeneic transplantation achieved or maintained hematologic CR, and five patients became PCR negative. None of the patients who became PCR negative after allogeneic transplantation has relapsed whereas all who remained PCR positive have relapsed [185]. In the setting of reduced intensity conditioning and allogeneic stem-cell transplantation, three patterns of MRD were observed: negative (31%), mixed (24%), and always positive (45%). The cumulative incidence of relapse according to the MRD status at 6 and 12 months after transplantation was significantly different between MRD-negative and -positive patients. Two-year DFS was 93% and 46% for MRD-negative and -positive patients, respectively, at 6 months after transplantation. Similarly, 2-year DFS was 100% and 57% for MRD-negative and -positive patients, respectively, at 12 months [186]. These studies demonstrate that in CLL, MRD negativity following allogeneic transplantation is strongly associated with a very low relapse risk; however, MRD positivity does not always evolve to overt relapse, and may remain low-level MRD positive with no progression to overt relapse, potentially due to graft-versus-tumor activity.

MRD testing in multiple myelomaMM

According to the International Myeloma Working Group (IMWG), complete remission in MM is defined as negative immunofixation of serum and urine, disappearance of any soft tissue plasmacytoma, and less than 5% plasma cells in BM [187]. Stringent complete response is defined as CR plus normal free light chain ratio and absence of clonal plasma cells by immunohistochemistry or two- to four-color flow cytometry, and molecular CR is defined as CR plus negative allele-specific oligonucleotide PCR with a sensitivity of 10^{-5} [187]. Despite the high rates of response following induction therapy and consolidation with autologous or allogeneic HCT, the majority of patients eventually relapse. The high rates of relapse among MM patients who initially respond to therapy suggest that MRD remains after therapy and proliferation of the remaining MM cells ultimately leads to clinical relapse [188]. With the most significant advances in the treatment of MM, including the use of autologous and allogeneic HCT in combination with the immunomodulatory drugs thalidomide and lenalidomide, and also the proteasome inhibitor bortezomib, albeit none curative, the major challenge will be to incorporate genetic and other MRD prognostic parameters into clinical trials that are designed to determine the best way, time, and sequence of administering these therapies.

Genomic aberrations evaluated by interphase FISH have been shown to play a major role in the prognosis and evolution of patients with myeloma. For example, three independent groups [3,189,190] reported that MM patients with t(4;14) or del(17p) fared worst when compared with the other genetic subgroups, especially when associated with high β_2-microglobulin levels or more than 60% plasma cells in the marrow. The poor prognosis associated with −13/del(13q) was further attributed to the fact that t(4;14) and del(17p) patients showed the chromosome 13 aberrations simultaneously, with few patients benefiting from thalidomide maintenance therapy. However, some studies suggest that bortezomib may overcome the poor prognosis conferred by chromosome 13 deletions at relapse in MM patients with high serum albumin [191,192]. Taken together, these data have broad implications for patient counseling, choice of therapy, and innovative molecular approaches to clinical investigations for both younger and older MM patients [193].

Studies have suggested that allogeneic HCT for MM results in CR rates of 22–67%, with molecular remissions in about one-third [194,195] and prolonged DFS in 25–33% of patients [196,197] (see Chapter 56). Martinelli et al. [195] noted that MM patients who achieved a molecular CR after allogeneic or autologous HCT had a significantly lower relapse rate (41% versus 16%) and a longer RFS (35 versus 110 months). Using a sensitive flow cytometry assay, Rawstron et al. [198] quantitated normal and neoplastic plasma cells and compared their results with standard immunofixation in 45 patients undergoing high-dose chemotherapy. By flow, neoplastic plasma cells were detected in 42% of the patients at 3 months after transplantation (compared with 27% by immunofixation). Patients with detectable MRD showed a significantly shorter progression-free survival (median 20 months) compared with those with no detectable disease (median >35 months), and a significantly higher risk of early disease progression. Sarasquete et al. [199] compared the clinical utility of MRD in MM by allelic-specific oligonucleotide RQ-PCR and flow cytometry. Owing to technical issues, allelic-specific oligonucleotide RQ-PCR was evaluable in only 75% of patients compared with 90% for flow cytometry; however, PCR detected residual myelomatous cells (median 0.014%, range 0.001–0.11%) in 17 patients compared with 11 patients by flow cytometry. This head-to-head comparison showed that the ability to predict outcome in MM is similar in PCR and flow cytometry (~0.01% level), with flow cytometry having the critical advantage of speed, possibly allowing for real-time clinical decisions, including dose escalation and de-escalation strategies or the timing of stem-cell harvests based on MRD detected during therapy.

Maintenance therapy, defined as extended drug therapy administered after achieving a remission or plateau phase with MRD, may be beneficial. Typically, maintenance therapy involves the use of a lower dose of an anti-myeloma agent and aims to provide sustained control of MRD while being well tolerated and easy to administer [200]. Although maintenance therapy is unlikely to achieve complete eradication of the malignant clone, it may provide sustained control of MRD and delay both relapse and the need for more intensive therapy. Ultimately, clonal selection will likely produce a resistant population that causes relapse; however, a meaningful alteration in disease course and delay in relapse are clinically beneficial [201].

Not all patients with detectable MRD progress to relapse

This phenomenon has sometimes been referred to as "dormancy," although it is not clear that the leukemia (if it is leukemia) is truly inactive. There are three clear demonstrations of dormancy. The first is in t(8;21) AML following conventional chemotherapy, and also in CML following HCT [14,202]. In t(8;21) AML, the *RUNX1/RUNX1T1* (*AML1-ETO*) messenger RNA can be detected in a minority of lymphoid and erythroid colonies taken from remission samples and long term survivors after HCT, suggesting that the disease involves a primitive leukemia progenitor cell [203,204]. As such, some MRD in this disease may arise from non-leukemia lymphoid cells. Furthermore, another example of dormancy is the observation that late relapses in pediatric ALL (>10 years after CR) have the same clonal IGH V-D-J gene rearrangement as at diagnosis [205].

How can such examples of dormancy be explained? Perhaps prolonged immune surveillance, most relevant after HCT, can control the expansion of a leukemia clone. Or, as in the example of t(8;21) noted above, the MRD assay may detect signal in a cell lineage not involved in the leukemia. Lastly, a residual leukemia cell may be "pre-leukemia," not having all the mutations necessary for malignancy, or "post-leukemia," whereby additional mutations have rendered the clone relatively inert. Regardless, it is likely that the clinical definition of "cure" does not mean the elimination of all cells involved in the leukemia process. Further studies of dormancy may clarify what it takes to "cure" leukemia.

Conclusion – the future of MRD

The detection of MRD is strongly associated with relapse in almost all clinical situations. Where do we go from here?

First, MRD will be used more extensively to guide treatment options. For example, patients with "favorable" risk acute leukemia will be "restaged" by their MRD status during therapy, with treatment adjusted as necessary (for example, moving a patient with CR by morphology but MRD positive to transplant). Patients with MRD after transplant will have their immunosuppression adjusted, or placed on trials using immunotherapy or drugs to slow or abort relapse.

Second, MRD will be used as a surrogate for outcomes. If the presence of MRD at 1 year is highly correlated with relapse and survival, why not have MRD be the early end-point of clinical trials, rather than the traditional 5–10-year survival outcome end-point? In recent phase III trials in CML, the MRD end-point of MMR at 12 months was used as the primary or secondary study end-point [206–208]. The acceptance of MRD as a trial end-point surrogate would greatly reduce study time and costs.

Third, MRD is another example of where technology shapes the questions that can be asked (and hopefully answered). Next-generation sequencing is allowing the study of the complexity of clonal structure in leukemia. It is clear that many (most?; all?) leukemia has many independent clones, which compete against each other before and during therapy, so that treatment, response, and relapse are a large exercise in Darwinian selection [209,210]. Hence, in the future, a combination of MRD detection, cell isolation, and genotyping will likely be used not only to identify the burden of MRD, but also understand which leukemia population has survived, so better to tailor therapy to eliminate the persistent clones.

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CHAPTER 26

Pathology of Hematopoietic Cell Transplantation

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Introduction

The hematopoietic cell transplantation (HCT) setting presents pathologists with four major types of complications: toxicities resulting from pre-transplant cytoreductive conditioning regimens, immunological problems [rejection or graft-versus-host disease (GVHD)], infections, and malignancy. Since the previous editions of this book, new biologic insights on the role of the endothelium as well as advances in diagnostic and treatment modalities have affected some of the attitudes regarding the role and application of surgical pathology in the HCT setting [1–3]. There has been a steady increase in matched, unrelated hematopoietic cell donors (using peripheral and cord blood), prolonged prophylaxis for GVHD beyond day 100, and reduced-intensity conditioning regiments with older recipients and patients with significant pre-transplant comorbidities. Quality of life indices [2] and combinations of serologic biomarkers [3,4], clinical parameters such as weight gain, and bilirubin [5,6] have been validated for predicting survival outcomes with GVHD. The frequency of severe liver complications [sinusoidal obstructive syndrome (SOS) and hepatic GVHD] has markedly declined as a result of changes in the pre-transplant conditioning regimen (see Chapters 20, 21, and 94) [7]. Severe viral infections have declined with earlier detection by sensitive molecular methods and from the widespread use of effective antiviral agents**.** The use of open lung biopsies for suspected infection has declined with the increasing use of bronchoalveolar lavage (BAL), newer CT imaging studies, and serologic and multiplex PCR tests for presumptive fungal or viral infections, respectively. The bronchiolitis obliterans syndrome of chronic GVHD now includes small airway lesions of lymphocytic bronchiolitis and constrictive bronchiolitis [1].

Pre-transplant evaluation

Hematopoietic system

Before a patient is accepted for HCT, the referral evaluation is used to confirm the diagnosis, review eligibility for available protocols, and identify potential complications that may influence transplant decisions. The pathologist reviewing material from the referring institution will need relevant flow cytometry, immunohistology, molecular, and cytogenetic data and also details of recent therapy. Data from the original malignancy are essential for planning therapy, subsequent disease monitoring, and minimal residual disease detection. The patient is often in complete remission at the time they arrive at the tertiary referral transplantation center and the essential molecular, immunophenotypic, and cytogenetic characteristics of the malignancy can no longer be determined. It is therefore very important to characterize the tumor fully at presentation to facilitate subsequent therapeutic planning and disease monitoring. The pathology report from the referring institution should provide a detailed description of all ancillary testing results.

Prior to undergoing HCT for hematologic malignancies, the marrow is evaluated to determine remission/relapse status. Patients who are transplanted in complete remission without any detectable minimal residual disease (MRD) have a much better prognosis. The pre-transplant detection of MRD by multiparameter flow cytometry, cytogenetics, or molecular tests is a strong independent negative prognostic indicator but does not exclude possible cure [8]. Chapter 25 provides an extensive review of the various modalities used to monitor and detect minimal residual disease. Although polymerase chain reaction (PCR)-based molecular testing is an extremely sensitive means of detecting minimal residual disease, it depends on the presence of a unique molecular signature (mutations or translocations) that can be targeted by PCR. Multiparameter flow cytometry, on the other hand, provides a very sensitive means of MRD detection. It can be easily applied to most hematologic malignancies and has the ability to detect as few as 0.01% malignant cells without a prior immunophenotype signature for comparison [9–11]. In our practice, we use clinical multiparameter flow cytometry incorporating panels with up to 10 antibodies that can be used to define cell lineage, detect abnormal immunophenotypes, and monitor maturation of hematopoietic cells [12]. The identification of malignancy is based on the fact that normal hematopoietic maturation demonstrates a characteristic and reproducible pattern of cell surface antigen expression that is altered in most hematopoietic tumors. Common tumor-specific antigen variation includes (1) the expression of unique antigens not usually associated with a given lineage, (2) the increased or decreased expression of certain antigens during maturation, (3) an abnormal immunophenotype maturation pattern, and (4) a homogeneous immunophenotypic population suggesting a block in maturation [11]. Although prior immunophenotype will aid the detection of MRD, many malignancies undergo immunophenotypic evolution. The immunophenotype of a given leukemia can change during the course of therapy, possibly due to selection of resistant clones. Clinical flow cytometry is a rapidly evolving field and there is enormous variability in methodology that impacts tumor diagnosis and MRD detection. For example, many laboratories report levels of blasts based on the number of CD34-positive cells within the “blast gate” without indication of whether the expanded immature population is abnormal or the result of a reactive process in a regenerating marrow. There is a developing international effort to standardize the practice of clinical flow cytometry in diagnosing and classifying leukemias and lymphomas [13].

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Compared with multiparameter flow cytometry, MRD detection by PCR analysis has enhanced sensitivity but requires a unique molecular target (mutations, translocations, or gene rearrangements) for detection. For this reason, it is important to determine potential molecular abnormalities at diagnosis for subsequent therapy and disease monitoring. The diagnosis of many B- and T-cell lymphomas involves PCR studies to determine clonality, targeting gene rearrangement or detecting potential translocations, including *t*(14,18) in follicular lymphoma or *t*(11,14) in mantle cell lymphoma that can then be used to monitor recurrent post-transplant disease. In the case of lymphocytic leukemia, the rearranged genes serve as ideal targets for molecular detection. However, it is difficult and expensive to design unique tumor-specific PCR primers and molecular evolution of the tumor may interfere with PCR-mediated detection. Wu et al. used high-throughput sequencing of the T-cell receptor to diagnose minimal residual T-cell acute lymphoblastic leukemia/lymphoma [14]. They sequenced the variable regions of T-cell antigen receptor genes amplified using multiplex PCR that targeted all potential V–J rearrangement combinations. They identified the expanded neoplastic clone in the diagnostic sample and subsequent post-therapy sample with MRD. In a subset of cases, high-throughput sequencing detected MRD that was not identified by the concurrent clinical multiparameter flow cytometry. Additional studies have reported a similar assay for monitoring B-cell neoplasms [15,16]. These studies demonstrate the enormous power that high-throughput DNA sequencing will have on the detection and monitoring of lymphocytic neoplasms. In contrast, myeloid neoplasms lack gene rearrangements, hence molecular detection of clonality is more problematic. The molecular detection of myeloid neoplasms is dependent on tumor-specific mutations and translocations including Bcr/Abl in CML, Jak2 (V617F) in myeloproliferative neoplasms [17], and NPM1, Flt3, CEBPA mutations, or other cytogenetic abnormalities in acute myeloid leukemias [18]. Many of these tumor-specific mutations represent therapeutic targets and sensitive molecular studies are being designed for the early detection of resistant mutations that can guide therapeutic decisions [19].

Proper disease classification is essential to direct pre-transplant conditioning, determine prognosis, and provide markers for disease monitoring. Many patients being considered for HCT have a long history of previous therapy and biopsies. Ideally, the original diagnostic sample and a recent biopsy should be reviewed since a single biopsy may not be sufficient to confirm both disease classification and recent disease activity. Prior and recent drug therapy can alter the morphology and immunophenotype of the neoplastic cells, making it difficult to evaluate a biopsy from a recently treated patient. Granulocyte colony-stimulating factor (G-CSF) and other cytokines may cause confusing left shifts in hematopoietic cells and abnormal monocytic hyperplasia [20]. Bisphosphonates given for multiple myeloma may thicken trabecular bone in pre-transplant marrow biopsies. Demethylating agents can induce cellular maturation and mask morphologic and immunophenotypic dysplasia [21].

In general, disease classification is made on the original diagnostic sample and recent disease activity is determined from subsequent biopsies. Disease subclassification of myelodysplastic syndromes by World Health Organization criteria relies predominantly on the marrow blast count. Post-transplant survival for both de novo and secondary myelodysplastic syndrome/transformed acute myeloid leukemia is related to the disease stage (blast count), cytogenetics, conditioning regimen, and donor relationship [22]. Myeloproliferative neoplasms tend to evolve over time with an increasing myeloid blast population. The timing of HCT is very important since many studies have shown that patients transplanted in chronic phase have a much better prognosis than those in the accelerated phase or blast crisis. While most patients with chronic myeloid leukemia can achieve complete clinical, cytogenetic, or molecular remission with targeted tyrosine kinase inhibitors, the disease often evolves with emergence of resistant mutations (clones). The determination of current disease activity is essential for planning when to proceed with a stem-cell transplant.

A detailed evaluation of myelofibrosis may be needed to distinguish chronic myeloproliferative disorders including essential thrombocythemia, primary myelofibrosis, and polycythemia from acute panmyelosis, myelodysplastic syndromes, and metastatic disease. In many cases, the marrow is inaspiratable, hindering the morphologic evaluation of potential dysplasia or elevated myeloid blast population. Preparation of touch preparations in the setting of myelofibrosis is essential for cytologic morphology since the marrow is frequently inaspirable. Subsequent immunohistochemical studies for the immature myeloid markers CD34 and CD117, and also potential metastatic carcinoma (cytokeratin), should be considered. When analyzing a severely fibrotic marrow, we find it helpful to perform flow cytometry on cells extracted from a minced bone marrow biopsy rather than a hemodilute bone marrow aspirate. Additional molecular testing for Bcr/Abl and Jak2 (V617F), cytogenetic studies, and a previous clinical history are important to determine the correct diagnosis. Severe myelofibrosis portends slower engraftment but is not a contraindication to HCT [23]. Magnetic resonance imaging of the pelvis and femur can be helpful to assess the extent and distribution of myelofibrosis and other disease [24]. The differential diagnosis of a markedly hypocellular marrow includes aplastic anemia, hypocellular myelodysplastic syndrome, or a reactive process including toxin exposure, drug reaction, or nutritional deficiency. Differentiation between aplastic anemia and hypocellular myelodysplastic syndrome (MDS) requires good morphologic, cytogenetic, and immunophenotypic studies that are often unavailable because of a hypocellular or inaspiratable marrow.

Toxicity from cytoreductive therapy

The cytoreductive therapy given for treatment of underlying malignancy, marrow ablation, and immune suppression may cause widespread multiorgan dysfunction and atypical changes. The frequency, the organ systems involved, and the time of onset are related to both the type and overall intensity of the conditioning protocol. The histopathology within some affected organs, such as the heart, may be disease "non-specific" (edema, hemorrhage, necrosis of myocytes, and interstitial fibrosis), yet the associated clinicopathologic entity may be highly characteristic of toxicity. Other changes due to toxicity, particularly those in the mucous membranes [25], skin, gastrointestinal (GI) tract and lung, cause diagnostic difficulties because of their clinical or histological overlap with GVHD and infection (Figures 26.1–26.4). Studies of these organs with serial biopsies demonstrate the diffuse distribution of conditioning injury and amelioration of these changes with time (Figure 26.5).

Pre-transplant hepatic disease

Pre-transplant liver dysfunction can have a major impact on outcome along with a substantial influence on the choice of conditioning regimen and donor [26]. Liver biopsies carried out to assess the severity and cause of abnormal liver function can identify processes that would jeopardize post-transplant survival and predispose to liver failure, fatal SOS, or fatal infection. Patients at increased risk of developing SOS include those with inflammatory liver diseases demonstrating sinusoidal fibrosis, significant necroinflammatory activity, or bridging fibrosis. Specific diseases include steatohepatitis (Figure 26.6), sinusoidal fibrosis associated with extramedullary

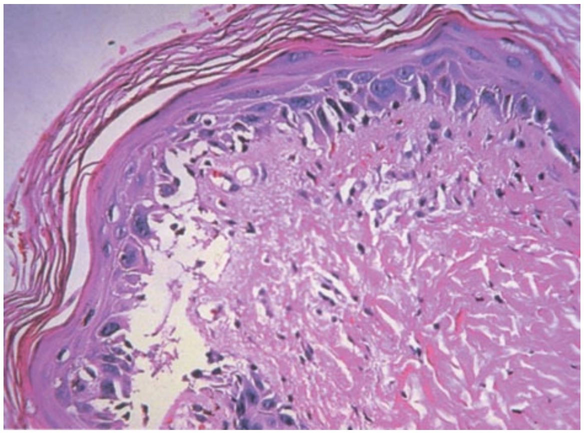

Figure 26.1 Skin biopsy from a recipient of identical twin marrow who had received a single 15mg/kg dose of dimethylbusulfan 15 days previously. The epidermal separation, keratinocyte atypia, and scanty lymphoid dermal infiltrate can also be seen in GVHD. (For a color version of this figure, please see the color plate section.)

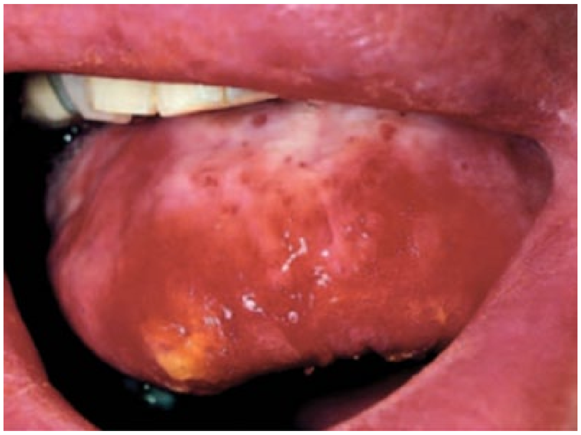

Figure 26.2 Acute glossitis 1 day post-HCT after a conditioning regime of 60mg/kg of cyclophosphamide (CY) followed by 12Gy of fractionated total body irradiation (TBI). There is early mucosal damage with severe erythema and focal atrophy. (For a color version of this figure, please see the color plate section.)

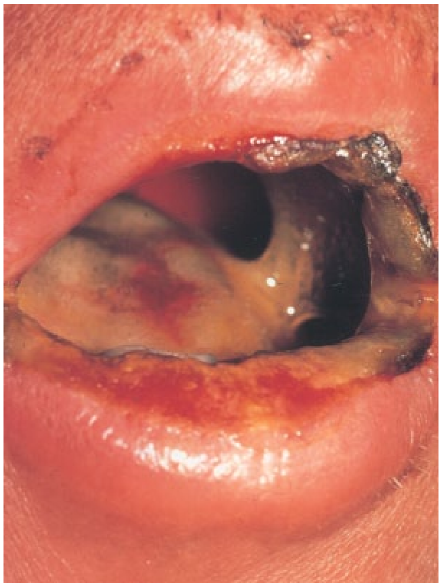

Figure 26.3 Severe mucositis of lips and tongue with pseudomembranous exudate 20 days after HCT following conditioning with TBI and chemotherapy. Similar changes may be present with severe acute GVHD or herpetic infection. (For a color version of this figure, please see the color plate section.)

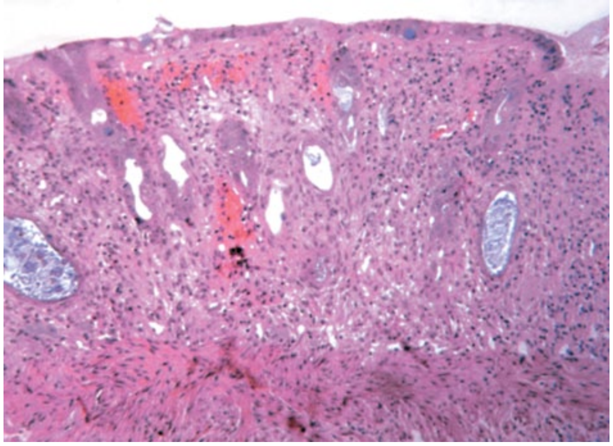

Figure 26.4 Effect of cytoreductive therapy on colonic mucosa, day 7. This biopsy specimen was taken following therapy with busulfan (BU), CY, and TBI (12Gy). There has been obliteration of almost all epithelial cells, leaving cystic crypt remnants. (H&E with Alcian Blue counterstain.) (For a color version of this figure, please see the color plate section.)

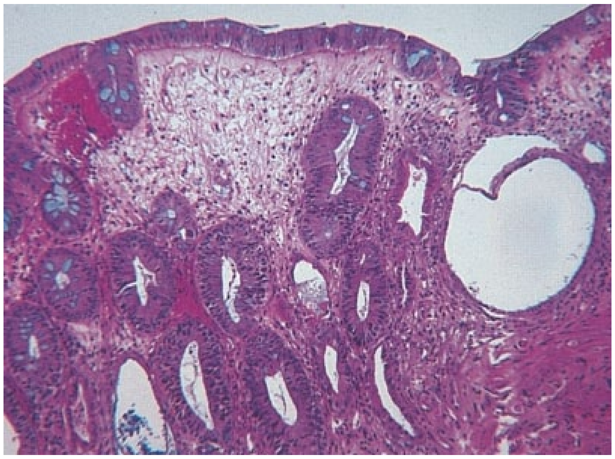

Figure 26.5 Recovery from the effects of cytoreduction therapy on colonic mucose, day 16. This repeat biopsy from the same patient as in Figure 26.4 indicates that regeneration is occurring, although some cystic crypts remain. (H&E with Alcian Blue counterstain.) (For a color version of this figure, please see the color plate section.)

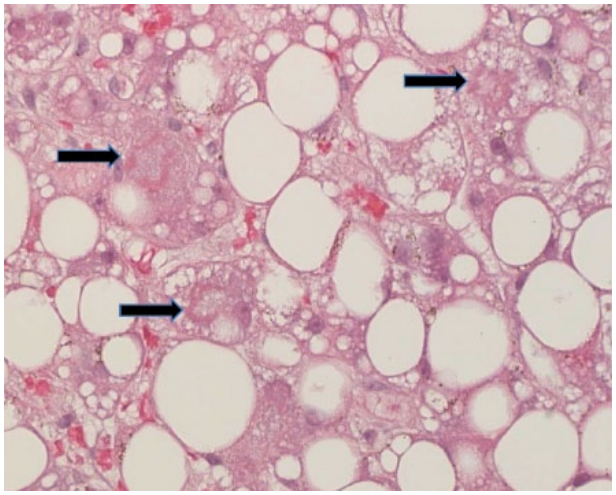

Figure 26.6 H&E-stained liver biopsy demonstrating changes of non-alcoholic steatohepatitis (NASH) with extensive macro- and lesser amounts of microsteatosis, hepatocyte ballooning, and focal Mallory body formation (arrow). (For a color version of this figure, please see the color plate section.)

hematopoiesis from myeloproliferative neoplasia (Figure 26.7), prior toxicity from gemtuzumab ozogamicin (Mylotarg™) (Figure 26.8) and rarely Gleevec® [27], active chronic viral hepatitis, and amyloidosis (Figure 26.9). Poorly compensated cirrhosis is a contraindication for HCT because of the prohibitive risk of developing SOS after myeloablative conditioning regimens. Even compensated cirrhosis has a high likelihood of hepatic decompensation after non-myeloablative regimens [28].

Hepatic sinusoidal obstructive syndrome (veno-occlusive disease)

The most frequent life-threatening regimen-related toxicity involves SOS, a clinical syndrome of jaundice, weight gain, ascites, and painful hepatomegaly that typically develops in the first few weeks after HCT. Initially, this syndrome was called hepatic veno-occlusive disease (VOD), referring specifically to the obliterative lesions within the lumina of small hepatic venules [26,29] (Figure 26.10). However, based on more recent experimental and molecular studies, the authors renamed it sinusoidal obstructive syndrome (SOS), because the primary site of injury occurs in the sinusoids before clinical signs appear. The term SOS reflects the proximate cause, which is toxic injury to zone 3 sinusoidal and venular endothelium related to specific drug metabolites and the intracellular depletion of glutathione stores. Secondary events include a decrease in nitric oxide associated with vasoconstriction and deposition of coagulants evinced by factor VIII staining within the widened venular subendothelial zone, and the perivenular zone corresponding to the pores that drain the sinusoids into the small venules (Figure 26.11). This sequence of events leads to subsequent deposition of extracellular matrix and collagens by activated and proliferated hepatic stellate cells (HSCs) (Figure 26.12). In a study of severe hepatocellular injury before day 100 [defined as alanine aminotransferase (ALT) >30 times the normal upper limit] conducted on 6225 consecutive patients, SOS was

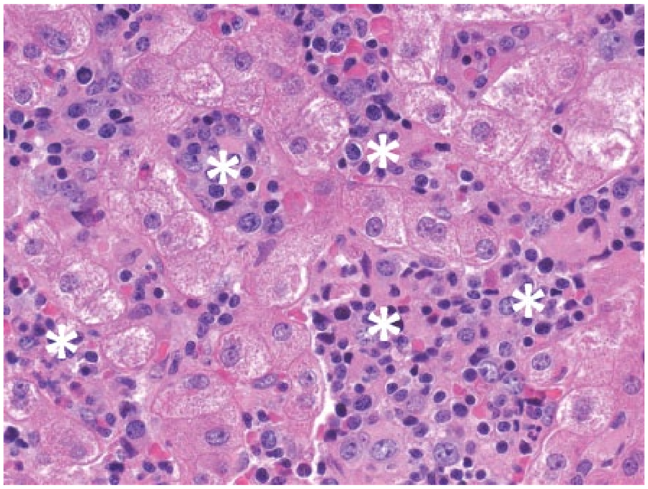

Figure 26.7 H&E-stained section of a pre-transplant liver biopsy from a patient with myelofibrosis. The hepatic sinusoids (white asterisk) are expanded by a mixed cellular infiltrate of hematopoietic precursors. (For a color version of this figure, please see the color plate section.)

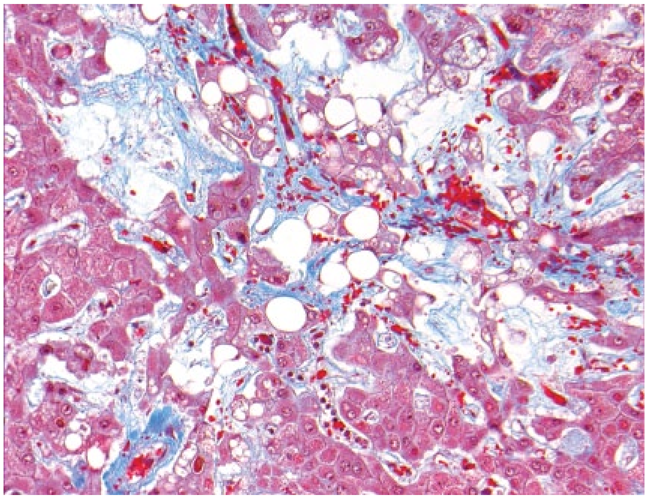

Figure 26.8 Fatal gemtuzamab ozogamicin (Mylotarg™)-associated sinusoidal obstruction syndrome. The obliterated central venule is surrounded by focal sinusoidal fibrosis. (Trichrome stain.) (For a color version of this figure, please see the color plate section.)

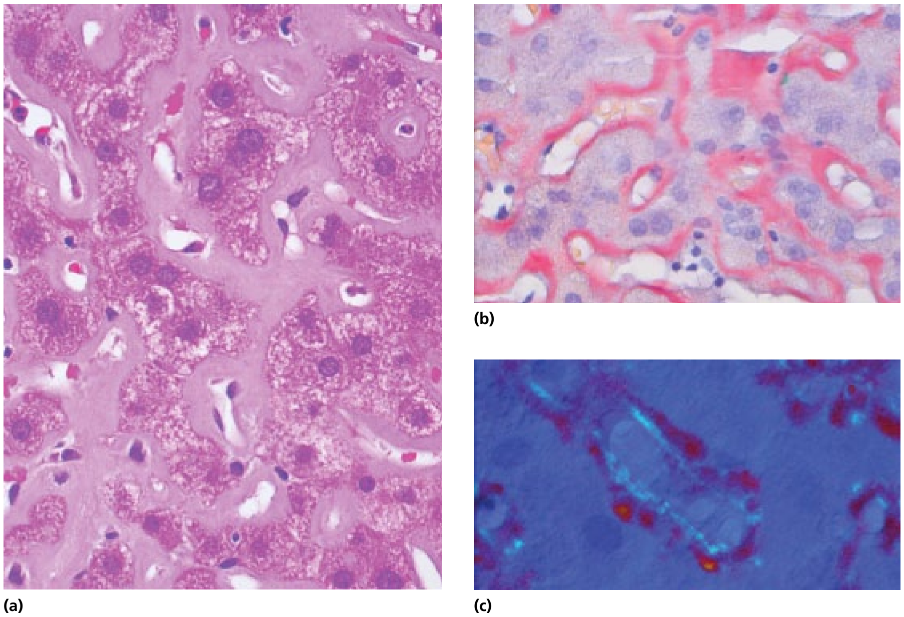

Figure 26.9 (a) H&E-stained section of a pre-transplant liver biopsy demonstrating extensive amyloid deposition in the liver sinusoids. (b) Congo Red stain of the same liver biopsy, with the sinusoidal amyloid deposit highlighted by salmon-pink staining. (c) Congo Red stain under polarized light: the sinusoidal amyloid deposit shows apple-green birefringence. (For a color version of this figure, please see the color plate section.)

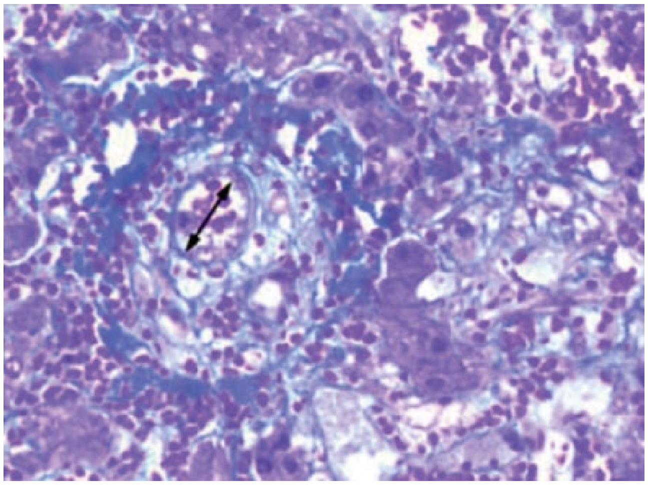

Figure 26.10 Hepatic sinusoidal obstructive syndrome (SOS) 95 days post-HCT for malignancy. The trichrome stain shows the blue outline of a hepatic venule with marked luminal narrowing (arrow shows lumen diameter) caused by subendothelial trapping of red cells. The hepatocytes of acinar zone 3 are severely disrupted. When the clinical diagnosis of hepatic SOS is uncertain, it may be confirmed by a transvenous liver biopsy with measurement of an elevated gradient between wedged and free hepatic venous pressures. (For a color version of this figure, please see the color plate section.)

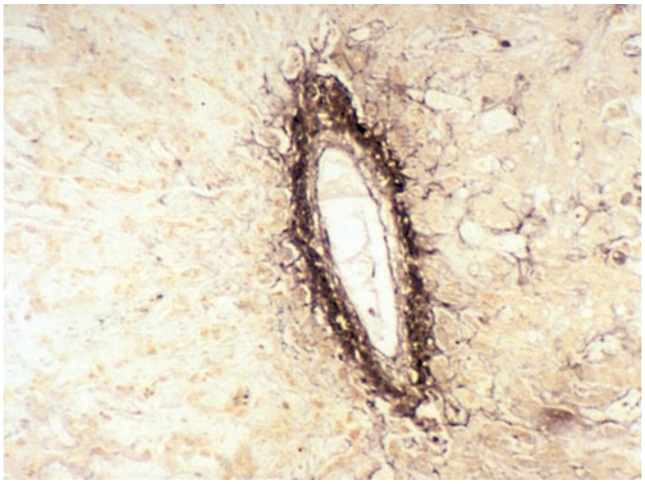

Figure 26.11 Early hepatic SOS showing perivenular staining for procoagulants. Immunohistochemical staining for factor VIII (von Willebrand) demonstrates post-sinusoidal obstruction corresponding to the endothelium-lined pores through which the sinusoids drain into the venules. Immunostaining for fibrin localizes in the same regions. These observations provide some of the rationale for using anticoagulation and anti-thrombotic therapy for hepatic SOS. (For a color version of this figure, please see the color plate section.)

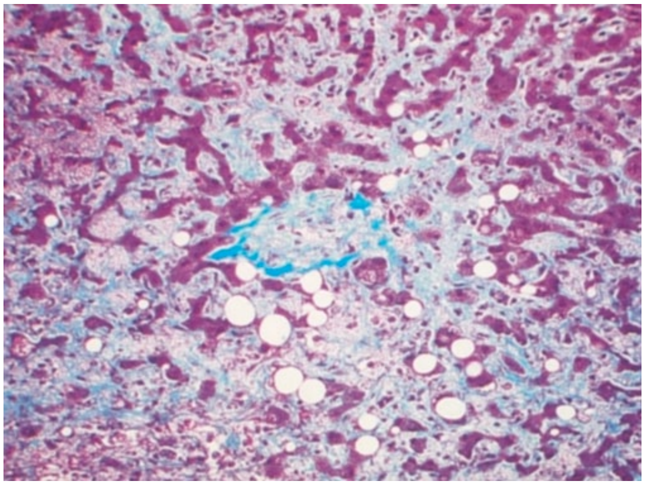

Figure 26.12 Late hepatic SOS, day 63. The trichrome stain demonstrates fibrotic obliteration of the venous lumen with extensive fibrosis in surrounding sinusoids. The resulting vascular obstruction produced intractable ascites. Immunohistochemical stains at this stage reveal extensive deposition of collagen, while fibrin and other blood procoagulants are no longer identifiable. (For a color version of this figure, please see the color plate section.)

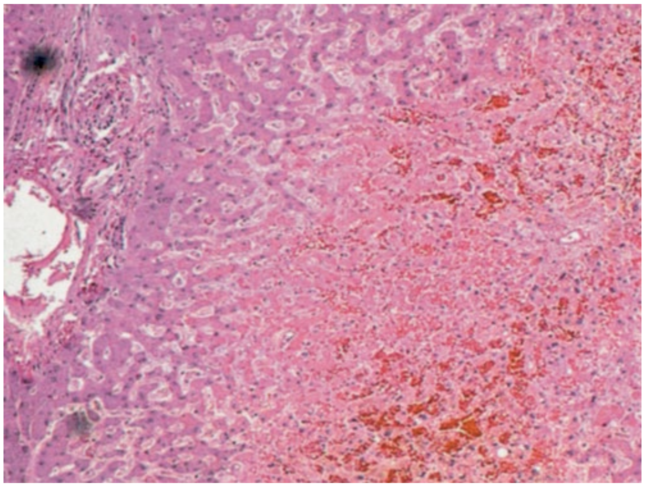

Figure 26.13 Fatal hepatic SOS at autopsy 12 days post BMT for leukemia. At this low magnification, the H&E-stained section demonstrates striking congestion, hemorrhage, and hepatocyte disruption in zone 3 (centrilobular) of the liver acinus toward the right. The portal space and surrounding acinar zone 1 on the left are well preserved. These early severe hepatic SOS lesions at times can be identified with the H&E stain after tissue fixation with B5 or methyl Carnoy's fixative. However, stains for connective tissue are more effective. (For a color version of this figure, please see the color plate section.)

the leading cause of severe hepatocellular injury in 48/88 patients with high ALT, followed by ischemic hepatopathy 32/88 [30]. The frequency of high ALT elevations declined markedly from 1.75% between 1993 and 1995 to 1.06% between 2005 and 2007, reflecting a marked reduction in the prevalence of SOS.

Autopsy specimens reveal that the histology of injury to sinusoids, hepatocytes and venules, best seen with a trichrome stain, may be rapidly progressive. In difficult cases, liver histology using transvenous liver biopsy should be done in conjunction with measurement of the wedged hepatic venous pressure gradient [31]. Examination of serial sections may be needed to identify characteristic histologic changes that may not be present in all levels. The initial injury is to the sinusoidal endothelial cells (SECs), resulting in death of cells which may then detach and embolize, leading to sinusoidal obstruction along with deposition of fibrin and procoagulants in the pores that drain into the venules, and hemorrhage in zone 3 and the space of Disse (Figures 26.10, 26.11, and 26.13). Immunostaining with CD31 demonstrates a loss of endothelium in the perivenular sinusoids and the central venule (Figure 26.14). Frank necrosis of perivenular hepatocytes, demonstrated by their absence of cytoplasmic cytokeratin immunostaining, is often more widespread and severe than the extent of venular injury. A consequence of the sinusoidal obstruction, elevated sinusoidal pressure, ischemia, and fragmentation of hepatocyte cords is the dislodgement of clusters of liver cells, including hepatocytes, which may flow retrograde into portal veins or embolize through disrupted pores into the damaged central venules (Figure 26.15). Within 3 weeks of myeloablative conditioning, the deposition of sinusoidal reticulin is accompanied by a marked linear sinusoidal pattern of α smooth muscle actin staining for HSCs and by an increase in monocytes/Kupffer cells at the interface of ischemic hepatocyte necrosis (Figure 26.16) [26,29]. Later changes include collagenous obliteration of the venules, thickening of the outer wall of the hepatic venules (phlebosclerosis) (Figure 26.17), extinction of zone 3 hepatocytes, and fibrotic replacement of the sinusoids adjacent to the terminal hepatic venules (zone 3) of the liver acinus (Figure 26.12). In prolonged and fatal SOS, the liver may take on a pattern of reversed cirrhosis with fibrotic linkage between destroyed perivenular zones.

Several additional pericentral vein (zone 3) lesions that follow cytoreductive therapy are associated with SOS. Two studies have demonstrated that zone 3 sinusoidal fibrosis and phlebosclerosis, a non-occlusive perivenular fibrosis, are also associated with the same clinical features as SOS [26]. An example of the histologic spectrum is a rapidly developing syndrome of SOS that occurs among some

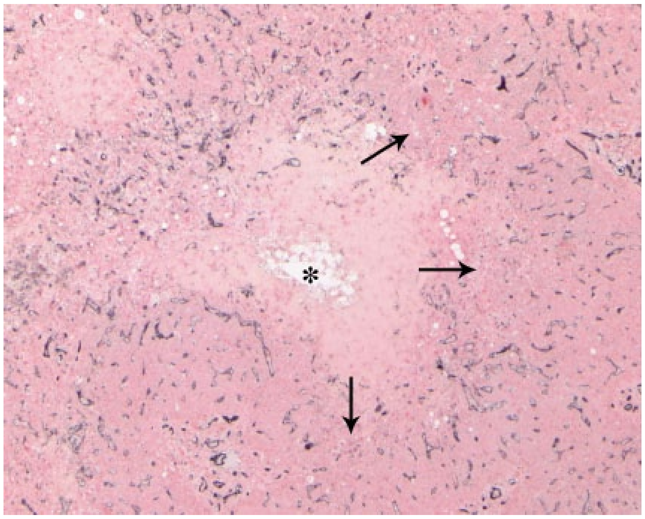

Figure 26.14 CD 31 immunostain of liver with fatal SOS. There has been partial to complete loss of sinusoidal endothelial staining of the hepatic cords in zone 3 (arrows) which surround the subtotally occluded venule lacking an endothelium (asterisk). (For a color version of this figure, please see the color plate section.)

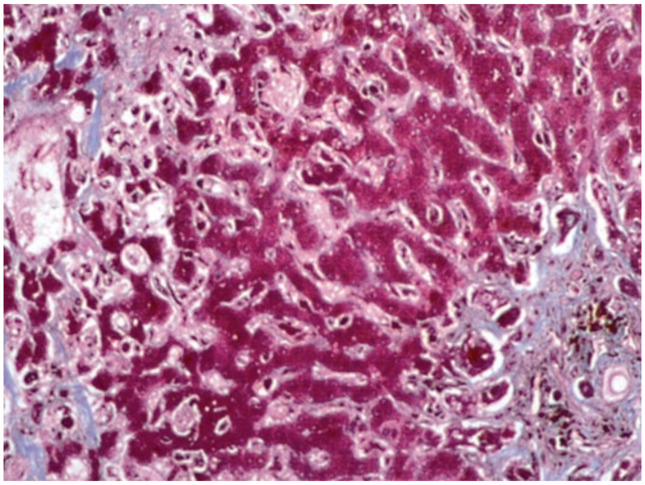

Figure 26.15 Hepatic SOS and coexisting GVHD, day 26. On this trichrome stain, the two most common liver problems after HCT can be differentiated by the zone of the liver acinus that they affect. The expanded portal space in the lower right demonstrates changes from GVHD, including fibrosis, proliferation of atypical cholangioles along the limiting plate, and destruction of small interlobular bile ducts. The SOS lesion along the left margin shows striking collections of embolized hepatocytes beneath the endothelial basement membrane. (For a color version of this figure, please see the color plate section.)

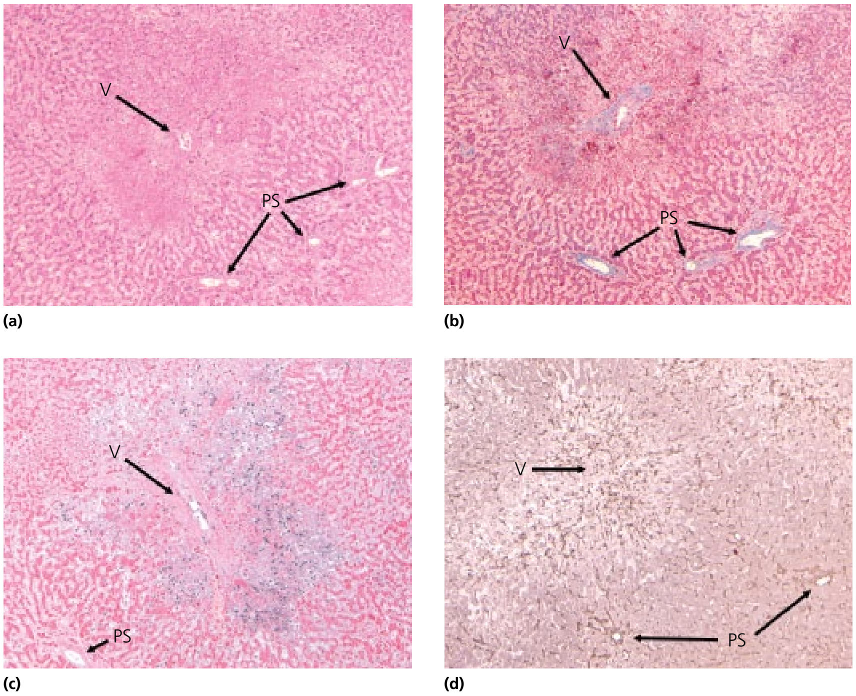

Figure 26.16 Fatal SOS. (a) H&E stain demonstrates a large perivenular zone of hemorrhagic necrosis with destruction of hepatocyte cords surrounding the damaged venule (V); the areas around the portal spaces (PS) are spared. (b) Trichrome stain demonstrates the subtotal occlusion of the venular lumen by collagen and extracellular matrix. (c) Immunostain Mac 387 for macrophage/monocytes demonstrates a zone of cells at the interface of the viable and necrotic hepatocytes. (d) Smooth muscle actin immunostain demonstrates marked expansion of hepatic stellate cell processes within the damaged sinusoids surrounding the venule. (For a color version of this figure, please see the color plate section.)

HCT patients treated with gemtuzumab ozogamicin (Mylotarg™), a humanized anti-CD33 monoclonal antibody conjugated to calicheamicin. The associated histology of gemtuzumab ozogamicin-associated SOS differs slightly from SOS occurring after myeloablative cytoreductive conditioning in displaying more sinusoidal fibrosis than venular occlusion (Figure 26.8) [32]. Late-developing ascites from nodular regenerative hepatocellular hyperplasia (NRH) alterations are not associated with symptoms of early liver toxicity. They most likely are secondary to disturbances in hemodynamics and serve as an indicator of compensatory repair and regeneration of hepatocytes in different zones of the liver acinus [33]. Several other conditions that may result in ascites or an SOS-like picture from diminished sinusoidal blood flow with increased sinusoidal resistance are GVHD, congestive cardiopathy [26], viral hepatitis with bridging fibrosis, occult cirrhosis, and steatohepatitis, both alcohol and non-alcohol related.

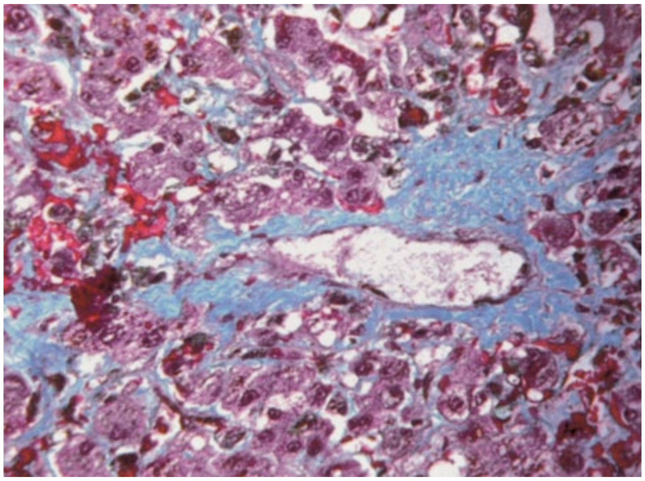

Figure 26.17 Hepatic phlebosclerosis, day 96. Eccentric thickening of the venular wall without striking luminal narrowing was associated with an early liver toxicity syndrome. The patient had received a 5-day infusion of high-dose cytosine arabinoside (Ara-C) followed by CY and TBI. Liver function abnormalities developed prior to the infusion of marrow. Changes of phlebosclerosis were widespread, but there were no identifiable hepatic SOS lesions at autopsy. (Trichrome stain.) (For a color version of this figure, please see the color plate section.)

Post-transplant hematopoietic evaluations

High-dose conditioning produces massive destruction of the host marrow, resulting in acute serous myelitis (serous atrophy) without necrosis of the bony trabeculae (Figures 26.18 and 26.19). The myelitis includes edema, hemorrhage, loss of most marrow elements except plasma and mast cells, and relative predominance of damaged and frequently necrotic fat associated with iron-rich macrophages. This damage gradually resolves over the first 4–6 weeks as colonies of donor myeloid, megakaryocytic, and erythroid precursors develop. Rarely, marrow necrosis occurs after intense cytoreduction or at the onset of leukemic relapse. The early analysis of the regenerating marrow focuses on marrow cellularity, trilineage maturation, and disease recurrence. Engraftment is usually simultaneous in the three major cell lines, although there can be considerable lag in one or more lineages. Failure or loss of donor engraftment may be manifest by the decline or absence of peripheral counts and marrow hypocellularity. Marrow engraftment following a cord blood cell transplant, especially in adults, is often delayed [34]. In rare patients with

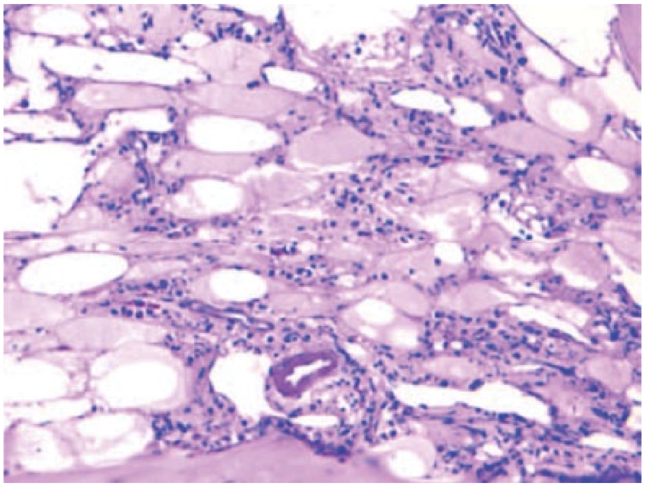

Figure 26.18 Markedly hypocellular marrow without evidence of engraftment, day 7. The interstitial fluid accumulation, fat necrosis, and iron deposition are consistent with chemoirradiation damage. (For a color version of this figure, please see the color plate section.)

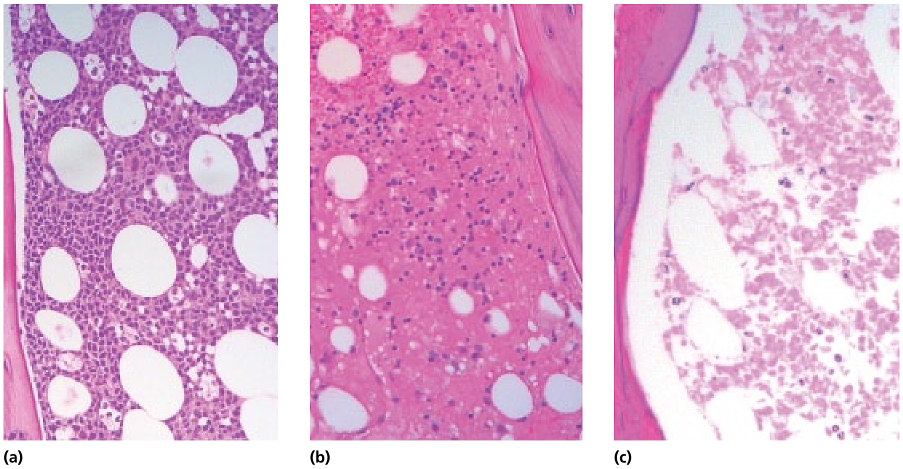

Figure 26.19 Same patient as in Figure 26.18 with acute myeloid leukemia: (a) diagnostic marrow with 96% blasts; (b) day 7 marrow after chemoreduction showing serous atrophy; (c) day 21 marrow with persistent pancytopenia showing extensive necrosis with only scattered neutrophils. (For a color version of this figure, please see the color plate section.)

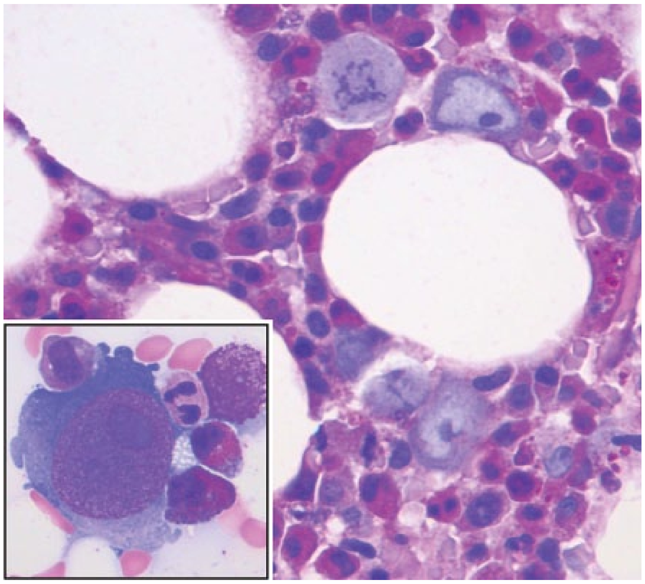

Figure 26.20 Pre-transplant marrow biopsy stained with periodic acid–Schiff (PAS) stain from a patient with diffuse large B-cell lymphoma with persistent anemia and quantitative Parvovirus virus PCR result of 13 billion. The PAS-stained marrow shows abnormally enlarged PAS negative proerythroblasts with prominent nucleoli and a marked erythroid hypoplasia. The inset at bottom left shows a Wright-stained aspirate smear with a markedly enlarged proerythroblasts with a prominent nucleoli. (For a color version of this figure, please see the color plate section.)

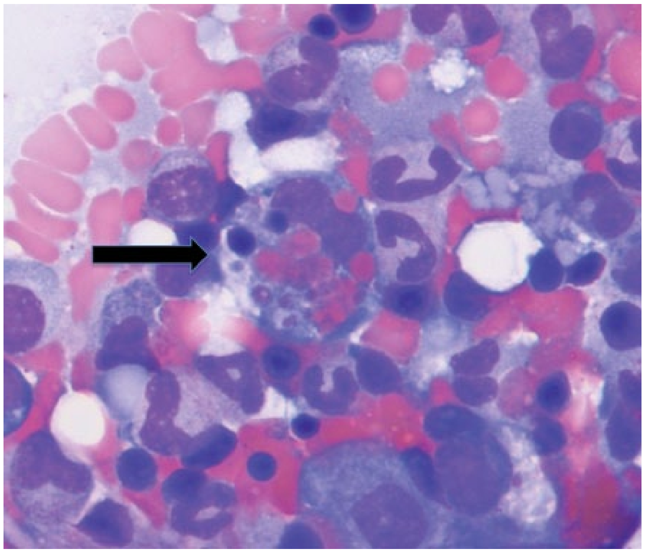

Figure 26.21 A Wright–Giemsa-stained marrow aspirate smear from a patient with large cell transformation of mycosis fungoides day 174 post-HCT with hemophagocytosis. A hematophagocytic macrophage with engulfed red blood cells and erythroid precursors in varying stages of degeneration is indicated by the back arrow. (For a color version of this figure, please see the color plate section.)

GVHD and absence of marrow hematopoiesis, there is apparently only lymphoid engraftment. Graft rejection has no specific histologic features and may be defined as transient engraftment with proliferation of at least one cell line followed by its loss. Rejection following trilineage engraftment occasionally involves loss of only a single line, analogous to pure red cell aplasia. Other causes of single- or bi lineage aplasia should also be considered in the differential for rejection, including parvovirus infection (Figure 26.20) and hemophagocytosis (Figure 26.21). Although rejection usually results in hypocellularity, this may not be true in patients being treated for some non-malignant conditions such as thalassemia or immunodeficiency. Lineage-specific chimerism testing and engraftment analysis are usually performed using PCR analysis against genomic polymorphisms termed short tandem repeat (STR) loci on lineage-sorted samples. Many laboratories fractionate the marrow by sorting separate myeloid (CD33), lymphoid (CD3), and immature (CD34) cell populations for detailed chimerism analysis (see Chapter 24). Chimerism testing is extremely important when monitoring donor cell engraftment in non-myeloablative and double cord transplants.

Despite numerous advances in the application of HCT, relapse remains the major cause of mortality [35]. Advances in MRD testing have facilitated the detection of early relapse that can then be treated with donor lymphocyte infusion (DLI) or other novel therapies. In many institutions, acute myeloid leukemia (AML) relapse is usually defined by the morphologic detection of >5% myeloid blasts. Relapse based on morphologic criteria has limited sensitivity and specificity compared with flow cytometry and molecular studies. In most cases, an increased population of malignant myeloid or lymphoid blasts cannot be distinguished from benign counterparts in a regenerating marrow by morphology alone. During marrow regeneration and with the use of hematopoietic growth factors, it is not unusual to detect an increased population of benign myeloid or lymphoid blasts (hematogones) [36]. In fact, some studies have shown that an increased population of hematogones is a good prognostic indicator in cord blood transplants of AML patients, possibly signifying a robust immune response [37]. Multiparameter flow cytometry is an essential tool since it can usually distinguish benign from malignant blasts and also identify rare aberrant blasts long before morphologic relapse is detectable [10,11]. It should be noted that in many cases there is clonal evolution and the morphology and immunophenotype of the post-transplant relapsed leukemia are different from those of the original primary pre-transplant leukemia [38]. Several studies have now shown that the presence of MRD post-transplantation is a poor prognostic indicator [39,40]. In addition, the regenerating marrow frequently shows left-shifted atypical to dysplastic features by morphology that should be interpreted with caution prior to diagnosing relapsed dysplasia. Ancillary studies including multiparameter flow cytometry, cytogenetics, and molecular testing can help determine if the atypical cells are due to marrow regeneration or relapse. In rare instances, leukemic relapse is donor derived [41,42]. Donor-derived leukemias can be easily overlooked because the relapsed leukemia may not be subjected to chimerism testing to determine donor or host origin. This is an important, but rare, distinction that impacts subsequent therapeutic decisions, since donor-derived lymphocyte infusions may not target donor-derived neoplasm.

Allogeneic HCT is the only potentially curative therapy for myeloproliferative neoplasms, including primary myelofibrosis. Some studies have shown that increased fibrosis and osteosclerosis can lead to delayed engraftment with diminution and reversal of fibrosis within 6–12 months [43]. However, most patients with primary myelofibrosis are older (median 60 years of age at diagnosis) and are frequently excluded from consideration for HCT because of regimen-related toxicity. Reduced-intensity conditioning regimens have enabled transplants to be performed in older patients, resulting in rapid regression of bone marrow fibrosis, osteosclerosis, and even molecular remission in JAK2-V617F-positive patients. One study reported a near uniform regression of fibrosis within 1 year post-transplant with most patients showing a marked reduction within 30 days of transplant [44].

Post-transplant lymphoproliferative disorder (PTLD) is a heterogeneous disease that arises in post-transplant immunosuppressed patients. PTLD is more common in solid organ transplants than in HCT patients, possibly related to immunosuppressive regimens. PTLD is defined as a proliferation of lymphocytes or plasma cells that ranges from a polyclonal reactive process to a monoclonal lymphoma. In most instances, PTLD is associated with Epstein–Barr virus (EBV) infection and is of B-cell lineage. In HCT recipients, the primary risk factor for developing PTLD is T-cell depletion, with the intensity and duration of immunosuppression being additional contributing factors. In practice, PTLD should be suspected in the setting of adenopathy or worsening of GI symptoms despite treatment for acute GVHD (Figures 26.22 and 26.23) [45]. Serial monitoring of plasma EBV DNA by PCR is used to detect increasing viral copy numbers, thus allowing early intervention [46].

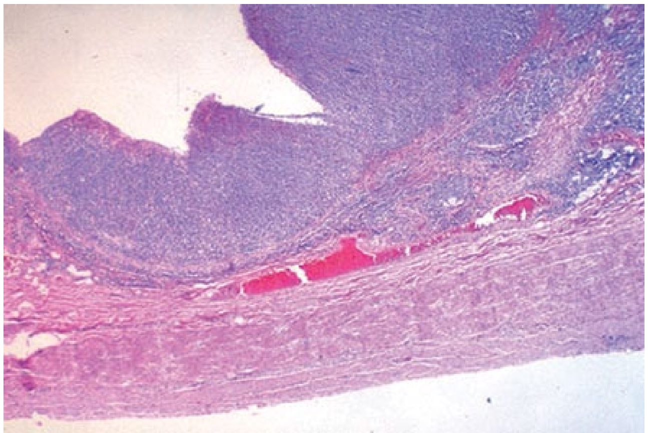

Figure 26.22 EBV-associated lymphoma infiltrating the intestinal mucosa in a boy treated for GVHD with cyclosporine and a monoclonal antibody to T-lymphocytes. Although the GVHD improved, large bizarre cells with atypical nuclei characteristic of a high-grade lymphoma rapidly formed tumor nodules in liver, spleen, lymph nodes, and mesentery. (For a color version of this figure, please see the color plate section.)

Role of the microvasculature in transplant complications

The microvasculature plays a major role in a number of post-transplant complications. Endothelial injury is the underlying cause of SOS, diffuse alveolar damage, alveolar capillary hemorrhage, immune-idiopathic pulmonary syndrome, and transplant-associated microangiopathic/hemolytic (TAM) syndrome (Figure 26.24) [47,48]. Visual methods for assessing endothelial injury include ultrastructure and immunostains for CD31, von Willebrand factor (vWF), vascular endothelial (VE) cadherin, TUNEL, and caspases. Additional blood markers in therapy-resistant GVHD include circulating endothelial cells, decreased vascular endothelial growth factor (VEGF), PAI-1, thrombomodulin, and adhesion molecules. Unresolved is whether the endothelium is damaged by cytotoxic T cells, inflammatory cytokines

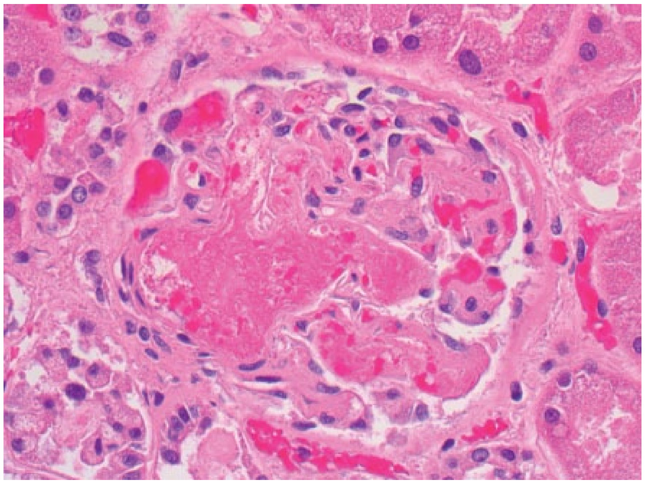

Figure 26.24 Kidney specimen stained with H&E showing transplant-associated microangiopathic/hemolytic (TAM) syndrome in a patient with AML progressed from MDS after HCT and died on day 21. There is thrombi formation in the capillary loops of the glomerulus, and characteristically showing involvement of the afferent vessel. (For a color version of this figure, please see the color plate section.)

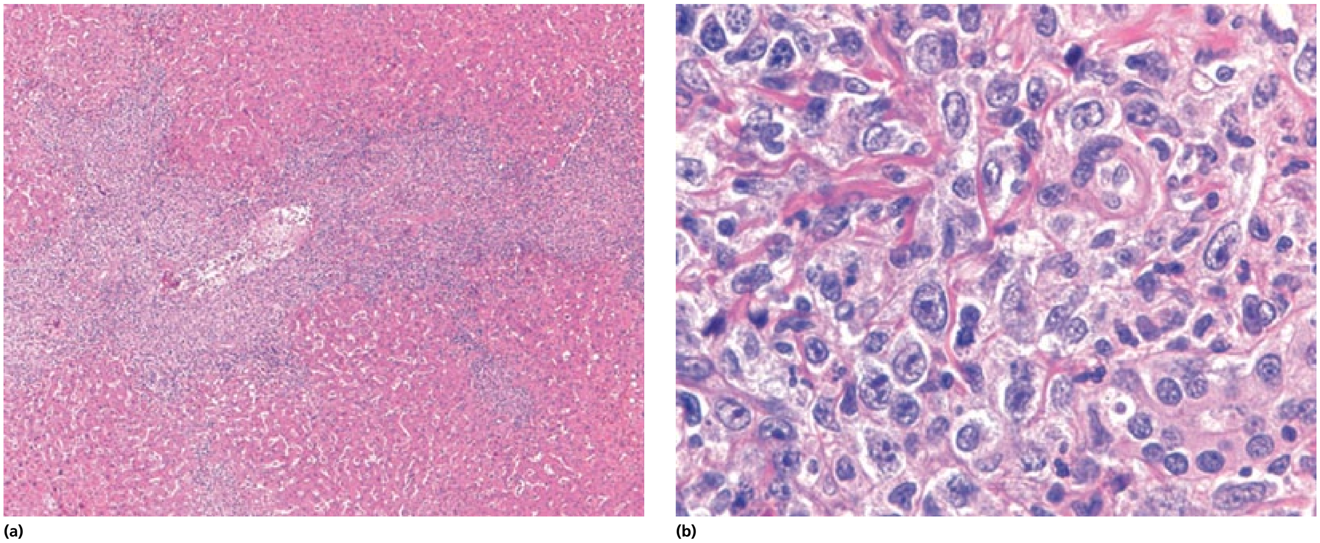

Figure 26.23 (a) Post-transplant EBV lymphoma. Following treatment for liver GVHD, the liver function abnormalities recurred. At low power, the portal space contains a prominent infiltrate that extends outwards into the periportal zone. (b) In the same case, higher magnification shows the large atypical immunoblastic cells that were positive for CD20 and EBER staining. (For a color version of this figure, please see the color plate section.)

from the inflammatory milieu and/or some combinations including the impact of certain immunosuppressive agents such as sirolimus on thrombotic microangiopathy [49,50] (see Chapter 96).

Graft-versus-host disease

The term graft-versus-host disease (GVHD) was introduced 60 years ago by Barnes and Loutit to describe diarrhea, skin disease, and wasting syndrome in irradiated mice after an allogeneic stem-cell transplant [51]. Today, GVHD continues to be the major complication of HCT that can lead to significant morbidities, infection, and death. Traditionally, GVHD has been classified into acute (occurring within the first 100 days post-transplant) and chronic (occurring after the first 100 days). The acute form is characterized by inflammation and epithelial cell apoptosis whereas the chronic form has a more pleotropic presentation, mimicking several autoimmune disorders such as scleroderma with increased tissue fibrosis. Recent changes in GVHD therapy and extended prophylaxis have created considerable overlap between acute and chronic GVHD, with late-occurring cases of acute GVHD resulting as "flares" following discontinuation of immunosuppressive regimens. Most investigators now favor the pathologic diagnosis to be based on histologic features (inflammation and apoptosis or increased tissue fibrosis) rather than time since transplant [52,53].

Immunopathogenesis

The pathophysiology of GVHD is commonly divided into two phases. In the afferent phase, there is initial tissue damage and immune activation related to the conditioning regimen. In the efferent stage, the activation of the effector cytotoxic T cells propagates the damage seen in GVHD. The classical scheme of GVHD can be subdivided into five basic steps, including priming of the immune response, T-cell activation and costimulation, alloreactive T-cell expansion and differentiation, activated T-cell trafficking, and the destruction of target tissue by effector T cells [54]. More recent studies have shown the importance of persistent B cell activation and loss of B cell tolerance contributing to and immunopathogenesis of chronic GVHD [55,56]).

GVHD can be thought of as an exuberant inflammatory response by the donor lymphocytes targeting selective host tissues and thus shares features with many autoimmune and other inflammatory conditions. The afferent or initiating stage is triggered by damaged or injured epithelium due to recent conditioning stimulating the donor lymphocyte-mediated inflammation. The damaged tissue produces proinflammatory cytokines and chemokines that result in the increased recruitment and activation of antigen-presenting cells (APCs). The APCs then present the host antigens including minor MHC antigens to the donor lymphocytes, triggering the initial phase of GVHD. Tissue damage to the GI epithelium is especially important because it results in systemic translocation of microbial products such as lipopolysaccharide (LPS) and other pathogen-associated antigens that further stimulate the APCs. The emerging influence of the intestinal microbiota and GVHD is an active area of investigation [57,58]. The observation that tissue damage from recent therapy contributes to GVHD has led to reduced-intensity conditioning as a strategy to decrease the incidence of GVHD.

The classical concept for the efferent stage in GVHD is that donor cytotoxic lymphocytes attack host cells, including some epithelial cells of the skin, intrahepatic bile ducts and gut. Electron microscopic and immunohistologic data demonstrate lymphocyte epithelial attachments and activation of infiltrating cytotoxic lymphocytes in human biopsy material. T-cell subsets, especially the regulatory T cells (Tregs), are thought to play an important role in modulating the expression and severity of GVHD. The addition of Tregs post-transplant has been shown to reduced GVHD and therapies based on this approach are currently in clinical trials [54]. In addition, patients with the rare disorder immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) lack functional Tregs owing to a dysfunctional FoxP3 transcription factor present with an autoimmune disorder, which mimics GVHD. Although HCT provides a potential cure for patients with IPEX, it is difficult to differentiate between GVHD and recurrent disease in post-transplant patients. Additional non-lymphoid cells of the innate immune system also play a role in the effector stage of GVHD, as two French studies have demonstrated that activated eosinophils [59] and apoptotic granulocytes [60] (along with TNF-α and Fas) are found in the lamina propria of GI GVHD. Similarly to many other inflammatory conditions, once triggered there are many pathways that serve to amplify and promote the inflammation, resulting in more tissue damage, apoptosis, and inflammation.

The targets of GVHD primarily involve selective epithelial cells in the skin, GI tract, liver, and lung. The specificity for these tissues is not known but may be related to the barrier function of the epithelium that is exposed to exogenous pathogens [54]. Preferential target cells reside in subregions of epithelium where cytokeratin 15 (K15) epithelial stem cells or their early progeny are located (e.g. the parafollicular bulge of the hair follicle and rete ridges of skin, and the latero-basal and neck regions in the crypt cells of gut) (Figures 26.25–26.27). In an experimental system using murine lingual epithelium, which resembles human cutaneous rete ridges, it has been shown that the genes that regulate apoptotic vulnerability are differentially expressed in a basal layer subpopulation distinguishable by K15 [61]. Recent studies by Zhan et al. demonstrated a cytokine-induced change in the CK15 stem cells from an anti-apoptotic profile to a proapoptotic profile [62]. Parallel studies have shown that the intestinal stem cells (ISCs) are targeted in GVHD, leading to an inhibition in the recovery and regeneration of the intestinal mucosa after injury. In addition, the injection of Wnt agonist R-spondin1 (R-Spo1) protected against ISC damage, enhanced restoration of injured intestinal epithelium, and inhibited subsequent inflammatory cytokine cascades. Therefore, protection of the epithelial stem cells during GVHD represents a novel strategy to decrease epithelial damage and stimulate regeneration, thus improving the outcome of allogeneic bone marrow transplantation (BMT) [63].

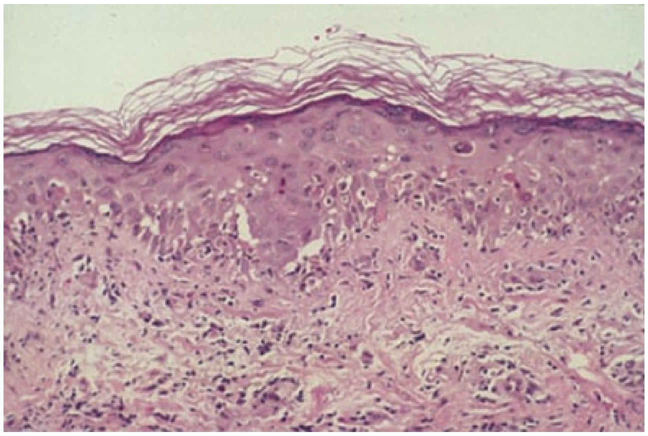

Figure 26.25 Acute skin GVHD, day 28 after mismatched allogeneic HCT. The epidermis demonstrates a prominent lichenoid reaction involving the tip of the rete ridge in the center of the specimen, a location of epithelial stem cells within the epidermis. (For a color version of this figure, please see the color plate section.)

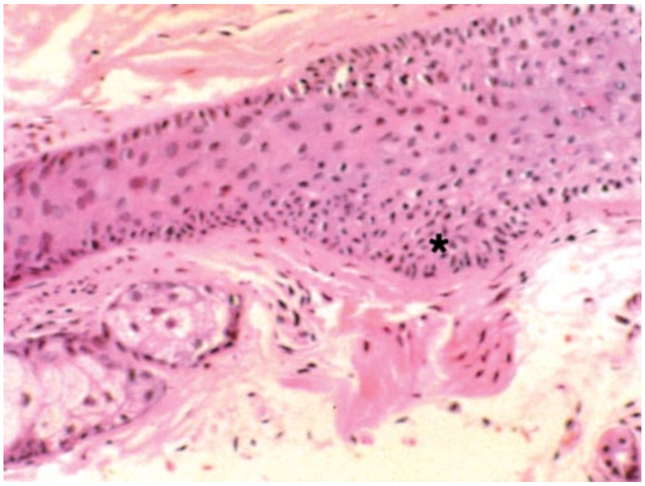

Figure 26.26 Early skin GVHD involving the parafollicular bulge region of the pilar unit. Note the small numbers of mononuclear inflammatory cells which infiltrate the parafollicular bulge area (*) near the arrector pilorum muscle in association with apoptotic bodies. (For a color version of this figure, please see the color plate section.)

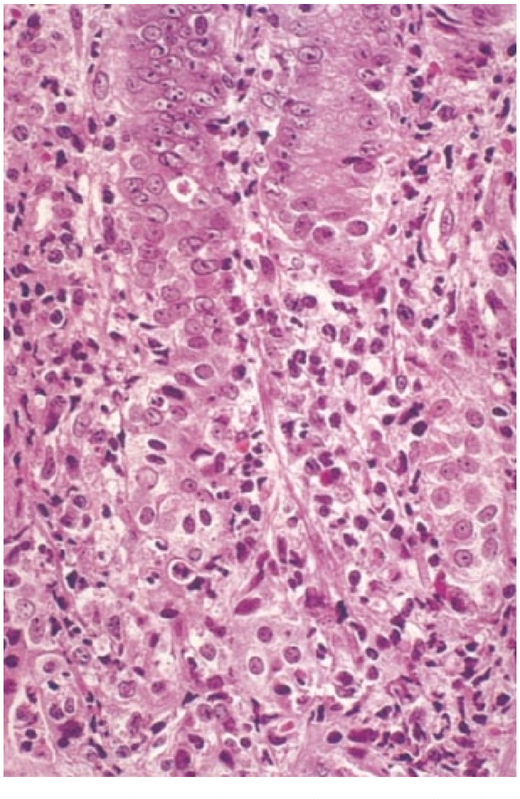

Figure 26.27 Gastric biopsy with GVHD, day 35. Several studies have shown that of all upper endoscopic biopsy sites, the stomach most frequently shows histologic changes of GVHD. In the area shown here, large numbers of lymphocytes infiltrate the lamina propria and the basilar portions of the gastric crypts. There is apoptosis of crypt epithelial cells and frank early crypt destruction. Inflammation and apoptosis of this intensity are not required for the diagnosis of gastric GVHD. More subtle yet still diagnostic alterations are illustrated in Figures 26.50 and 26.51. (For a color version of this figure, please see the color plate section.)

The initial investigations of acute GVHD focused predominately on T-cell-mediated injury of target epithelia while largely ignoring the possible involvement of B cells [56] and the endothelium, a major target in solid organ rejection. While assessing endothelial injury, it is difficult to separate non-specific activation by standard injury from selected targeting of the vessels. Early studies of dermal post-capillary venules in human cutaneous GVHD noted that endothelial damage was more marked in mismatched than matched sibling recipients [64]. The vascular endothelium serves as the gateway for lymphocytes to home through specialized addressins into the organs targeted by GVHD. The endothelial surface and homing are influenced by cytokine-mediated damage. In both the afferent and efferent stages of acute GVHD, the trafficking of naïve or programmed efferent T cells is directed by the requisite combination of homing receptors and chemokine receptors that engage the endothelium and the target tissue [54,65]. The release of serum factors affecting endothelial activation occurs in both the allogeneic and autologous setting. It reflects the collective effects of activation by chemoattractants and cytokines from conditioning and engraftment, upregulation of proinflammatory, prothrombotic, and proapoptotic state, resulting in increased permeability and cellular migration [66]. Of relevance, some drugs, such as lovastatin, may interfere with the expression of GVHD by blocking T-cell adhesion, proliferation, and cytokine production [67,68].

Immunohistology

In general, T-cell infiltrates, predominately CD8+, have been found in immunohistochemistry (IHC) studies of skin biopsies. Those few groups who have studied blood and tissue ratios of T-cell subsets simultaneously have found similar CD4/8 ratios in the peripheral blood and affected tissue. Later studies of GVHD in the skin and lip have shown that the infiltrates are primarily cytotoxic T cells as defined by CD8, the cytotoxic marker TIA-1, and perforin. Two studies found that increased numbers of CD163+ cells (macrophages) in human skin GVHD were variably associated with steroid refractoriness [69,70]. Increased numbers of donor and host dendritic cells and donor macrophages in the skin may facilitate antigen presentation and cytokine stimulation of CD8+ T cells by sustaining the response of previously activated T cells [71]. A combined histologic, ultrastructural, and IHC study of human upper endoscopic mucosal biopsies found both TNF-α and Fas expressed in the infiltrate and a significant correlation between the number of apoptotic cells and day 90 transplant-related mortality [60]. Human studies of mucosal FOXP3 regulatory T cells reported contradictory findings. Rieger et al. [72] reported numerically reduced Tregs in GI GVHD whereas Lord et al. [73] found no relationship between the frequency of Tregs in the gut and blood and the severity of GVHD. These conflicting results may be explained by a physiological heterogeneity of Tregs; following ex vivo fucosylation of human Tregs, there is both enhanced persistence and anti-GVHD potency [74]. In a study focusing on repair and regeneration of GVHD-induced damage, Murata et al. demonstrated both keratinocytes and dermal endothelial cells of donor origin by using TUNEL and XY fluorescence in situ hybridization (FISH) staining of skin biopsies from sex-mismatched HCT recipients [75]. The donor-derived cells were most numerous in the areas of severe GVHD damage, and only present in patients with a history of GVHD. However, the implications for any subsequent repair of GVHD-damaged tissues is unclear since the switch from recipient to donor phenotype was present only in biopsies taken early and not later during the course of GVHD.

In the clinical setting, the use of IHC for routine diagnosis of GVHD is limited by the sparseness of lymphoid infiltrates, additional delay and expense, lack of control data for other inflammatory conditions in the transplant milieu, and variations in the GVHD effector cell immunophenotype. There are limited data comparing apoptosis markers such as activated caspase 3 to hematoxylin and eosin (H&E) stains in both GVHD and relevant non-GVHD inflammatory conditions [76]. Little has been published on the use of IHC staining with C4d, a marker of complement activation in GVHD [77].

Surgical pathology of GVHD

Certain problems or controversies in the pathology of GVHD persist. There is still a histologic overlap between early conditioning toxicity, certain infections, and GVHD in the skin and gut. Defining the minimal threshold for early GVHD diagnosis or after prolonged immunosuppressive therapy remains a challenge. There is variance in the criteria for minimal activity consistent with active GVHD, particularly for the gut, when biopsies are obtained early after the onset of symptoms or during ongoing immunosuppressive therapy. In addition to false-positive diagnostic interpretations from concurrent viral infections and drug or conditioning toxicity, the inverse situation of false-negative biopsies exists, particularly when biopsies are obtained soon after the onset of rash or GI symptoms. Furthermore, in the GI tract there is discrepant opinion on whether the gold standard for GVHD diagnosis should be based on the broad visual survey seen by endoscopy, clinical features, or the microscopic examination of small biopsies [78].

The 2005 NIH Pathology Consensus Committee's minimal histologic criteria for GVHD by organ system are reproduced in Table 26.1 [53]. The updated table in the 2014 NIH consensus includes minor changes in the lung and inclusion of the kidney [1]. While the basic pathology of GVHD is apoptosis and eventually destruction of selective targeted epithelia with or without fibrosis, there are a number of factors that can influence or cause difficulty in interpretation. These include residual toxicity from conditioning, immunosuppressive treatment that blunts inflammation, a key indicator of activity, coexisting infections, and drug reactions that

Table 26.1 Histological Criteria for GVHD by Organ System

Organ or System	Minimal criteria for acute/active GVHD*	Specific criteria for Chronic GVHD[†]
Liver	Global assessment of dysmorphic or destroyed small bile ducts ± cholestasis, lobular and portal inflammation	Ductopenia, portal fibrosis, chronic cholestasis reflect chronicity but are not specific for chronic GVHD
Gastro-intestinal	Variable apoptotic criteria (≥1/piece) in crypts	Destruction of glands, ulceration or submucosal fibrosis may reflect severe or long-standing disease but are not specific for chronic GVHD
Skin, in general	Apoptosis in epidermal basal layer or lower Malphigian layer or infundibulum/outer root sheath/hair bulge of hair follicle or acrosyringium/sweat ducts ± lichenoid inflammation ± vacuolar change ± lymphocytic satellitosis	
Skin lichen planus-like		Combination of epidermal orthohyperkeratosis, hypergranulosis and acanthosis resembling lichen planus ± lichenoid inflammation and / or vacuolar changes of eccrine units
Skin morpheic (localized or diffuse		Thickening and homogenization of collagen bundles throughout reticular dermis or pandermal sclerosis with overlying interface changes ± thickening and homogenization of subcutaneous septa
Skin lichen sclerosus- like		Homgenization ± sclerosis of papillary dermal collagen with overlying interface changes including melanophages in the papillary dermis and sparse lymphocytic infiltrate
Skin fasciitis		Thickening of fascial septa with adjacent inflammation ± sclerosis of subcutis
Oral/ oropharyngeal mucosa and conjunctiva	Lichenoid interface lymphocytes with infiltration of mucosa (exocytosis) and variable apoptosis[‡]	
Minor salivary or lacrimal gland		Periductal lymphocytic infiltrate with infiltration and damaged intralobular ducts, fibroplasia in periductal stroma, mixed lymphocytic and plasmacytic inflammation with destruction of acinar tissue[§]
Lung		Constrictive bronchiolitis obliterans: dense eosinophilic scarring beneath the respiratory epithelium, resulting in luminal narrowing or complete fibrous obliteration. May be preceded by lymphocytic bronchiolitis without intraluminal fibrosis[¶]
Kidney		Membranous nephropathy, Minimal Change Disease
Lesions of Uncertain Pathogenesis		
Lung		Cryptogenic organizing pneumonia
Skeletal Muscle		Myositis

*Conditions that result in lesser degrees of change include immunosuppressive treatment, biopsy very soon after onset of signs, suboptimal or small tissue sample, insufficient serial sectioning, confounding infection, drug reaction or inflammatory conditions.

[†] After the diagnosis of chronic GVHD has been established or following immunosuppressive treatment, the histological manifestations of active disease may meet only minimal diagnostic criteria for activity. Different manifestations of cutaneous chronic GVHD may all be present together in one biopsy or in separate but concurrent biopsies

[‡] Inflammation of the oral mucosa and within the minor salivary glands may persist from prior chemo-irradiation or prior inflammation. The distinction between acute and chronic GVHD requires the addition of distinctive oral manifestations [81].

[§] The distinction of past acinar destruction and fibrosis from ongoing chronic GVHD activity can be difficult and relies on assessing lobules that are not completely fibrotic. Acinar and periductal inflammation with features of damage to ducts, such as vacuolar change, lymphocytic exocytosis nuclear dropout, dyspolarity or apoptosis, and resultant fibroplasia indicate chronic GVHD activity.

[¶] Constrictive bronchiolitis obliterans (CBO) [179,189] should be distinguished from cryptogenic organizing pneumonia [176,177], which is also associated with GVHD but has a different clinicopathologic presentation and a more favorable outcome.

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can mimic GVHD. The timing of the biopsies, sampling error (especially with gut biopsies), and technical factors can cause a false-negative histologic assessment. Furthermore, the diagnostic standards for minimal criteria for GVHD are inexact regarding the minimum required number of apoptotic epithelial cells, whether their presence is necessary in mucosal biopsies with inflammation, the number of serial sections needed to exclude a false-negative diagnosis, and the extent of dysmorphic change for a positive diagnosis in salivary and bile ducts. As a result, histologic examination may not always be the gold standard for diagnosis. In reality, the histologic changes, especially in patients undergoing immunosuppressive treatment for GVHD, are often not pronounced; a biopsy can be interpreted as GVHD with mild activity and confounding conditions. The pathology report can convey these issues by integrating the histologic findings with the relevant clinical details in a comment and by inserting qualifying phrases, the implication of which is understood within that institution, into the final diagnosis (e.g. possible, consistent with, favor). In the final analysis, the decision to treat for GVHD is made by the clinician based on a number of factors, including the likelihood of relapse and the desirability of a graft-versus-tumor effect, clinical stage of the target organ abnormality, rapidity of progression, the toxicity of treatment, and the histologic findings [79].

Acute versus chronic GVHD

Advances in HCT practice have altered the manifestation and separation of acute and chronic GVHD [52,53,80] (Table 26.1). The clinical manifestations [52,80] and certain unique histologic changes [53] listed in Table 26.1 are the minimal criteria that define acute and chronic GVHD. The NIH clinical classification identifies an overlap syndrome, which is a composite of both acute and chronic features. The classification also includes acute GVHD that is persistent, recurrent, or occurs after day 100 without meeting additional diagnostic criteria for chronic GVHD [81,82]. Tissue biopsies are used as a primary biomarker for establishing the diagnosis and type of GVHD, for assessment of activity to monitor therapy, and as a tool for prognosis. Pathologists should be familiar with the changing histologic spectrum of GVHD [53,80], and have an awareness of relevant clinical data and questions surrounding a biopsy. Protocols for guiding histologic evaluation and clinical information relevant for interpretation from skin, liver, gut, and mucosal biopsy sites are available online at the American Society Blood and Marrow Transplantation: http://www.asbmt.org/?page=Histopathology; http://www.asbmt.org/?page=MeasureCGVHD.

The incidence of chronic GVHD over a 12-year period has shown a steady significant increase to 37% between years 2004–2007 [83]. Physical evaluation of the skin in chronic GVHD has been standardized [84] and assessment of progression and prognosis have been validated through a combination of the NIH composite skin score and the Lee patient symptom score [2]. The histologic changes specific for chronic GVHD occur in the biopsies from skin and fascia, salivary and lacrimal glands, muscle, and lung. There is no clear histologic dichotomy between acute and chronic GVHD in the liver or gut. From the pathologist's viewpoint, chronic GVHD is a multiorgan inflammatory disorder that resembles a mixture of GVHD and several autoimmune diseases and rejection after solid organ transplantation.

Clinical and experimental data indicate that clonotypic T-cell effectors that mediate chronic GVHD have a unique specificity that includes tissue targets against donor and self [85]. One explanation for this observation is the destruction of thymic epithelium by acute GVHD effector T cells resulting in failure to detect autoreactive T cells (Figure 26.28). The contribution of microvascular damage in the genesis of the dermal fibrosis of chronic GVHD is controversial. Biedermann et al. found a correlation between elevated blood vWF levels and reduced capillary density identified by immunostaining for *Ulex europaeus* to the dermal sclerosis in chronic GVHD [86]. They concluded that reduced vascularity was responsible for the fibrosis and further inferred that the presence of T cells in the perivascular zone suggested that the endothelium was the target. In contrast, Fleming et al. did not find a correlation between the dermal fibrosis in chronic GVHD and a reduction in dermal capillary density. They also showed that the microvasculature in chronic GVHD did not have the abnormal endothelial phenotype of reduced VE cadherin and vWF [87] nor the abnormal capillaroscopic appearance characteristic of capillaries in systemic sclerosis [88].

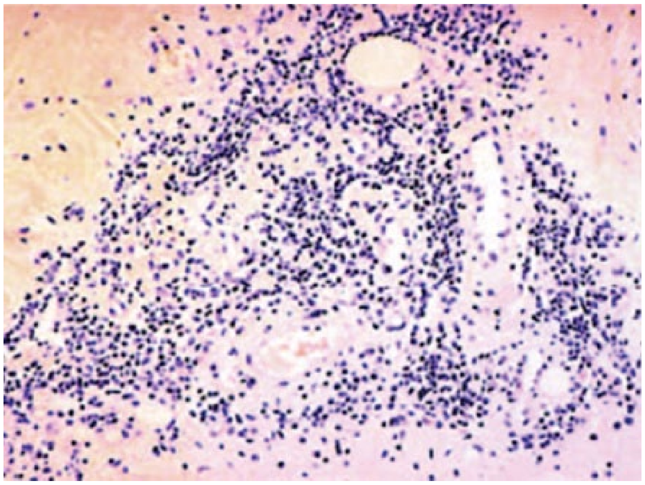

Figure 26.28 Atrophy and fibrosis of thymus associated with chronic GVHD in a 13-year-old, day 350. The patient had not received immunosuppressive drugs, so the destruction was presumably the result of chronic GVHD activity. (For a color version of this figure, please see the color plate section.)

The importance of screening biopsies at day 80–100 to identify histologically active GVHD of skin or lip in asymptomatic patients still on immunosuppression is less relevant. A positive screening biopsy (after day 100) with evidence of active GVHD (i.e. apoptotic epithelial cells) but devoid of the histologic features of chronic GVHD is no longer considered to be chronic GVHD. Likewise, a positive screening oral biopsy unaccompanied by distinctive gross oral features of chronic GVHD is no longer classified as chronic GVHD [52,53]. Accordingly, there is a wide spectrum of utilization of oral and skin biopsies in the diagnosis and management of late acute or chronic GVHD [82,89]. Biopsies are recommended to confirm active chronic GVHD when alternative diagnoses are entertained, the clinical signs are confined to internal organs, or the clinical assessment of activity is obscured by prior changes. In one tertiary care center, 7% of patients sent for consultation regarding chronic GVHD had been incorrectly diagnosed and treated for active chronic GVHD before referral [90].

Grading of GVHD

The initial histologic grading system of Lerner et al. was devised in 1974, with conditions far different from those today [91]. The scheme had four histologic grades for acute GVHD in the skin, liver, and gut, which attempted to use the chronologic progression of injury as a scale of severity based on the amount of inflammation, apoptosis, and the degree of tissue damage. In fact, GVHD histology is a snapshot in time such that mild (grade I) or severe (grades III–IV) changes may primarily be a reflection of timing and duration of activity (stage) and degree of immunosuppressive treatment. The Lerner scheme and other subsequent histologic grading schemata for GVHD were created without first being verified against useful clinical end-points. The lower histologic grades, II in the skin and I in the gut, still serve as benchmarks for defining minimal diagnostic criteria. In contrast, the higher grades of skin GVHD are rarely encountered. Serologic liver tests may provide a more accurate gauge of response to treatment than histologic damage to the biliary system, which is much slower to show improvement. A histologic grade with a high level of inflammatory and/or apoptotic activity is highly relevant information that will impact current treatment decisions [30,53]. Persistent grade IV-gut histology, particularly in the small intestines, portends refractoriness and potentially lethal GVHD [78,92]. Nonetheless, an accurate prediction on overall outcome can be made using non-invasive clinical signs of persistent bloody diarrhea with refractoriness to steroids [93]. In addition to avoiding the need to repeat the endoscopic biopsy, it also avoids the interpretive issue of whether the gut has had adequate time to show histologic improvement. Evidence-based observations indicate that time to recovery is proportionate to the severity (stage) of the destruction of the gut mucosa and/or bile ducts [26,53]. Whereas skin histologic grading schemata have limited independent prognostic value in the current milieu, the stage of cutaneous GVHD, acute or chronic, carries important therapeutic implications. These comments and those in the sections below are not meant to dismiss the use of biopsies for GVHD but rather to point out the nuances in their interpretation, and how they are currently used as a diagnostic modality or to complement clinical data.

Skin

Utility of skin biopsies in GVHD

The primary utility of skin biopsies in patients who have undergone recent HCT is to aid clinical diagnosis for GVHD and infection from other conditions such as drug eruption. It is important to identify pre-transplant diseases since GVHD has morphologic overlap with autoimmune disorders, connective tissue diseases, dermatomyositis, and many other skin rashes. In the early post-transplant setting, skin rashes are common and considered to be multifactorial. The management of early skin rashes is influenced by a set of variables, including the sex and degree of donor match, whether conditioning is myeloablative, desirability of graft-versus-tumor effect, extent of rash, rapidity of progression, and protocol requirements. In a study with a large cohort of 809 consecutive patients, 265 (33%) developed clinical grade II–IV GVHD; 27% of these had an early-onset acute GVHD manifested by day 14, referred to as hyperacute GVHD, and 88% of the patients with hyperacute GVHD had severe skin involvement with a higher non-relapse mortality, especially in recipients of unrelated donors (matched or mismatched) [79].

In previous editions, we advocated serial skin biopsies to aid in the interpretation of early skin rashes and GVHD was considered likely if the changes progressed without treatment or remained grade II or higher. Although this approach still has validity, a common alternative approach is to give a short course of steroids without first obtaining a biopsy. Sustained resolution implies that the cause is not GVHD, whereas no response or a recurrence suggests GVHD. There is no simple answer to this conundrum of when to use skin biopsies to diagnose early GVHD. The predictive utility of skin biopsies rests primarily in their display of continued histologic signs of activity rather than grade per se. Firoz et al. addressed this conundrum in a study using a decision analysis model to estimate the value of skin biopsy in the evaluation of a hypothetical case of a morbilliform rash covering 60% of the body surface status post allogeneic stem-cell transplant [94]. Ten experts provided their estimates of the prevalence of GVHD, the sensitivity and specificity of the skin biopsy, and the potential outcomes from treatment or observation. Only 25% of the experts chose an intervention consistent with their estimates of the prevalence, test characteristics (histologic results), and outcome evaluations. In this decision analysis modeling, the best patient outcome occurred if biopsy was done when the prevalence of GVHD was <30%, whereas treatment for GVHD was given without biopsy if the prevalence of GVHD was >30% [94]. The NIH consensus development project on criteria for clinical trials in chronic GVHD recommends that skin biopsies should be used to confirm active chronic GVHD when the clinical picture is unclear, another diagnosis such as infection is suspected, clinical signs of chronic GVHD are confined to other organs, only distinctive features of GVHD are seen, or the clinical assessment is obscured by prior changes [53].

The use and timing of skin biopsies for diagnosing GVHD have wide institutional variation. Depending on the patient population and, thus, the pretest probability for GVHD, the use of skin biopsies to aid in confirming GVHD may be of high importance. Some centers use skin biopsies routinely for GVHD screening, diagnosis of GVHD, and as a tool to track improvement with therapy or to detect treatment-refractory GVHD. However, other centers avoid using skin biopsies based on studies that conclude acute GVHD is histologically indistinguishable from other causes of rashes [95,96], viral infections, drug reactions, engraftment syndrome associated with lymphocyte recovery [97,98], and toxicity from pre-transplant cytoreductive conditioning [95,96,99].

Grading of GVHD in the skin

The cutaneous histologic grading system of Lerner et al. [91] has provided a useful framework for discussing minimal diagnostic criteria and the specificity of histology for GVHD. Grade I is superficial perivascular dermatitis with epidermal basal cell vacuolization; grade II is an interface dermatitis with scattered apoptotic or dyskeratotic keratinocytes in the basilar or lower spinosum layers that sometimes is in close contact with infiltrating lymphoid cells (lymphocytic satellitosis) (Figures 26.29 and 26.30); grade III has extensive apoptosis with reticular degeneration, destruction of the basal layer with nuclear dyspolarity and atypia, and suprabasilar bulla formation (Figures 26.25); and grade IV is represented by ulceration of the epidermis [92] (Figure 26.31). Distinction of GVHD from a variety of conditions is problematic and not always resolved by using grade II change for the minimal diagnostic criteria [80,95,96,99]. Clinical images of acute skin GVHD before and after PUVA therapy are illustrated in Figure 26.32.

Differential diagnosis of Grade I/II GVHD

Skin biopsies in HCT patients with grade I and II GVHD may show minimal cellular infiltrates and infrequent apoptosis (Figure 26.30). The most common differential of lower grade GVHD includes drug reaction, eruption of lymphocyte recovery syndrome, another pre-existing skin condition, or infection. Although various

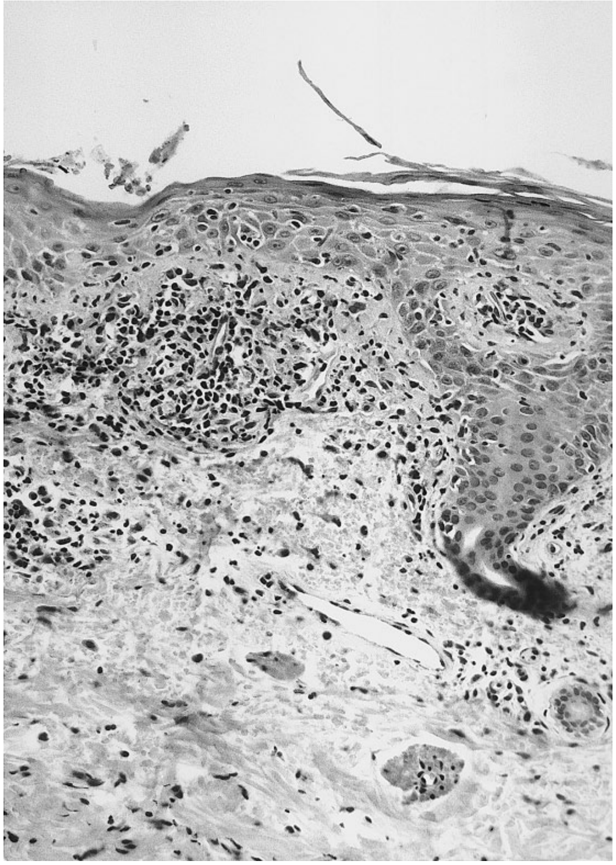

Figure 26.29 Skin, acute GVHD, day 30. Marked lichenoid reaction involving the epidermis and acrosyringium with intraepidermal lymphocytes, apoptosis, and destruction of rete ridges. (H&E.)

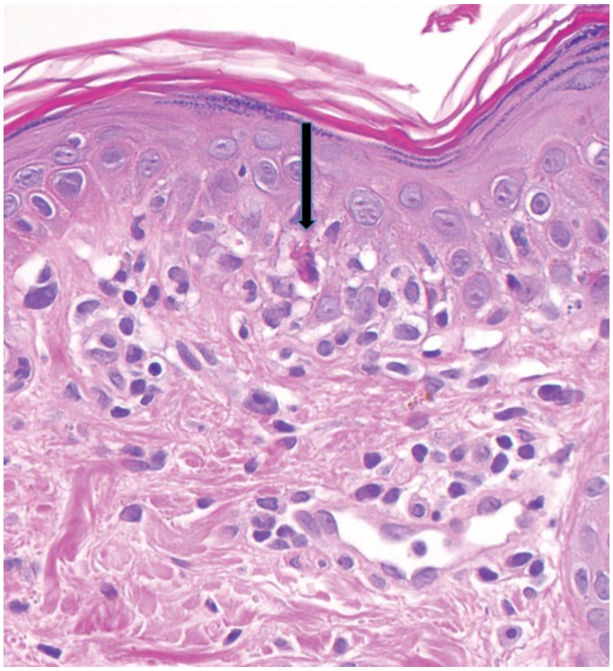

Figure 26.30 H&E stain of a skin biopsy from a, HCT patient day 25 with GVHD. There is mild spongiosis, basal vacuolization, lymphocytic inflammatory cells, and a few scattered apoptotic cells (arrow). (For a color version of this figure, please see the color plate section.)

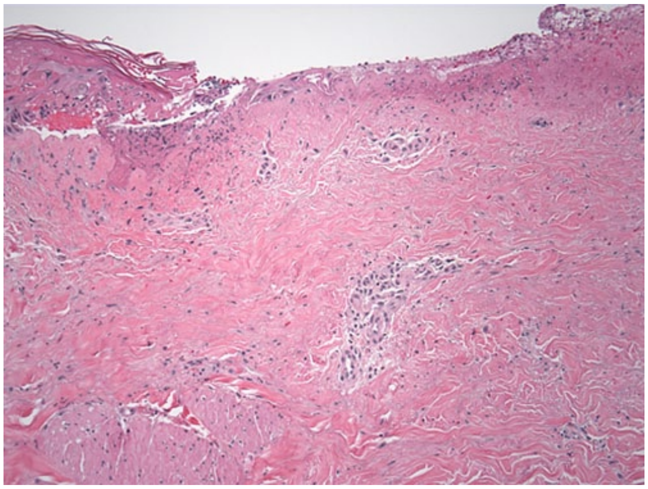

Figure 26.31 Skin biopsy with ulcerated epidermis from a patient with severe combined immunodeficiency disease, post-allogeneic stem cell transplant, day 475. The histology of this skin biopsy raised the differential of acute graft versus host disease or toxic epidermal necrolysis. (H&E.)

viral etiologies, including HHV 6, have been suggested for some of these early rashes, studies have shown evidence of increased association between HHV 6 and GVHD [100–102].

To favor a diagnosis of GVHD, it is important to review serial sections looking for apoptosis along the outer root sheath of hair follicles, the basilar layer of the epidermis, and at the tips of rete ridges. Additionally, apoptosis and architectural disarray in the eccrine units also provide support for the diagnosis of GVHD.

Drug hypersensitivity should be considered a possibility, especially if the rash has an atypical pattern or distribution for GVHD. An eczematous pattern of spongiosis with abundant lymphocytes and little or no apoptosis suggests a drug reaction rather than GVHD. Frequently, drug reaction presents with a pronounced perivascular inflammation in the superficial dermis without significant epidermotropism. Tissue eosinophils in the infiltrate are not an indication of a drug eruption as they are often also found in GVHD [103]. Drug eruption rashes frequently resolve spontaneously, and the exact causative agent is difficult to identify owing to polydrug regimens. Histology is non-specific, with perivascular inflammation edema and dilatation of the vessels.

Patients with eruption of lymphocyte recovery syndrome present with a maculopapular rash approximately 14–21 days after treatment with cytotoxic therapy that coincides with recovery of lymphocytes in the peripheral blood [104]. This rash will spontaneously improve without treatment or following withdrawal of medications [97,98]. Histology of these skin biopsies consists of scant superficial perivascular lymphocytic inflammation and vascular dilatation, with little or no keratinocyte apoptosis.

In patients who have received a busulfan-conditioning regimen, severe keratinocyte dysplasia (SKD) is a common finding, reported in up to 92% of HCT recipients [105]. SKD has been reported in HCT patients from day 28 to day 84 post-HCT; and has distinct histology compared with GVHD, although they may be seen concurrently [106]. The histologic appearance of SKD is characterized by keratinocytes with large and irregular nuclei, but may be seen in the background of GVHD (Figure 26.33).

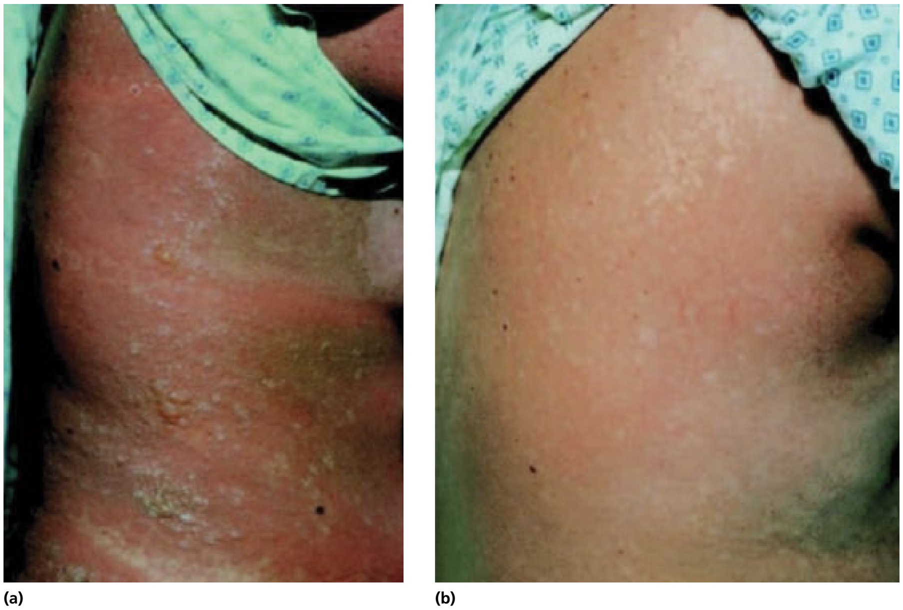

Figure 26.32 Before and after therapy photographs of the right lateral torso from the two different time points in an HCT patient: (a) erythema and desquamation before treatment for acute GVHD, day 27; (b) virtually normal skin with focal depigmentation after treatment for acute GVHD, day 74. (For a color version of this figure, please see the color plate section.)

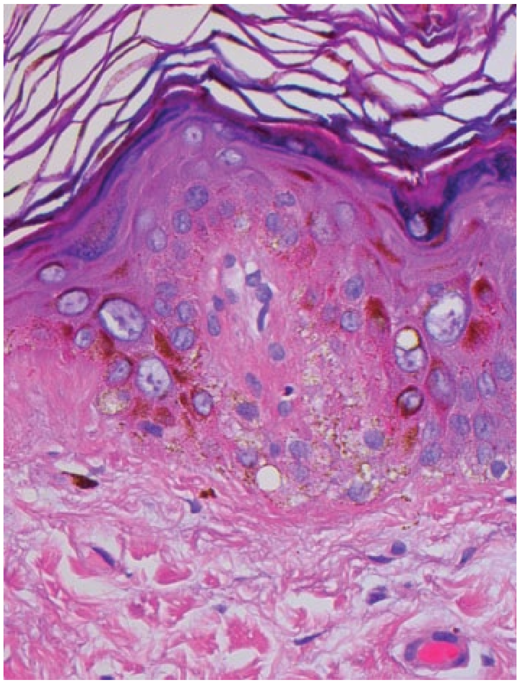

Figure 26.33 Severe keratinocyte dysplasia in a patient with myelodysplastic syndrome who is day 76 after myeloablative HCT with busulfan-based chemoreduction therapy. (For a color version of this figure, please see the color plate section.)

Differential diagnosis of Grade III/IV GVHD

The features associated with grade III/IV GVHD include extensive keratinocyte apoptosis, destruction of the basal layer causing separation of the dermal–epidermal junction, erythema, desquamation, or ulceration of the epidermis (Figure 26.24). Varying degrees of keratinocyte atypia may also be seen. These histologic findings raise the differential of erythema multiforme, toxic epidermal necrolysis (TEN), or severe drug reaction/Stevens–Johnson syndrome (SJS). A clear histologic distinction between these entities has not been established. Chemotherapy-induced acral erythema is also a differential that pathologists encounter in the early post-transplant period with a similar histology to higher grade GVHD. HHV 6 reactivation causes a rash with overlapping morphology to GVHD [107].

Erythema multiforme is an episodic self-limiting rash with varied clinical presentation ranging from macules and papules to vesicles and bullae. As expected, the variation in gross morphology also translates into varied histology with many overlapping features of higher grade GVHD, including frequent apoptosis, epidermal spongiosis, basal vacuolar change, lichenoid lymphocytic infiltrate, and clefting of the dermal–epidermal junction. In contrast to GVHD, eosinophils are generally not considered a prominent component of the inflammatory infiltrate in erythema multiforme. However, isolated case reports have shown that cases of eosinophil-rich erythema multiforme are possible [108]. The severe drug eruption producing a clinical picture of SJS or TEN with suprabasilar bullous formation and denudation can be difficult to distinguish from severe GVHD (Figure 26.34).

Chemotherapy-induced acral erythema is a syndrome of painful cutaneous acral erythema involving the palms and soles that is a distinct clinical entity caused by cytoreductive conditioning [109]. Histologically, this toxic injury to the epithelium cannot be clearly distinguished from GVHD (Figures 26.1 and 26.25). Both may display varying degrees of epidermal keratinocyte atypia, vacuolar damage, dyskeratosis, apoptosis, and interface inflammation, and depending on the particular myeloablative-conditioning regimen used, waiting 20–30 days before using the skin biopsy was advocated [96,99].

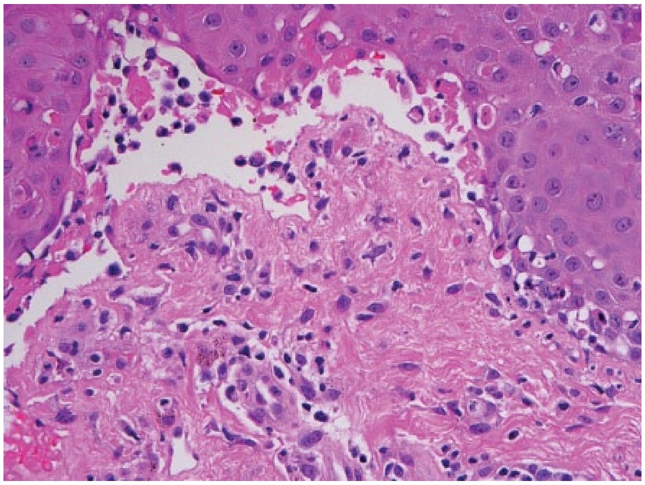

Figure 26.34 Skin biopsy from a patient who is day 36 after HCT with an erythematous non-pruritic rash involving 40% of the total body surface including the upper trunk, arms, head, and a few spots on the legs. This biopsy shows confluent basal cell necrosis with separation at the dermal epidermal junction, a histologic pattern more consistent with Stevens–Johnson type of drug eruption. (For a color version of this figure, please see the color plate section.)

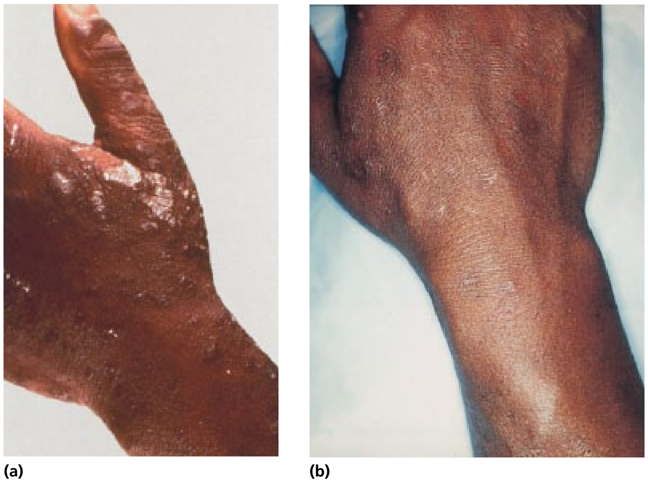

Figure 26.36 Photographs from a 24-year-old African American male who received an human leukocyte antigen (HLA)-identical HCT: (a) hand with lichen planus-like chronic GVHD day 220 post-HCT; (b) forearm after treatment, day 770 post-HCT. (For a color version of this figure, please see the color plate section.)

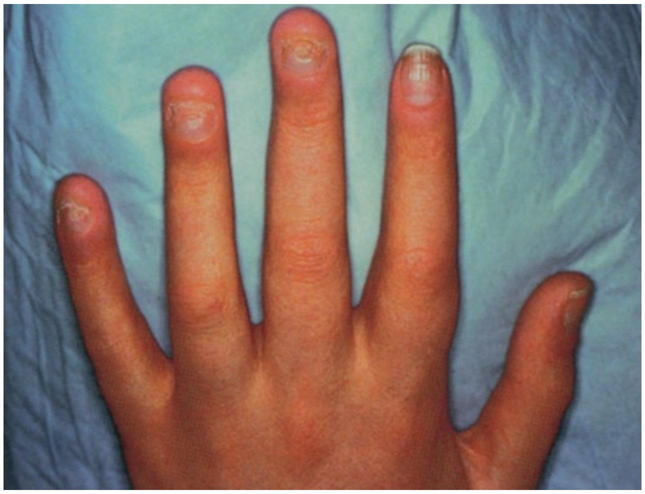

Figure 26.35 Periungual erythema and onychodystrophy 100 days after allogeneic BMT in a patient with ocular sicca and oral lesions of chronic GVHD. (For a color version of this figure, please see the color plate section.)

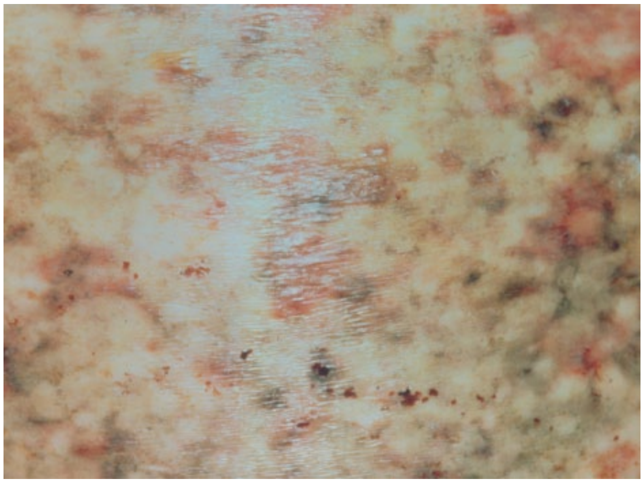

Figure 26.37 Poikiloderma in late chronic GVHD in a 2-year-old girl, day 450. Thinning of the epidermis and dermis is present along with telangiectasia and reticulated pigmentation. (For a color version of this figure, please see the color plate section.)

Chronic GVHD of the skin

Chronic GVHD of the skin has a polymorphous gross appearance potentially involving all regions, including the nails (Figures 26.35–26.37). A detailed glossary of the gross cutaneous manifestations along with a standardized nomenclature and their likely underlying histopathology may prove useful in correlating appearances with the outcome and response to therapy [80,110] The pathologist should be mindful of several caveats pertinent to the histopathology of chronic GVHD:

1 The histopathology changes over time. Usually, the predominant features of the early manifestations, referred to as lichen planus-like GVHD (Figure 26.38), are lymphoplasmacytic inflammation involving the epithelium and tubuloalveolar glands in the eccrine, lacrimal, and aerodigestive tracts. Unlike acute GVHD, chronic GVHD causes extensive destruction of these tubulo-alveolar glands and ducts with corresponding clinical sicca syndromes (Figure 26.39).
2 In later stages, referred to as sclerodermoid GVHD, there may be widespread fibrosis, stenosis, obliteration, or atrophy of the tissues involved (Figure 26.40; see also Figure 26.41). As a result, it may be difficult to distinguish active disease from residual fibrotic damage (Figure 26.42; see also Figure 26.43) in the absence of chronic inflammation or serial biopsies showing progression of the fibrosis. However, the initial presentation of cutaneous chronic GVHD may be primary with changes associated with the later stages of dyspigmentation and dermal sclerosis [110].

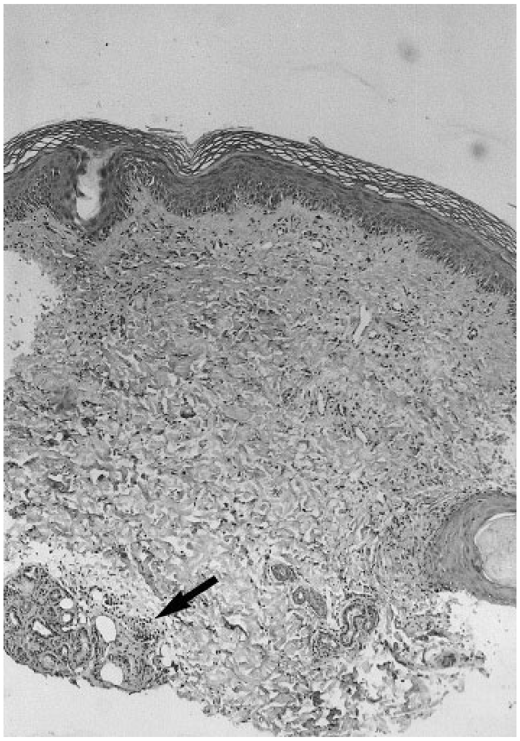

Figure 26.38 Skin, day 314, with the extensive or generalized type of early chronic GVHD. The so-called lichen planus-like chronic GVHD refers to the acanthotic and hyperkeratotic epidermis that has inflammatory and destructive changes along the dermal–epidermal junction. An eccrine unit at the base of the dermis (arrow) and a follicle deep in the dermis are also being destroyed. The dermal collagen is unaltered.

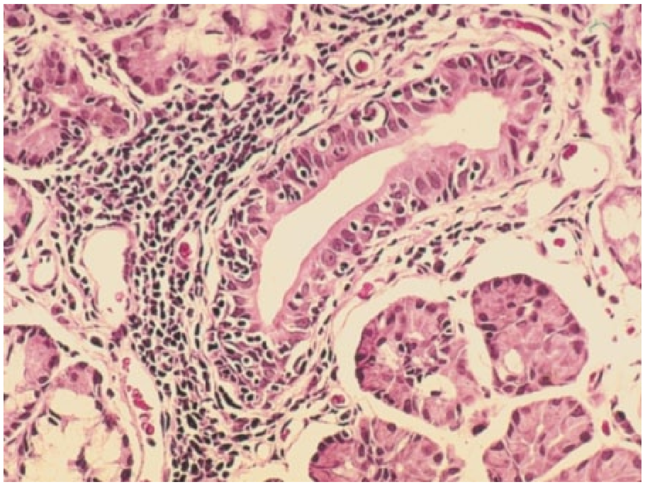

Figure 26.39 Oral labial biopsy with early chronic GVHD, day 92. The minor salivary gland exhibits a periductal mononuclear inflammatory infiltrate involving the duct epithelium, in which there is focal epithelial apoptosis. There is inflammation of adjacent acini. This process leads to fibrosis with acinar atrophy in lobules drained by the affected ducts. The squamous surface epithelium (not present here) showed grade II lesions similar to those previously illustrated in skin (see Figure 26.25). The lip biopsy is valuable in the diagnosis and staging of chronic GVHD because it frequently mirrors destructive inflammatory changes in lacrimal, tracheal, and esophageal glands as well as bile ducts. (For a color version of this figure, please see the color plate section.)

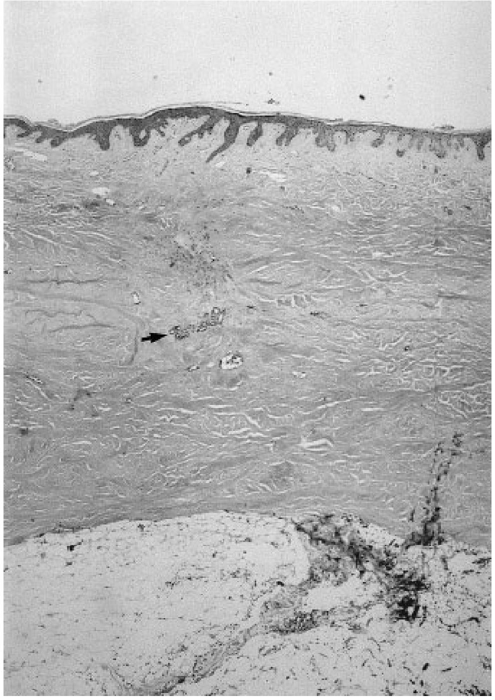

Figure 26.40 Late sclerodermatous chronic GVHD, day 910. The epidermis is atrophic. The dermal collagen is diffusely sclerotic with homogenization of the collagen, and the dermis–subcutis border is straightened. The dermis below the entrapped eccrine unit (arrow) represents acquired fibrous tissue resulting from chronic GVHD.

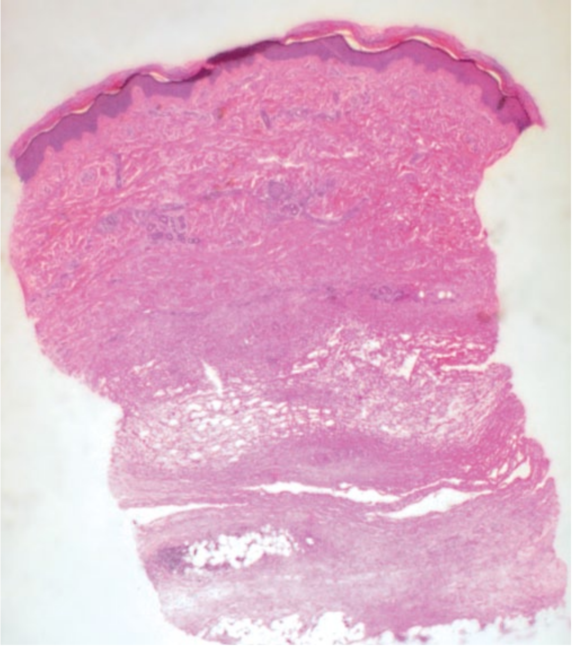

Figure 26.41 Late chronic GVHD of skin, day 959. As this patient's GVHD progressed, the epidermis became atrophic, with straightening of the dermal–epidermal junction. All dermal appendages were destroyed. The reticular dermal collagen became increasingly sclerotic. A panniculitis seen at the base of the specimen resulted in fibrosis of the subcutaneous fat and large vessels. This histologic picture corresponds to the dense hidebound and sclerodermatous changes of late chronic GVHD. (For a color version of this figure, please see the color plate section.)

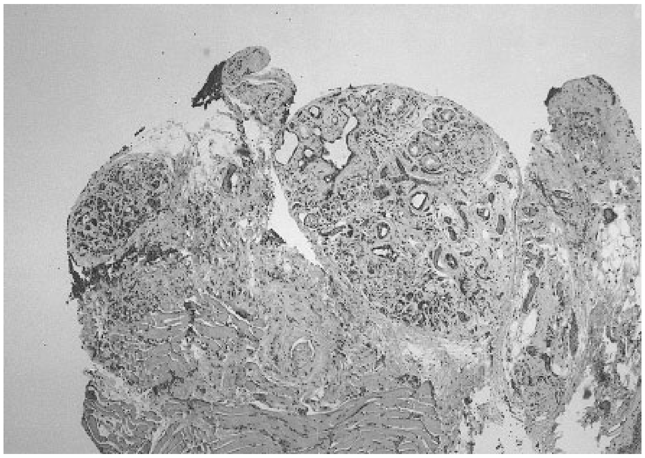

Figure 26.42 Lip biopsy, day 376. Fibrotic and destroyed minor salivary gland contains only ectatic ducts. These changes reflect previous damage from chronic GVHD. Unless they are accompanied by some inflammatory component in other glands or in the mucosa, they should not be taken as a sign of active chronic GVHD.

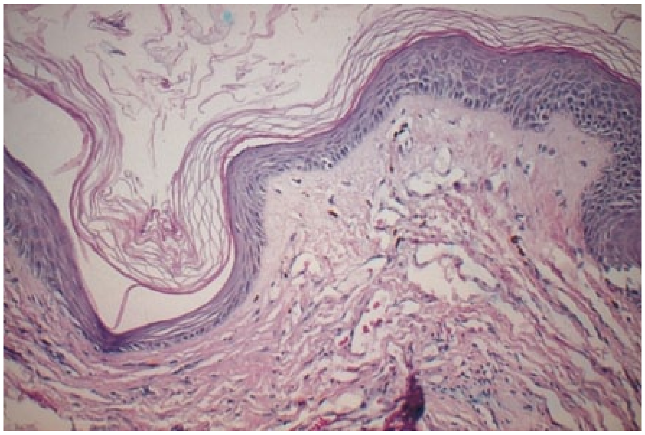

Figure 26.43 Poikiloderma, day 719. The patient was treated vigorously for extensive chronic GVHD with corticosteroids plus several other immunosuppressive agents. Although he had extensive dyspigmentation, telangiectasia, and atrophy of the skin, there were no contractures or scleroderma. Histologically, the epidermis is atrophic, with loss of rete ridges. The fibrotic papillary dermis contains many telangiectatic vessels, and there is intracellular melanin pigment. The deep dermal sweat glands remain despite earlier inflammatory involvement, and the reticular dermal collagen is normal. (For a color version of this figure, please see the color plate section.)

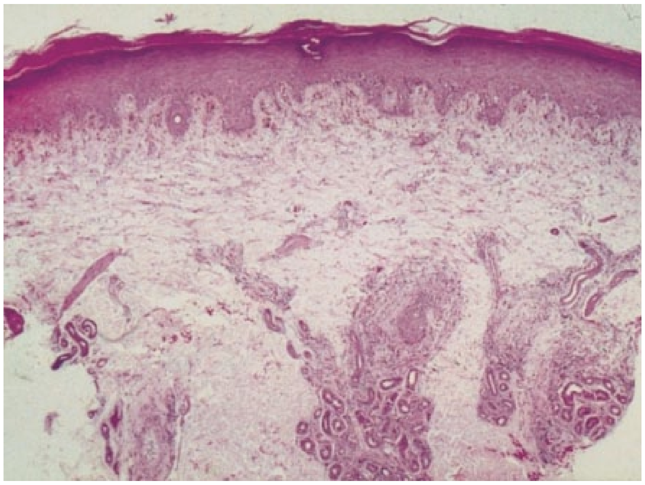

Figure 26.44 Lichenoid early chronic GVHD of skin, day 233. Biopsy of a papulosquamous lesion from the posterior neck demonstrates the characteristic features of the lichen planus-like type of chronic cutaneous GVHD. The thickened epidermis displays hyper- and parakeratosis, hypergranulosis, and acanthosis. The extensive destruction along the dermal–epidermal junction results in sawtooth-like changes of the rete ridges, mimicking lichen planus. The papillary dermis has considerable perivascular inflammation. The lichenoid reaction also involves the hair follicles and eccrine glands seen deep in the reticular dermis. At this stage, treatment can prevent progression to fibrosis (see Figure 26.41).
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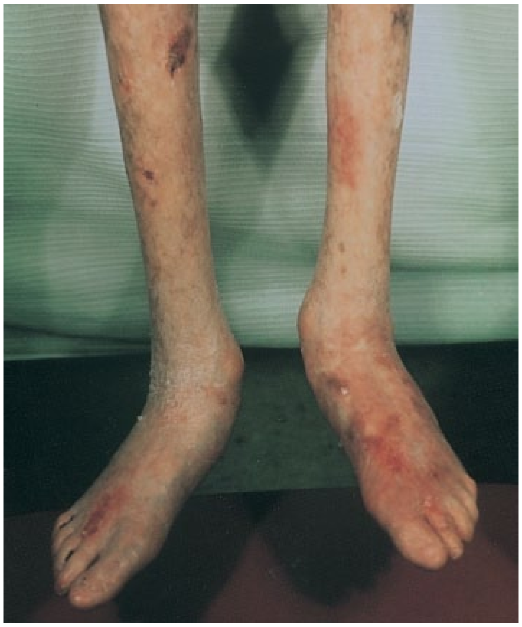


Figure 26.45 Generalized scleroderma with atrophy, sclerodactyly, and joint contracture, day 540 after HLA-identical BMT. (For a color version of this figure, please see the color plate section.)

3 Following a flare of chronic GVHD, the histologic changes may be more closely akin to those of acute GVHD. Lichenoid, sclerodermoid, and acute histologic features can coexist.

The initial dermatopathologic descriptions of cutaneous chronic GVHD were of a biphasic disorder, initially resembling a combination of widespread lichen planus and lupus profundus (Figure 26.44), which later progressed to scleroderma-like gross and histologic changes (Figures 26.40 and 26.45). Smaller subsets of patients initially present with poikiloderma, localized morpheic lesions, lichen sclerosus-like or fasciitis [111] (Figures 26.37, 26.43 and 26.46). In the current milieu, one may see various combinations of these changes plus superimposed skin lesions that only satisfy criteria for acute GVHD [52,80,90,112].

Full-thickness skin biopsies are essential to assess fully the possible changes in the eccrine units, subcutaneous fat, and fascia, and to delineate the degree of dermal fibrosis (Figure 26.41). The specific criteria for the early lichen planus-like stage are a combination of hyperkeratosis, hypergranulosis, and irregular acanthosis with

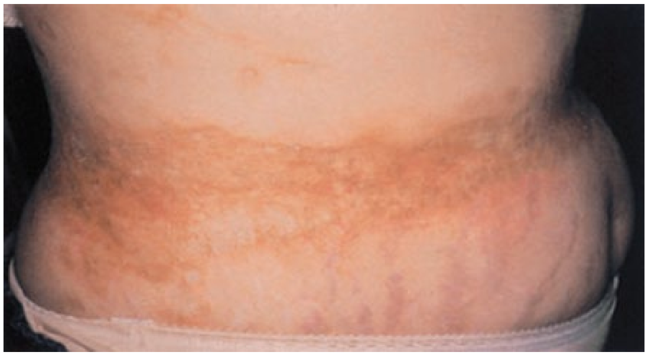


Figure 26.46 Localized late phase of chronic GVHD resembling morphea with atrophic plaque formation, induration, and peripheral hyperpigmentation, day 684. (For a color version of this figure, please see the color plate section.)

lichenoid basilar layer changes with or without syringitis (inflammation within eccrine units) or lobular panniculitis (inflammation of the subcutaneous fat) [53] (Figure 26.44). Serial biopsies demonstrate progression of fibrosis from the papillary dermis downwards, consistent with an etiology related to ongoing inflammation and release of cytokines (Figure 26.41 and 26.43). Patients with cutaneous sclerodermatous chronic GVHD, like those with progressive systemic sclerosis, have an autoantibody to the platelet-derived growth factor receptor which stimulates the activation of skin fibroblasts in vitro, suggesting a direct role in cutaneous sclerosis in both conditions [113]. However, the role of autoantibodies to platelet-derived growth factor receptor in promoting cutaneous sclerodermatous chronic GVHD is a controversial topic and not supported by all studies. The remodeling of dermal collagen progresses from straightening of the dermal–epidermal border with loss of the rete ridges and dermal appendages, along with sclerosis of the papillary and adventitial dermis with variable degrees of fibroblastic stroma. If progressive, the reticular dermis becomes thickened by smudgy collagen bundles. Eventually, the dermal–subcutaneous border becomes straightened and fibrotic septae may form in the subcutaneous fat (Figure 26.39; see also Figure 26.41). In patients receiving immunosuppressive treatment, the inflammatory changes may be minimal, with evidence of active GVHD limited to epithelial vacuolar degeneration or apoptosis in the basilar layers of the skin and its appendages, oral mucosa, or minor salivary glands of the lip.

Morpheic GVHD presents as localized dyspigmented macules with variable degrees of induration (Figure 26.46). A full-thickness biopsy demonstrates nodular fibrous remodeling in the deeper reticular dermis with variable inflammation in the dermis and adjacent subcutaneous fat. The epidermis may have minimal apoptosis or vacuolar changes. Eosinophilic fasciitis of the skin presents with an acute painful deep groove, or rippling cellulite appearance. A deep incisional biopsy shows edema and fibrosis of the fascia and subcutaneous septae with a mixed infiltrate of lymphocytes, histiocytes, and eosinophils [53,111]. A lichen sclerosis atrophicus type of chronic GVHD leads to scarring and stricture when it affects the female genitalia. Histologically, there is epidermal or mucosal atrophy with basal vacuolar changes, a band-like infiltrate associated with small numbers of apoptotic cells, and a subepidermal zone of pale homogenized collagen. Several additional forms of cutaneous chronic GVHD include eruptive angiomas, vitiligo, hyperpigmentation, destructive nail dystrophies, and ichthyosiform change [110] (Figures 26.37 and 26.45).

In contrast to assessing the development and progression of dermal fibrosis in chronic GVHD, the opposite situation pertains when hematopoietic cell transplantation is carried out for progressive systemic sclerosis. Both the clinical Rodnan dermal mobility score and the histologic scoring system for dermal sclerosis demonstrated agreement in the regression of dermal sclerosis [114,115].

Oral GVHD

Pathology of chronic oral GVHD has mucosal changes with varying degrees of lichenoid interface inflammation, exocytosis, and apoptosis. The typical differential diagnoses to consider include lichen planus, lichenoid drug reaction, and lichenoid hypersensitivity.

The assessment of GVHD in minor salivary glands should focus only on lobules that are not completely fibrotic. Active GVHD changes include lymphocytic exocytosis into intralobular ducts and acini, and lymphocytic periductal inflammation with or without plasma cells (Figures 26.40, 26.47, and 26.48). Fibroblastic periductal and periacinar stroma is indicative of chronic GVHD activity, whereas dense fibrosis indicates only previous damage (Figure 26.42). Because of residual non-allogeneic baseline inflammatory changes from myeloablative conditioning [116], there are institutional variations in how the threshold for minimal activity is defined. One such proposal with more stringent criteria requires more than three apoptotic cells in the mucosa and >10% loss of acinar tissue or ductal epithelial necrosis [117] The same criteria for oral biopsies also apply to esophageal, vulvar, conjunctival, and lacrimal biopsies.

Gastrointestinal GVHD

The diagnosis and monitoring of GVHD in the GI tract require a multidisciplinary approach including the clinician, gastroenterologist, and pathologist. Patients frequently present with GI symptoms including nausea, vomiting, anorexia, and secretory diarrhea. Severe cases can evolve to GI bleeding and protein-losing enteropathy. GI GVHD is a patchy and focal disorder and the primary site of activity does not always correlate with symptoms. Imaging studies are frequently non-specific and show diffuse mucosal

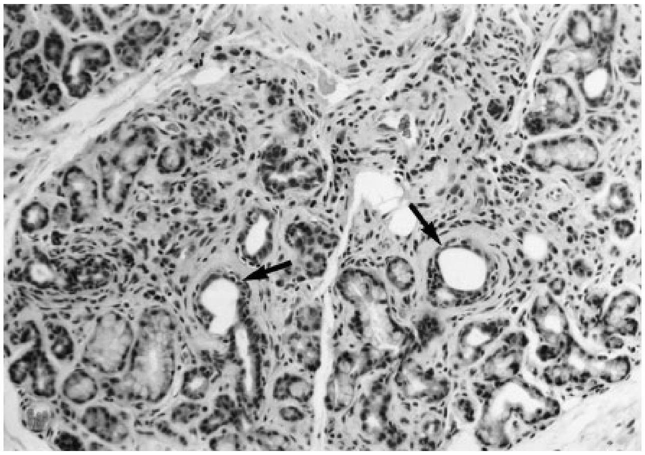


Figure 26.47 Lip biopsy, minor salivary gland. The lymphoplasmacytic inflammatory cells are centered around small ducts. Signs of ductal epithelial cell destruction, irregularity of outline, nuclear stratification and hyperchromatism, and apoptosis (arrows) are similar to GVHD changes in small bile ducts. The glandular interstitium is becoming fibrotic and the acini atrophic, resulting in a sicca syndrome.

edema with effacement of the normal mucosal folds involving the stomach, and entire small and large bowel (Figures 26.49–26.51). Endoscopic analysis provides a visible survey of the entire surface mucosa and facilitates GI biopsies for culture and histologic analysis. The endoscopic appearance of GI GVHD varies from diffuse edema and erythema including friable mucosa to focal erosions, ulcers, and sloughing of the epithelial mucosa. There are multiple studies that show a significant discordance between endoscopy and histology attributed to sampling bias and sensitivity. The gross appearance of GI GVHD usually involves edema of the intestinal wall that is readily apparent to the radiologist who performs imaging tests of the intestine and to the endoscopist who can also see mucosal erythema, friability, and microerosions. However, edema and erythema are not often appreciated by the pathologist, whose specimens are limited to tissue above the muscularis mucosa and dehydrated during tissue processing (Figures 26.49 and 26.50). The endoscopic diagnosis of GVHD is based on findings that the pathologist cannot appreciate [92,118] (Figure 26.51). Likewise, the diagnosis of GVHD by histology is based on microscopic findings and isolated apoptotic cells that are not detected during endoscopy. Multiple studies have reported a lack of visible endoscopic lesions in many cases that were diagnosed as GI GVHD by histology [78]. It is important to emphasize that the endoscopist can visually inspect large areas of GI mucosa and the most severely involved lesions may not be amenable to biopsy. On the other hand, histologic examination is limited to a small tissue biopsy and is therefore affected by sampling bias. Hence the gold standard for GI GVHD may not be histology alone [119,120], but a composite of clinical signs,

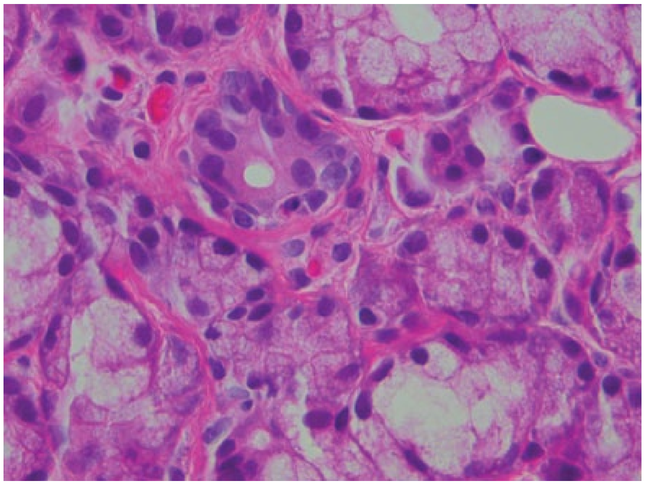


Figure 26.48 GVHD in a salivary gland biopsy from a patient with aplastic anemia day 81 after HCT on with apoptotic cells in the intercalated ducts and architectural distortion with nuclei disarray. (For a color version of this figure, please see the color plate section.)

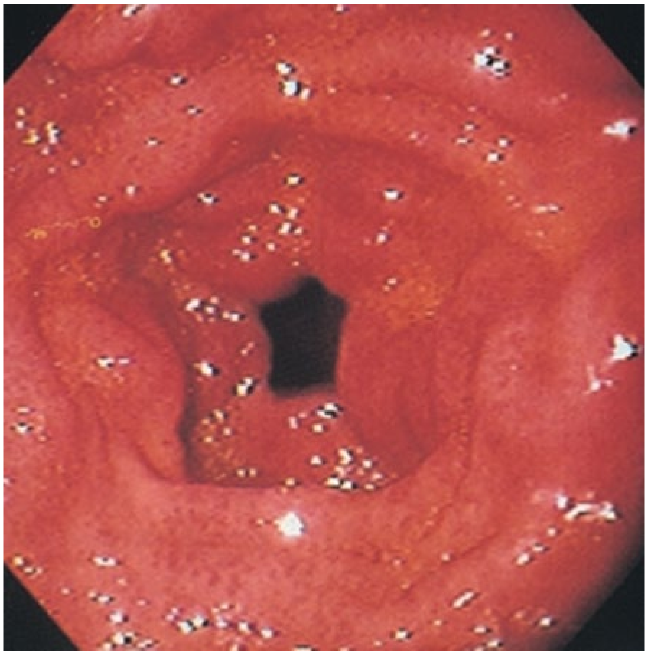


Figure 26.49 Endoscopic appearance of acute GVHD in the stomach, day 32. The pyloric channel is in the center of the picture. The mucosa of the gastric antrum is edematous, reddened, and friable. (For a color version of this figure, please see the color plate section.)

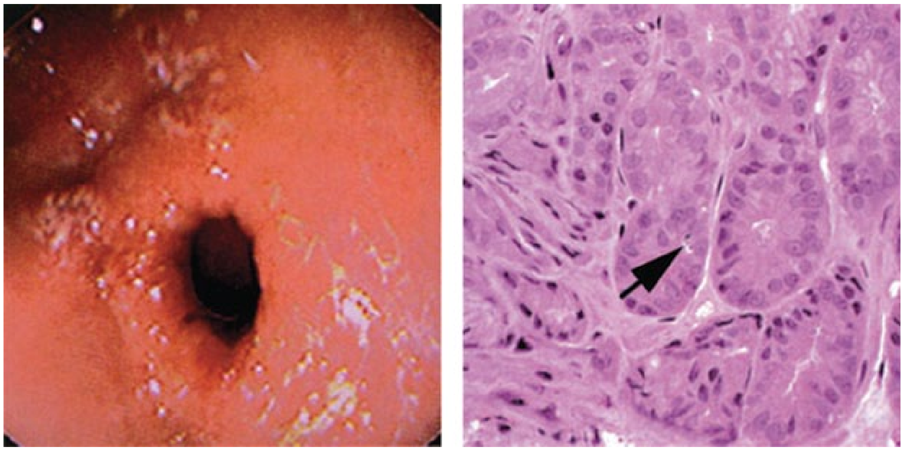


Figure 26.50 Gastric GVHD in which the severe mucosal erythema, edema, and erosion seen endoscopically on the left are more striking that the focal, mild, epithelial cell apoptosis (arrow) in the histological section on the right. Although a lymphocytic infiltrate is absent, apoptosis in multiple crypts is consistent with graft-versus-host activity. Inflammation may have been partially controlled by immunosuppressive therapy. Source: Ponec et al. 1999 [118]. Reproduced with permission of Elsevier. (For a color version of this figure, please see the color plate section.)

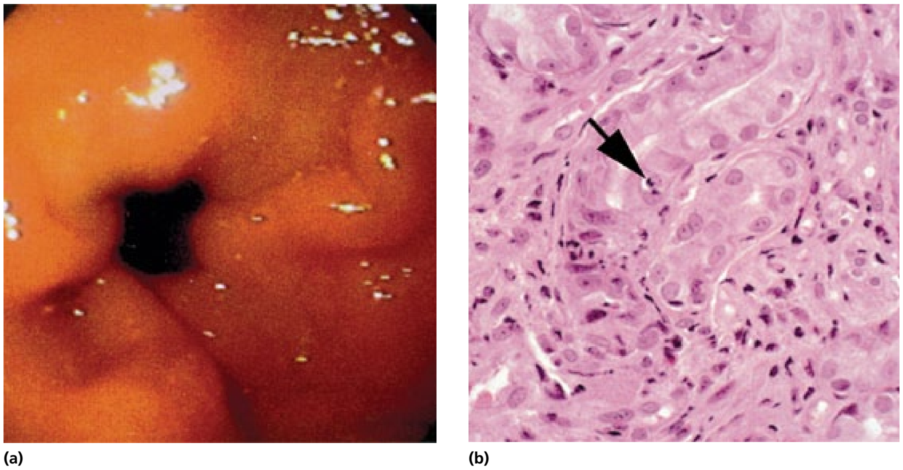


Figure 26.51 Gastric GVHD in which the antral mucosa is endoscopically normal aside from edema (left). With air insufflation through the endoscope the antrum did not distend and there was no motor activity. Biopsy of such a bland area can be diagnostic. In this case, as shown in the photomicrograph on the right, there was epithelial cell apoptosis (arrow) and moderate lymphocytic infiltration diagnostic for GVHD. Source: Ponec et al. 1999 [118]. Reproduced with permission of Elsevier. (For a color version of this figure, please see the color plate section.)

biomarkers, endoscopic appearance, histologic findings, and absence of infection by culture and histology [4,6,78].

The histologic diagnosis of GI GVHD is based on the identification of apoptotic epithelial cells that are usually found in the regenerative or stem-cell areas (i.e. the neck region of the gastric glands, the basal layer of esophagus, and the lower lateral basilar portion of the crypts in the small and large bowel) (Figure 26.27). Severe cases with frequent apoptotic cells and glandular disruption are easily identified with good inter-observer correlation. However, the minimal histologic criteria for GI GVHD have not been established and there is considerable debate on the number and frequency of apoptotic cells required for the diagnosis. There is good intra-observer identification of apoptotic cells but significant discordance on the diagnostic interpretation, especially in mild cases of GI GVHD. In our practice, we usually set a minimal threshold of one apoptotic epithelial cell per tissue fragment for a diagnosis of GI GVHD once other possible etiologies including conditioning, infection, and drug reaction have been eliminated. Individual apoptotic surface epithelial cells are ignored, interpreted as non-specific changes attributed to other etiologies including bowel preparatory regimens.

The location, timing, and frequency of endoscopic biopsies are highly dependent on institutional bias and past experience with complications. Usually patients with gastric GVHD present with nausea and vomiting and those with colonic GVHD present with diarrhea. Some institutions target the endoscopy to the patient's symptoms. However, one study showed that gastric biopsy provided the highest diagnostic yield even when the predominant symptom was diarrhea [120]. The combination of rectal biopsy and gastric biopsy provided the highest diagnostic yield for GVHD [121]. To avoid potential complications associated with upper endoscopic biopsies, a pediatric study advocated the use of the less invasive rectal biopsy alone as an initial survey for GVHD [122]. In general, upper endoscopic biopsies are more difficult to interpret than rectal biopsies because of the disagreement on the minimal diagnostic threshold criteria for apoptosis and the overlap with reactive and inflammatory conditions. There are also differing opinions on whether a biopsy from the duodenum [59], gastric fundus [123], or antrum [120] provides the greatest sensitivity. However, in our experience, GI GVHD is a patchy disorder and can frequently be limited to isolated gastric or colonic involvement, necessitating additional endoscopy if a more limited study is negative.

Spectrum of gastrointestinal GVHD

The sequence of damage in gut GVHD has been conceptualized into a four-tier grading system that is a modification of the original Lerner histologic grading schema [91]:

1. *Grade I:* Individual cell necrosis in basal and lateral crypts, with sparse lymphocytic infiltrate (Figure 26.50–26.53). The minimal criterion is a single or rare apoptotic crypt cell, referred to as an exploding crypt cell. These alterations may involve only a few scattered crypts and can be missed unless the specimen contains properly oriented biopsies with multiple serial sections. In the grade I lesion, the exploding crypt cell may not have an accompanying lymphocytic infiltrate. Histologic grade I does not mean that the entire gut has the same grade; it is not uncommon for gastric and/or rectal biopsy histology to underestimate the severity of GVHD in the midgut, particularly the ileum.
2. *Grade II:* An apoptotic crypt abscess involves at least 1/3 of the glandular circumference. The dilated crypt is lined by thin epithelium devoid of mucin, and infiltrated by lymphocytes. The crypt abscesses contain apoptotic debris. A scattering of lymphocytes, neutrophils, and eosinophils may be present in the interstitium, crypt walls, and crypt abscesses. They may be present in considerable numbers, especially after withdrawal of immunosuppression in a fully engrafted recipient (Figure 26.54; see also Figures 26.27 and 26.53).
3. *Grade III:* Crypt loss resulting in stretches of mucosa devoid of crypts with some focal ulceration (Figure 26.55).
4. *Grade IV:* Mucosal denudation. Widespread ulceration with loss of the surface epithelium and crypts (Figure 26.56; see also Figures 26.57 and 26.58). This results in an empty lamina propria devoid of surface epithelium and crypts. There may be granulation tissue and scattered areas of hemorrhage. In grades II–IV, the dilated small vessels in the lamina propria are lined by reactive-appearing endothelium and infiltrated by mixed cell populations. Multiple representative samples should be obtained and correlated with the endoscopic locations since areas of mucosal destruction and ulceration can be adjacent to large stretches of regenerative mucosa with little or no apoptotic activity.

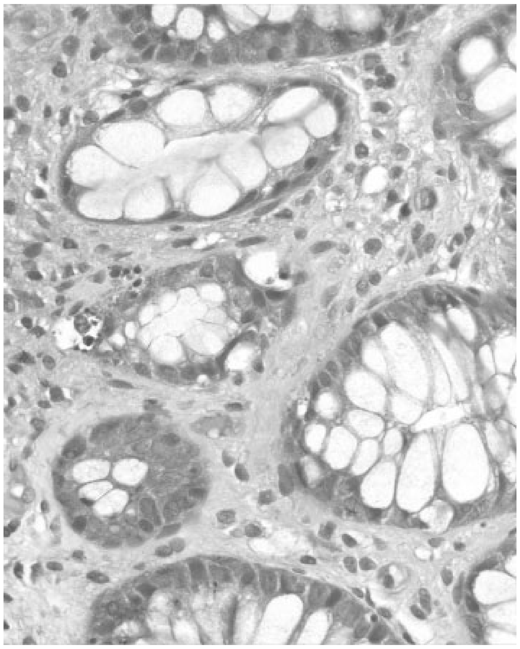


Figure 26.52 Colonic GVHD, grade I. A single crypt displays individual epithelial cell apoptosis (“exploding crypt cell”).

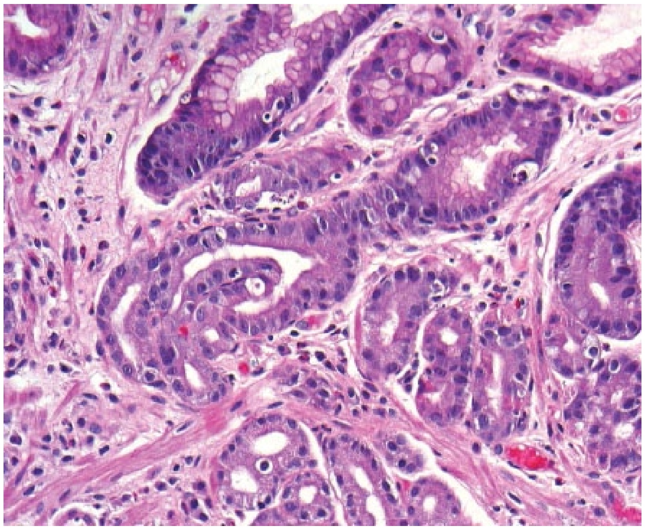


Figure 26.53 Gastric GVHD with architectural disarray and edematous antral mucosa. There are numerous intraepithelial lymphocytes infiltrating glands which also demonstrate reactive atypia. Apoptosis is widespread along with segmental disruption of the glands. (For a color version of this figure, please see the color plate section.)

Although the grading system is a useful descriptor, it is not intended to reflect severity since all four grades may be associated with extensive involvement and severe symptoms. Grade I covers a wide spectrum of damage, ranging from rare individual exploding crypt cells to widespread apoptosis, both single and confluent within a single crypt (Figure 26.53). In practice, identifying the minimal diagnostic threshold for grade I can require analyzing many serial sections. Focally enhanced gastritis, a focal mixed lympho-histiocytic infiltrate with or without neutrophils surrounding a small group of glands without apoptosis, can be associated with

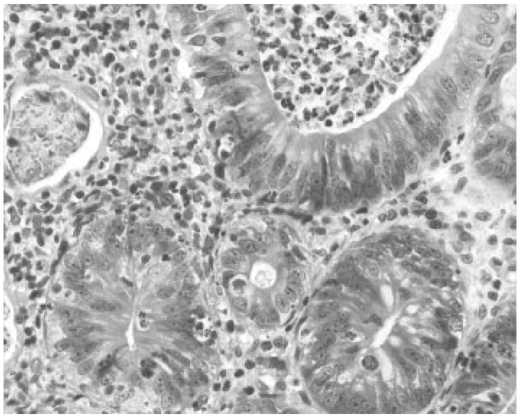


Figure 26.54 Colonic GVHD, grade II. Destruction of crypts with formation of crypt abscesses containing apoptotic debris.

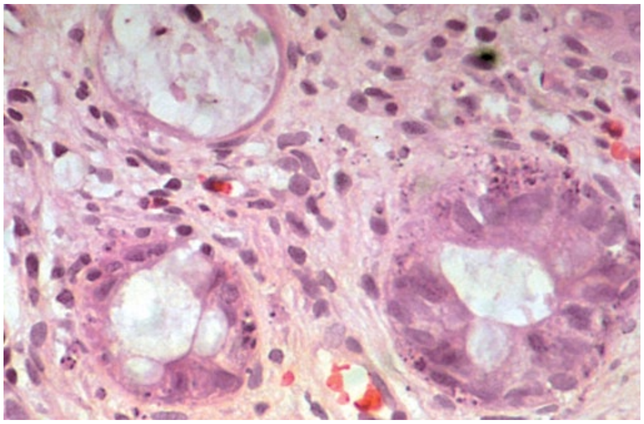


Figure 26.55 Rectal biopsy with GVHD, day 53. Three mucosal crypts display varying degrees of damage. The crypt on the right shows many apoptotic cells. More extensive cell damage is present in the crypt on the lower left. The upper crypt has lost all enterocytes. Note infiltrating lymphocytes in the lower left crypt associated with nuclear debris characteristic of apoptosis. Source: reproduced with permission of Dr. Robert C. Hackman. (For a color version of this figure, please see the color plate section.)

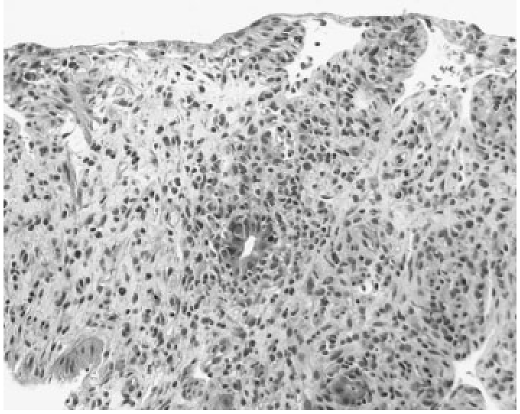


Figure 26.56 Grade IV GVHD in a day 48 duodenal biopsy. Mucosal ulceration with loss of villous epithelium and subtotal destruction of crypts. The features that indicate the cause as GVHD are the two small remaining degenerative crypts showing apoptosis and infiltration by lymphocytes and eosinophils. Concurrent biopsies from the stomach and colon had less severe damage: focal grade III and grade II changes, respectively.

GVHD but, by itself, is non-specific [124]. The cumulative damage in grade II and III GVHD is typically widespread, whereas in grade IV there may be no crypts left to demonstrate the apoptotic destruction of the earlier grades and is therefore difficult to monitor continued activity (Figure 26.56). It is important to realize that all four grades may occur in different biopsies from concurrent samples. In contrast to the colon and small bowel, the stomach rarely develops diffuse grade IV changes. Grade IV GI GVHD is slow to heal and often refractory, even with secondary immunosuppressive treatment or aggressive surgical management of obstruction (Figure 26.57). The inability to re-epithelialize, even with prolonged secondary treatment for gut GVHD, may result from a loss of the mucosal stem cells (Figure 26.58). In a recent study, Takashima et al. demonstrated that stimulation of the ISCs with a Wnt agonist (R-Spo-1) protected the cells against GVHD damage and enhanced restoration of the injured intestinal epithelium [63]. Since GVHD is a patchy disorder, serial colonoscopic biopsies obtained to monitor therapeutic response and epithelial regeneration frequently show discordant results attributed to various stages or activity and repair. Rarely, refractory gut GVHD may develop collagenous deposits within the lamina propria, submucosa, and serosa or a segmental intestinal stenosis with ulceration and fibrosis confined to the mucosa and submucosa (Figures 26.57 and 26.59).

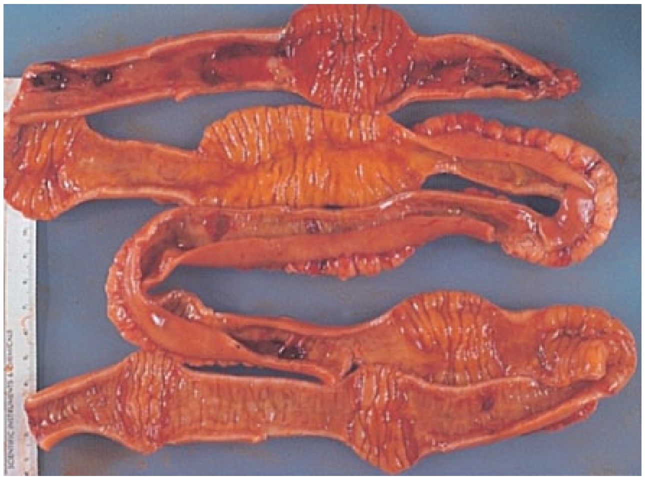


Figure 26.57 Surgical resection of severe long-standing intestinal GVHD, day 207. Scarred, ulcerated mucosa alternates with dilated areas of mucosal regeneration. The ulcerated portions demonstrate complete loss of epithelium and severe submucosal fibrosis. There is very high risk of invasive fungal or bacterial infection. Some patients have responded well to intestinal resection for localized GVHD. (For a color version of this figure, please see the color plate section.)

Differential diagnosis

One reason why there is a lack of consensus on the minimal diagnostic criterion for gut GVHD is because low levels of apoptosis are not specific to GI GVHD and can occur with various medications, including non-steroidal inflammatory inhibitors, mycophenolate mofetil (MMF), proton pump inhibitors [53,125], and with some infectious and/or reactive causes such as cryptosporidiosis and phosphate enemas [119]. In general, reactive gastropathy does not produce epithelial apoptosis. The tissue biopsies for GVHD evaluation must come from flat normal-appearing mucosa since

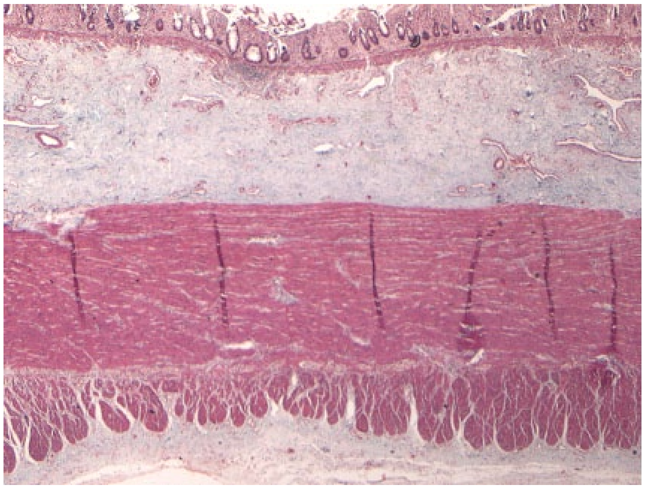


Figure 26.59 Collagenous colitis with long-standing refractory extensive chronic GVHD. The thickened bowel has extensive deposition of collagen in the submucosa, serosa as well the lamina propria. (For a color version of this figure, please see the color plate section.)

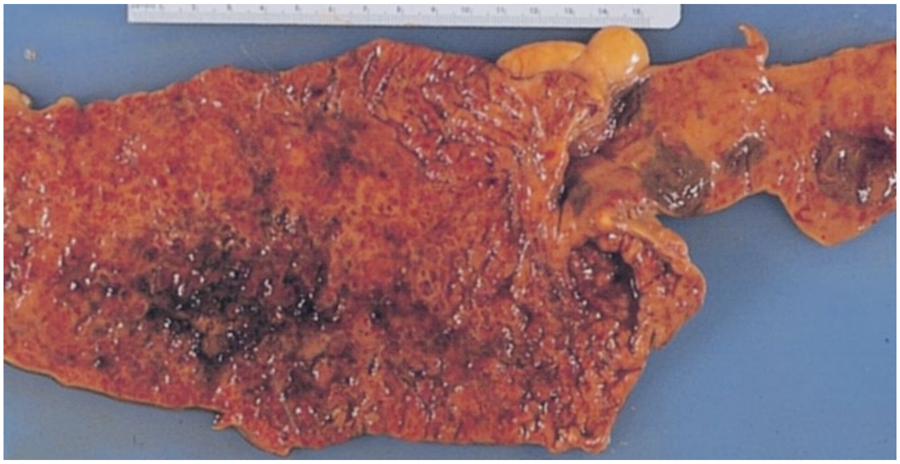


Figure 26.58 Severe GVHD of ileum and cecum, day 80. The mucosal lining and folds are replaced by a friable, beefy red, diffusely ulcerated lamina propria. The numerous exposed capillaries ooze blood and serum, a major cause of morbidity from intestinal GVHD. The endothelial cells and fibroblasts within the lamina propria are often infected with CMV, as was true in this patient. (For a color version of this figure, please see the color plate section.)

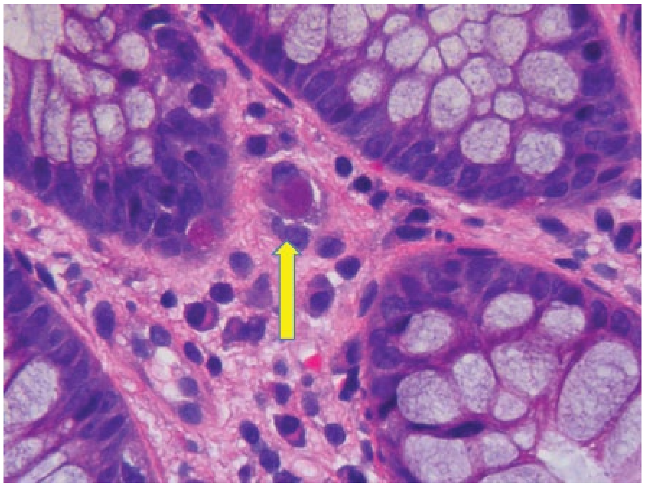


Figure 26.60 CMV infection in a patient with T-cell prolymphocytic leukemia from day 516 after HCT with chronic diarrhea. The yellow arrow indicates the classic large eosinophilic inclusion in a stromal cell. (For a color version of this figure, please see the color plate section.)

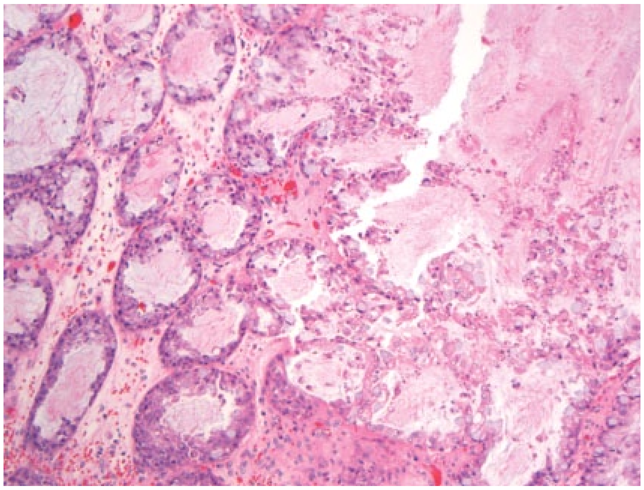


Figure 26.61 Fatal pseudomembranous colitis. An acute abdomen developed 15 days post-transplant. The degenerative colonic crypts were covered with a cap of mucin admixed with sloughed cellular debris containing large colonies of bacteria consistent with *Clostridium* sp. (For a color version of this figure, please see the color plate section.)

hyperplastic polyps and adenomas frequently have scattered apoptotic epithelial cells due to the increased epithelial proliferation. The differential diagnosis of grade II–IV changes includes cytomegalovirus (CMV) enteritis that produces a range of changes from shallow circular ulcers to an inflammatory pseudotumor mass involving multiple layers of the gut (Figure 26.58). The viral inclusions typically involve the endothelium but may also affect stromal cells and gut epithelium (Figure 26.60). There is a long-standing controversy as to whether the presence of CMV enteritis (a well-known cause of apoptosis in gut biopsies) precludes a diagnosis of GVHD [119]. Studies have addressed this issue using IHC and in situ hybridization for CMV and caspase 3 IHC for GVHD [76,126]. In CMV enteritis, apoptosis was largely confined to the cells with immunoreactive CMV antigens, whereas apoptotic cells were much more numerous in GVHD. These studies concur with the NIH chronic GVHD consensus [53] that multiple apoptotic crypt cells remote from CMV infected cells indicate the co-presence of GVHD. Antibiotic-related pseudomembranous colitis is related to the toxin produced within the colonic lumen by *Clostridium* species, especially *Clostridium difficile*. If severe, it can produce ischemic changes with apoptosis. Pseudomembranous colitis is distinguished from GVHD by the characteristic superficial pseudomembranous cap of neutrophils, mucin, and necrotic cells with or without bacterial rods and by a positive assay for the *C. difficile* toxin (Figure 26.61).

The immunosuppressive drug MMF can produce colitis and even upper gut damage [127,128] with focal ulcerations, marked apoptosis, and intense acute and chronic inflammation. In practice, the distinction of MMF colitis from GVHD is often established by improvement in bloody diarrhea after drug withdrawal [127]. There are insufficient data on whether MMF often produces lesions in the upper gut. Immune checkpoint blockade with antibodies that target cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and the programmed cell death protein 1 pathway (PD-1/PD-L1) provides a novel way to augment antitumor immunity by effector T-cells. Immune related adverse events arise triggering inflammatory conditions in the skin and colon that mimic GVHD, with frequent apoptotic epithelial cells, ulceration, and loss of colonic mucosa [129,130,131]. However, a dose escalation trial using ipilimumab to treat relapsed malignancy after HCT demonstrated induction of a graft-versus-tumor response without stimulating significant GVHD [132].

In 2011, Herrera [133] described a newly defined syndrome cord colitis (CCS) consisting of a colitis confined to cord blood recipients with late-onset secretory diarrhea, absence of GVHD or detectable infections with mucosal features of chronicity [134] and some cases with multicentric granulomatous inflammation that was responsive to antibiotics but not immunosupppression. Two subsequent studies disproved the CCS as a distinct entity since the histologic features of chronic mucosal damage or granulomas were not restricted to cord blood recipients [135,136].

Esophageal GVHD

Like most squamous epithelia, that of the esophagus may be involved with GVHD. The diagnosis of acute GVHD presents considerable diagnostic difficulty since a variety of conditions, including acid peptic reflux and infections, can produce mucosal damage. Unless there is a combination of lichenoid interface changes with apoptosis along the basilar portion of the mucosa, the diagnosis of esophageal GVHD should be made with some qualification. Accurate diagnosis requires special stains and often IHC studies for microorganisms. Bacterial, viral, and fungal cultures should be obtained endoscopically from lesions suggestive of infection (Figures 26.62 and 26.63).

Autologous GVHD

Autologous GVHD was first described by Hood et al. as an autoimmune syndrome similar to acute GVHD that occurred in patients receiving an autologous stem-cell transplant [137]. Autologous GVHD or autoaggression syndrome is frequently a self-limited and mild disorder that responds well to therapy; nearly all patients show durable responses to one or two courses of prednisone. Traditionally, autologous GVHD is most commonly seen in the skin, although one study reported that 13% of autografted recipients developed a GI syndrome that was both clinically and histologically indistinguishable from acute GVHD occurring in allogeneic recipients [138]

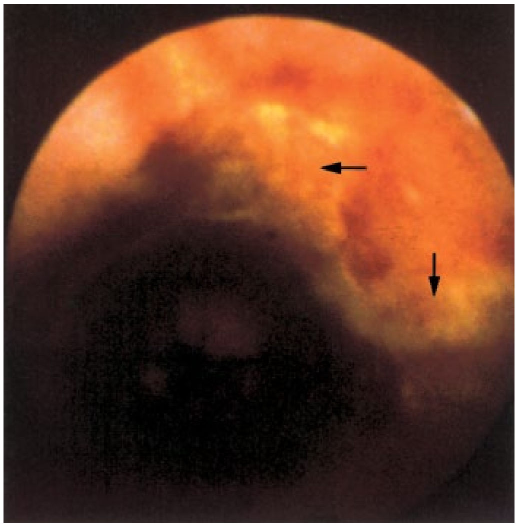


Figure 26.62 Endoscopic appearance of CMV esophagitis, day 61. A large, shallow ulcer with serpiginous, reddened borders is seen in the distal esophagus. The ulcer base (arrows) has a yellow, reticulated appearance. (For a color version of this figure, please see the color plate section.)

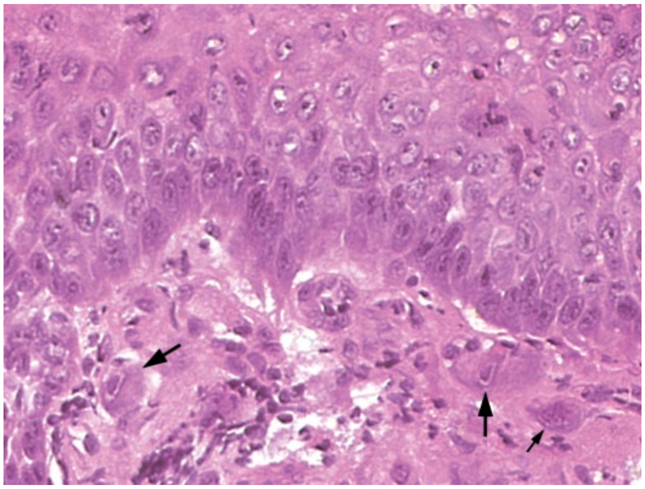


Figure 26.63 Esophageal biopsy showing CMV infection, day 61. Beneath the epithelium are two cytomegalic cells (large arrows), each containing a single, large, oval intranuclear inclusion surrounded by a clear halo (Cowdry type A). A third cell (small arrow) is probably also infected but is morphologically non-diagnostic. It would presumably be positive by immunocytochemistry and in situ hybridization techniques. Studies of cell morphology and also viral DNA and antigen expression indicate that the esophageal squamous epithelium is never infected with CMV. (For a color version of this figure, please see the color plate section.)

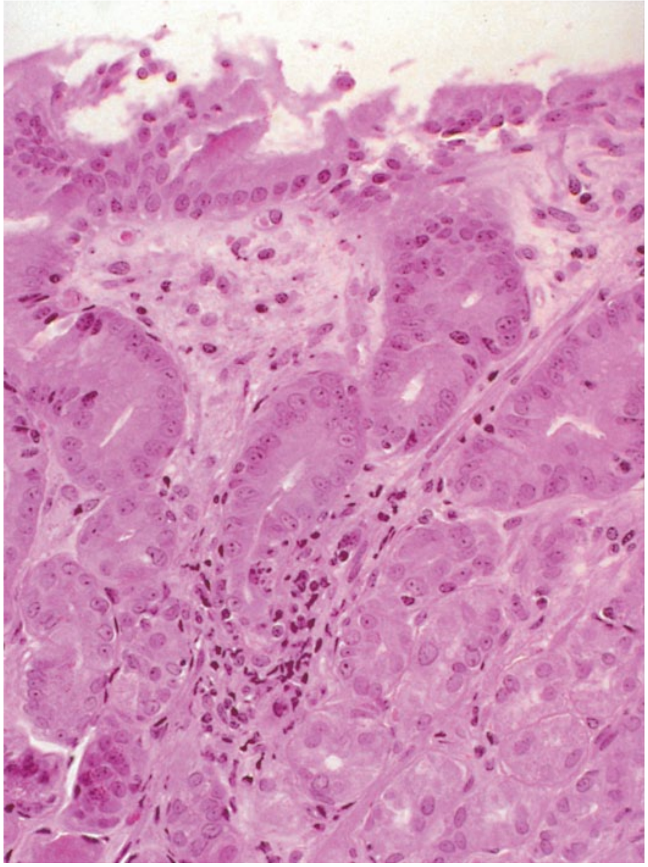


Figure 26.64 Autologous gastric GVHD or lymphocytic gastritis. The focal mononuclear inflammatory cell infiltration of the lamina propria and mucosal crypt associated with crypt epithelial cell apoptosis is histologically indistinguishable from GVHD in an allograft recipient. This inflammation may develop after autologous or syngeneic BMT if tolerance to self-MHC antigens is not re-established and autoreactive cells are allowed to persist. (For a color version of this figure, please see the color plate section.)

(Figure 26.64). Autologous transplantation is an effective therapy for treating plasma cell myeloma, although relapse remains a major problem. In a recent study, Lazarus et al. reported a cohort of myeloma patients that spontaneously developed autologous GVHD predominantly limited to the GI tract within 1 month after autologous transplant. These patients received high-dose melphalan conditioning that may have contributed to the onset of autologous GVHD [139]. Many clinical studies have been performed to determine whether induced autologous GVHD may also promote an autologous graft-versus-tumor response, but there is as yet no convincing evidence for this effect. The genesis of autologous GVHD is thought to be related to both compromised thymic function resulting in failure to delete autoreactive T cells and the elimination of regulatory T cells in the hematopoietic autograft [140].

Hepatic GVHD

The liver is a major target of allogeneic GVHD [26], and occasionally also autologous GVHD [141]. With improvements in clinical management, the overall prevalence of severe grade III–IV hepatic GVHD has markedly decreased, from 11.9% in 1993–1997 to 2.1% in 2003–2007 [7]. Liver involvement is heralded by a gradual rise in bilirubin and alkaline phosphatase and aminotransferase enzymes. It typically follows acute cutaneous or intestinal GVHD; but can present as the sole manifestation of GVHD. The initial elevation in liver tests is likely mediated by cytokine induction of the Fas/Fas ligand system leading to bystander death of hepatocytes. In both the experimental and clinical settings, histopathologic and ultrastructural studies demonstrate that the small interlobular bile ducts are the preferential targets of the alloimmune reaction (Figure 26.65; see also Figure 26.66). The characteristic dysmorphic interlobular bile ducts of GVHD have a withered, shrunken appearance with a collapsed lumen, vacuolated cytoplasm, and irregular outlines. Individual or groups of epithelial cells are flattened and often missing

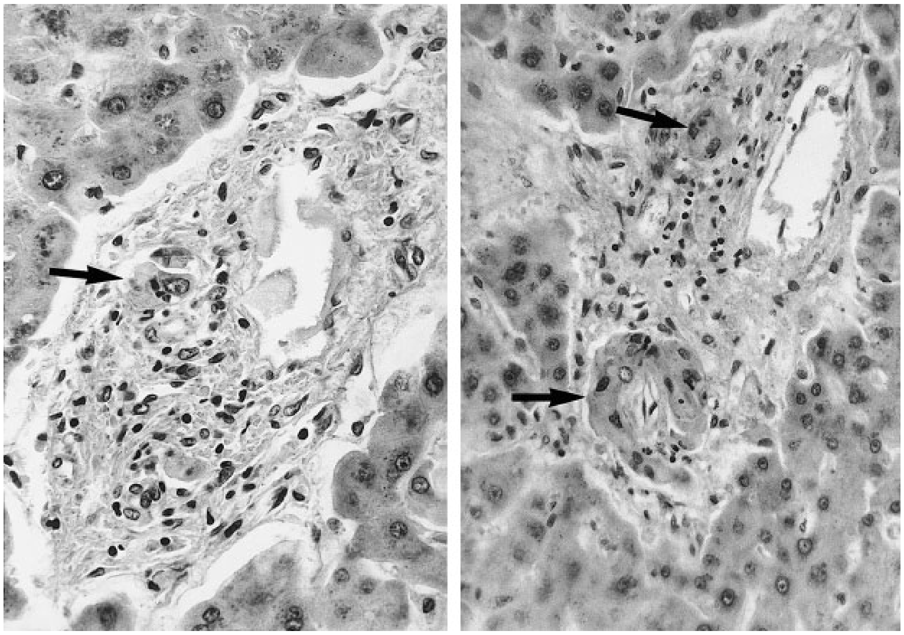


Figure 26.65 Hepatic GVHD, day 53. Despite the minimal inflammation within the portal space, the bile ducts (arrow) are reduced to degenerative cytoplasmic masses with a few remaining hyperchromatic nuclei.

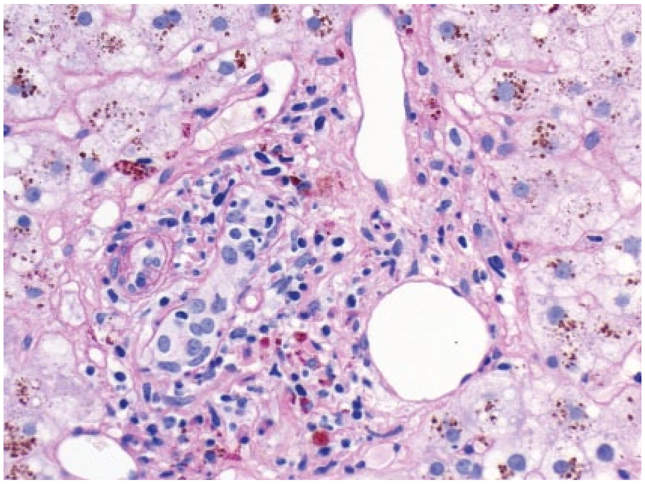


Figure 26.66 Hepatic GVHD. The PAS-stained section highlights a damaged interlobular bile duct that is infiltrated by lymphocytes. The nuclei vary in size and are irregularly arranged. The syncytium-like segment reflects nuclear loss. (For a color version of this figure, please see the color plate section.)

nuclei, creating segments of anucleate eosinophilic cytoplasmic syncytia. Remaining nuclei display atypical features with anisonucleosis, hyperchromasia, and dyspolarity. Lymphocytic ductitis is frequently present in the damaged portions of the biliary epithelium but apoptosis of bile duct epithelium is infrequent.

The histologic interpretation is directly affected by the quality, size, and timing of the sample. Thin core needle biopsies may be difficult to interpret owing to limited sampling of portal areas or crush artifact. The timing of the liver biopsy influences the histologic finding. When liver biopsies to diagnose GVHD are obtained soon after the onset of liver dysfunction, the characteristic bile duct changes may be absent or affect a minority of portal spaces. In a study that included serial liver biopsies, the initial biopsies taken at the onset of symptoms failed to show the characteristic bile duct damage, whereas follow-up biopsies taken 10 days later showed histologic signs of hepatic GVHD [142]. The amount and extent of the inflammatory component in liver GVHD are dependent on the degree of immunosuppressive treatment. The portal infiltrate is typically lymphocytic but may include a minor component of loosely scattered neutrophils, eosinophils, monocytes, and plasma cells. In a typical biopsy from a patient on therapeutic levels of immunosuppressive agents, inflammation is typically mild to scant, even with extensive bile duct damage (Figure 26.65).

Hepatic GVHD is no longer classified as acute or chronic [53,143]. The histology is largely unrelated to the period when the GVHD arose but is rather a reflection of the duration of active liver GVHD, the anti-inflammatory effects of immunosuppression, and the anticholestatic effect of ursodeoxycholic acid. A late clinical presentation of isolated liver GVHD with an acute hepatitic onset may develop as the solitary manifestation of GVHD, following tapering or cessation of immunosuppressive therapy or after donor lymphocyte infusion. The "hepatic onset of GVHD" is characterized by marked elevation of aminotransferase enzymes with prominent periportal inflammation that may include plasma cells and a lobular hepatitic component with abundant necroinflammatory activity (pyknotic shrunken hepatocytes, acidophilic bodies) [144]. In some cases, the bile duct damage may be less conspicuous owing to the marked inflammation and hepatocyte necrosis related to the accompanying cytokine storm (Figures 26.67 and 26.68).

The histologic threshold for diagnosing liver GVHD rests on qualitative rather than quantitative changes, given the enormous variability in the size and quality of liver biopsies. Special stains, including trichrome, periodic acid–Schiff (PAS)/diastase, reticulin, iron and immunohistochemical stains for cytokeratin 19 or 7, should be included in the workup. With prolonged liver GVHD, bile duct destruction is best appreciated by stains other than H&E (e.g. cytokeratin 7 or 19, trichrome, or PAS) (Figure 26.66). Hepatic

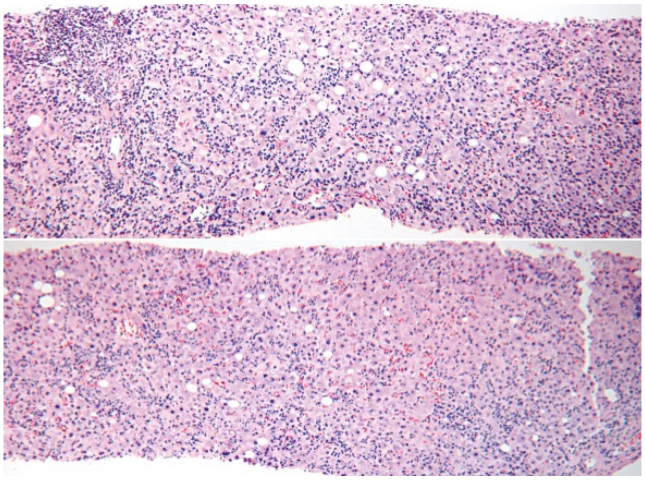


Figure 26.67 Acute hepatitic onset of GVHD at 10 months post-transplant. Three months after tapering off immunosuppression, the aminotransferases rose to 2000 u and alkaline phosphatase three times normal with normal bilirubin. This biopsy 1 was performed 26 days from the onset and shows an extensive lobular hepatitis with intense lymphocytic inflammation of sinusoids and portal spaces and hepatocellular necrosis. (For a color version of this figure, please see the color plate section.)

GVHD produces a variety of cholestatic changes, including canalicular bile plugs, hepatocyte ballooning, and dropout of hepatocytes in the perivenular zone along with a scattering of lymphocytes and collections of pigment-laden macrophages (Figure 26.68). Rarely, pseudo ground-glass PAS diastase-resistant hepatocytes develop from accumulation of abnormal glycogen [145,146]. Frequently, there is some degree of ductular reaction, a proliferation of small ductules along the periphery of the portal space that is common in inflammatory liver diseases. In addition to being a reparative response to duct injury, the proliferated ductules also appear to become a secondary target of liver GVHD because the destructive lymphocytic ductitis and cytologic changes in the ductules are similar to those in the damaged interlobular bile ducts. By analogy, in the immune mediated cholangiopathy of primary biliary cirrhosis, proliferated ductules and mature cholangiocytes displaying aberrant expression of I-CAM-1 were found in close association with lymphocytes expressing LFA-1, suggesting a similar lymphocytic targeting of the bile ductile epithelial cells [147]. A particularly striking form of cholangiolar cholestasis with dilated periportal bile-filled ductules occurs in patients with concomitant intestinal GVHD (Figure 26.69; see also Figure 26.70). This resembles cholangitis lenta associated with septicemia and results from the coexisting influence of gut GVHD, showering the liver with endotoxin which,

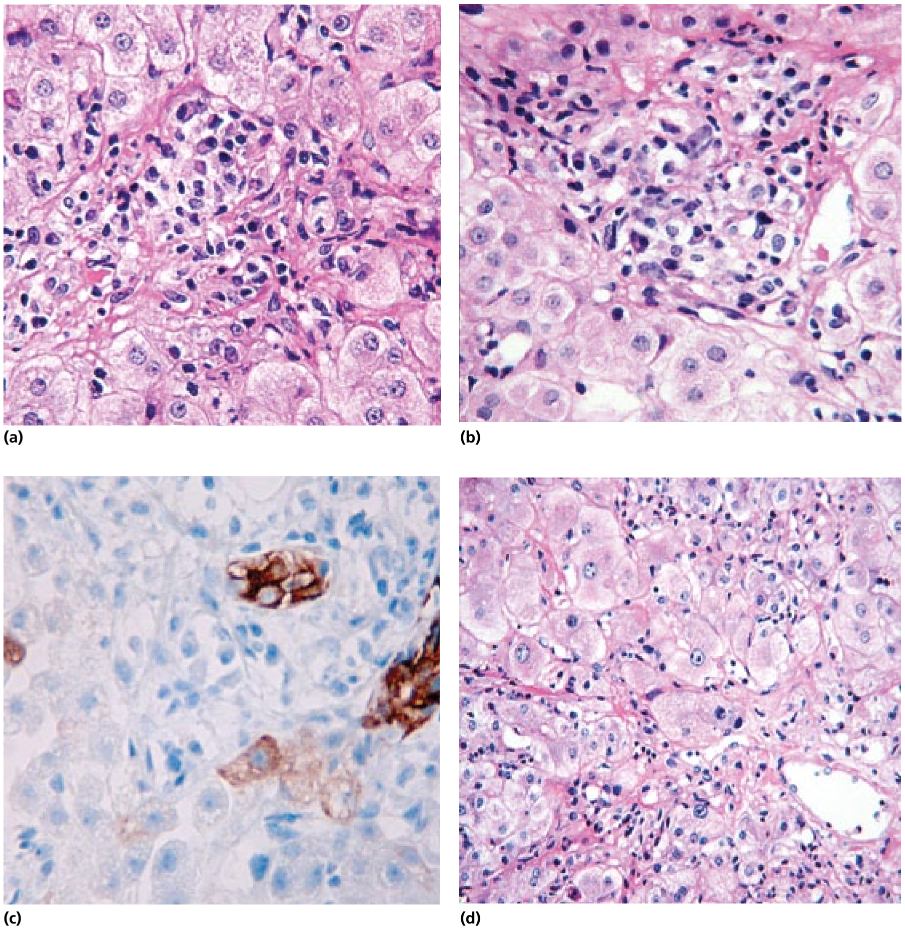


Figure 26.68 This biopsy 2 was done 1 month later (see Figure 26.67) when the total bilirubin was 30mg/dL. Images (a) and (b) show a lymphoplasmacytic portal infiltrate which obscures the damaged interlobular bile ducts. In (c), immunostaining with cytokeratin 7 demonstrates that damaged bile ducts are still present. In (d), there is perivenular (zone 3) cholestasis. Hepatocytes show ballooning, and there is focal dropout associated with a patchy lymphocytic infiltrate. The patient made a full recovery after immunosuppressive treatment for GVHD was initiated. (For a color version of this figure, please see the color plate section.)

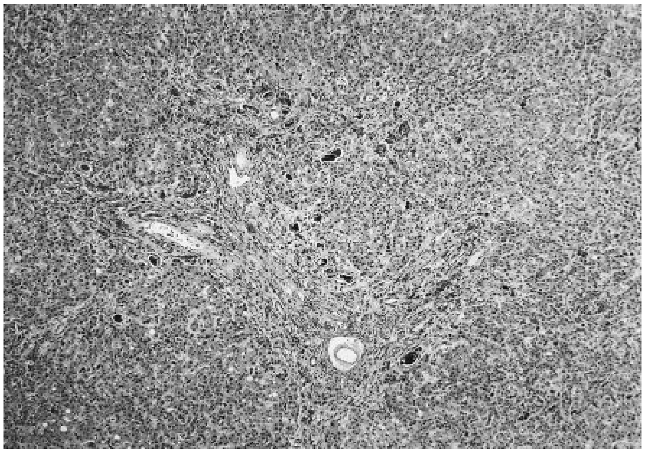


Figure 26.69 Hepatic GVHD, day 56. There is pronounced hepatocellular and cholangiolar cholestasis with portal-to-portal bridging and parenchymal collapse. Cholangioles have proliferated along the limiting plate.

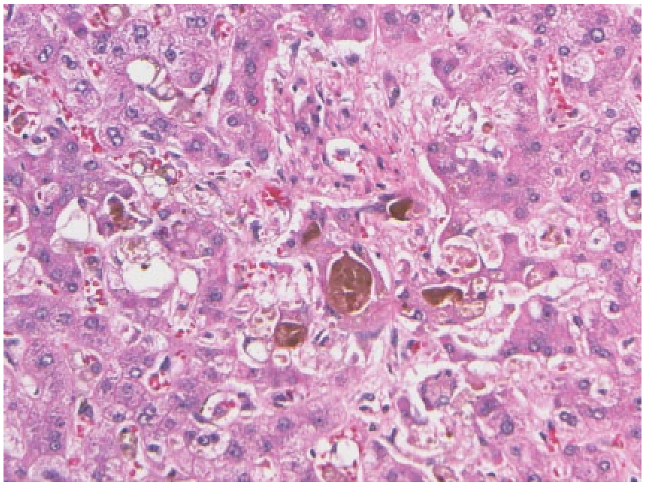


Figure 26.70 Hepatic GVHD with severe cholangitis lenta-like cholangiolar hepatocellular cholestasis. The interlobular bile duct is no longer recognizable. A ductular reaction with large bile-filled cholangioles lies along the periphery of the enlarged irregular portal space. The adjacent hepatocyte cords display marked unrest, disarray, and cytoplasmic ballooning. (For a color version of this figure, please see the color plate section.)

in turn, stimulates TNF-α and Il-6, a promoter of bile ductule proliferation and cholestasis [148]. A consequence of ductular reaction is the development of stellate fibrosis, periportal fibrous septae which extend out into the liver acinus, and rarely bridging fibrosis [26] (Figure 26.69). However, even in prolonged refractory liver GVHD with loss of bile ducts, cirrhosis rarely occurs. The few anecdotal case reports of cirrhosis related to GVHD were tainted by inadequate immunosuppression or the previous high prevalence of hepatitis C virus infection [26]. Nearly all cirrhosis developing in allogeneic recipients after 10 years post-HCT is attributed to hepatitis C virus (HCV) infection [149].

Despite the similarities of liver GVHD to rejection of orthotopic liver allografts, there have been no studies of human liver GVHD showing that the bile duct loss is caused by ischemia related to the loss of peribiliary capillaries or obliterative arteriopathy, as has been described in rejection [150].

Differential diagnosis

The timing, profile, and rate of rise of liver test abnormalities provide good indicators as to the likely diagnostic possibilities (see Chapter 94). The histologic differential diagnosis of liver GVHD includes drug liver injury, nodular regeneration, fatty liver disorder, infection, and malignancy, including PTLD. Sinusoidal obstruction syndrome may overlap with GVHD developing later, but it has different histologic features from GVHD. Most of the candidate antimicrobial drugs for causing liver injury do not produce the typical clinical or histologic patterns seen with GVHD. In one study, the frequently used antifungal agent voriconazole produced mild reversible enzyme elevations in 34% of treated patients [151]. In contrast, trimethoprim/sulfamethoxazole can produce prolonged cholestasis. If the clinical situation permits, it is prudent to discontinue the candidate hepatotoxic drug rather than trying to exclude it as a cause by histology. A common finding in patients biopsied after HCT is marked hepatic iron overload within both hepatocytes and Kupffer cells. Although this has been associated with elevated aminotransferases that could be confused with GVHD [26,152], this association has been challenged because although high blood iron measurements per se may be a cofactor in liver disorders, they are rarely the sole cause of serious liver disorders [153]. The iron overload may be multifactorial: early post-transplant it may result from prior multiple transfusions, but later on it is more often the consequence of anemia of chronic disease leading to increased intestinal iron transport coupled with retention of iron in the liver [154]. Infections, especially viral, are an important diagnostic consideration because failure to treat them can lead to liver failure (Chapter 94). CMV should not be considered in the diagnosis of severe hepatitis or jaundice since it results in mild anicteric hepatitis [155] whose principal histologic findings in immunosuppressed patients are microabscesses that contain some neutrophils. However, in a study of CMV in orthotopic liver allografts, the positive predictive value of the occurrence of a microabscess to predict CMV infection in the liver was very low (19%) [156]. The life-threatening adenovirus and herpes group of viral infections that have declined markedly in frequency are discussed in the Infection section below. Surprisingly, in a study of severe hepatocellular injury [aspartate aminotransferase (AST) >1500 U/L] in 6225 HCT recipients, adenoviral infections were not found among the causes [30]. If the portal spaces are greatly expanded by an infiltrate rich in plasma cells, the differential diagnosis and workup should include PTLD (Figure 26.23) (see Chapter 104), the hepatitic onset of GVHD [144], and, rarely, autoimmune hepatitis [26]. The development of positive tests and/or immunohistologic stains for hepatitis B virus (HBV) and HBC does not exclude the coexistence of GVHD. It may be difficult to distinguish bile duct changes in cases of severe reactivation of viral hepatitis B or C from prolonged GVHD with a ductular reaction. We have observed a handful of cases of fibrosing cholestatic hepatitis (FCH) occurring in patients with chronic HBV and HCV [157] (Figure 26.71). In the presence of the characteristic FCH findings of serpiginous bridging fibrosis with marked ductular proliferation, it is not possible to make a morphologic distinction of the viral-induced changes from concomitant GVHD.

The use of the liver biopsy in monitoring the therapeutic response and prognosis of patients with GVHD should utilize a clinicopathologic approach. Biopsies used to evaluate GVHD should indicate whether the histologic changes show progression in comparison with earlier biopsies and indicate how widespread and complete the significant biliary damage or destruction from GVHD is. When viewed as a single picture in time, the pathologist may not be able to

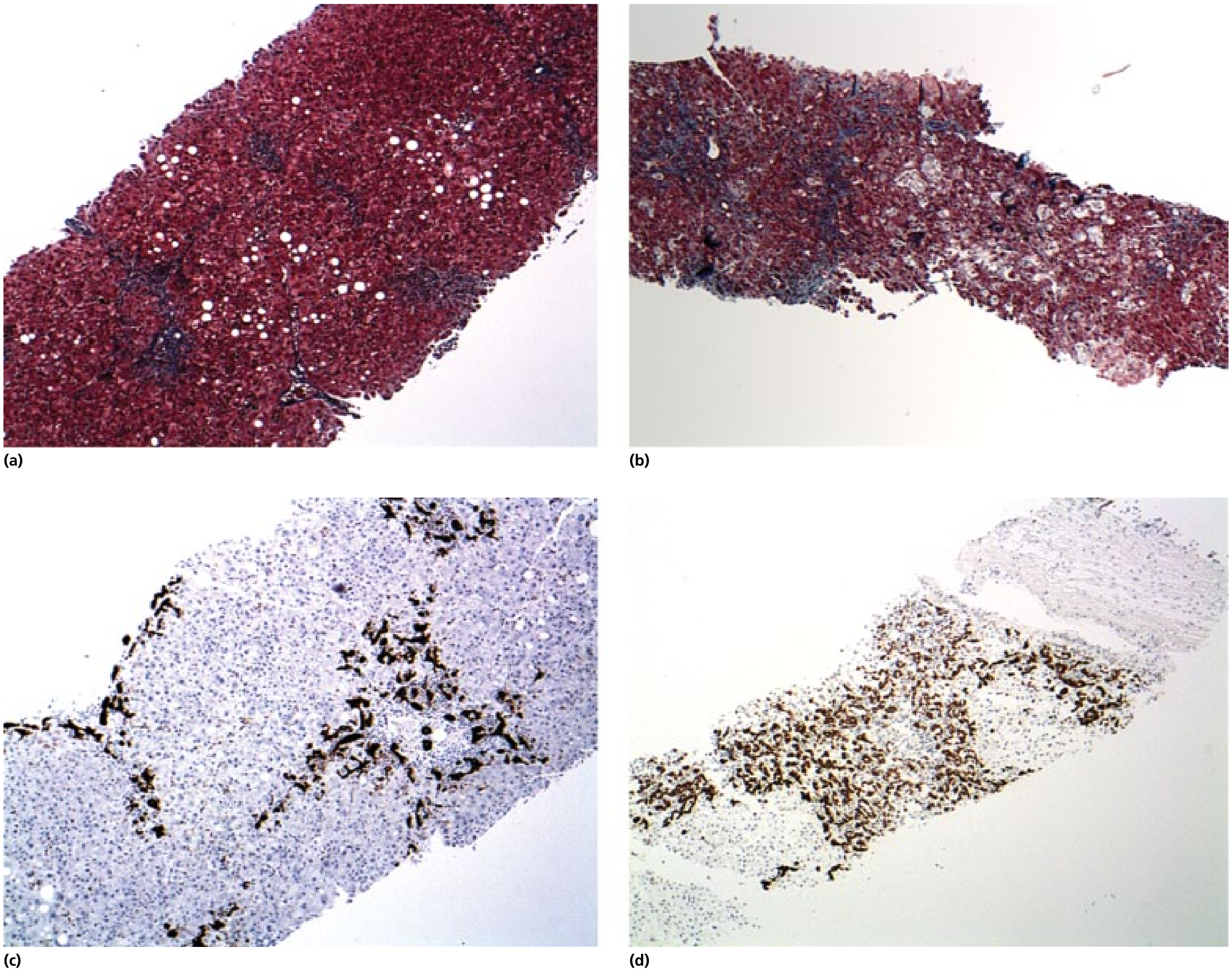


Figure 26.71 Rapidly evolving fibrosing cholestatic hepatitis C. Donor was positive for hepatitis C. Liver biopsies from day 70 [(a) and (c)] and day 88 [(b) and (d)] showing expanding portal fibrosis, hepatocyte necrosis and dropout, and marked bile ductule proliferation seen with cytokeratin 19 IHC [(c) and (d)]. (For a color version of this figure, please see the color plate section.)

determine if the process is inactive or improving since it may take months for the damaged biliary system to recover. Although there is no validated histologic grading system for liver GVHD, evidence-based observations of protracted GVHD indicate that time to recovery from jaundice is proportional to the extent of bile duct destruction.

Pulmonary complications

Pulmonary complications continue to develop in HCT patients and are most frequent in those receiving allogeneic stem cells. In addition to the major role of infection, other mechanisms of injury may include drug toxicity, irradiation damage, GVHD, and cytokine storm. The resulting histologic alterations are often non-specific and may include diffuse alveolar damage, alveolar hemorrhage [158], interstitial and alveolar inflammation, alveolar proteinosis, and pulmonary venous occlusion [159,160] (Figure 26.72). Several of these may coexist in the same patient and interact to cause significant tissue damage and symptoms. Diagnostic evaluation by the pathologist is closely coordinated with the clinical care team. Consideration is given to the underlying major disease and its treatment, possible exposure to infection or toxic damage, prior pulmonary complications, the pre-transplant conditioning regimen,

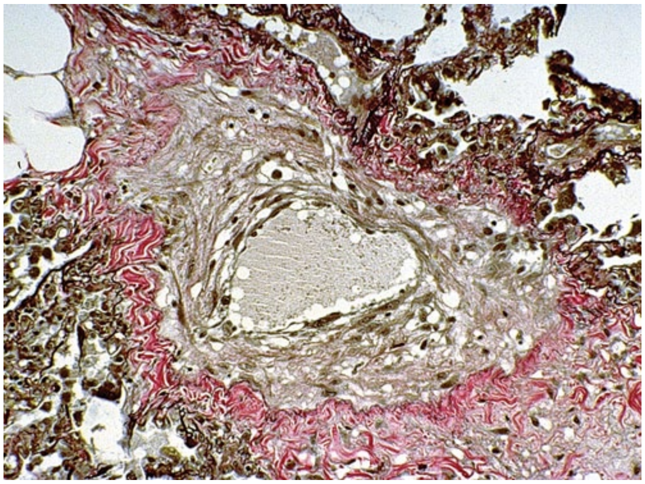


Figure 26.72 Pulmonary veno-occlusive disease (VOD) in a lung biopsy from a patient with pulmonary hypertension 48 days after second allogeneic BMT for acute lymphoblastic leukemia. Loose intimal fibrosis partially occludes the interlobular vein. This complication probably results from chemotherapy and may be more frequent than previously suspected. (Verhoeff–van Gieson elastin stain.) (For a color version of this figure, please see the color plate section.)

the post-transplant interval, the degree of immunosuppression, whether GVHD is present, the results of surveillance cultures and molecular tests for systemic infection, the evolution of signs and symptoms, and the results of imaging studies in addition to pulmonary function tests.

Typically, the first involvement for the pathologist is the immediate analysis of BAL cytospin preparations for possible infection (see the Infection section below) or malignancy. Either negative or positive findings for these two complications will often allow clinicians to focus and thereby simplify therapy. Because the diagnostic yield for BALs is low, tissue is often needed to analyze histological patterns and again carry out detailed morphological, microbiological, and molecular studies for infection. Transbronchial biopsies can usually be obtained safely and may fortuitously demonstrate infection, but they provide too little tissue to interpret and categorize the histological changes confidently. Ideally, the patient is sufficiently stable to undergo video-assisted thoracoscopic surgery (VATS). This provides tissue that the pathologist can immediately dissect under sterile conditions so that focal lesions can be cultured for bacteria, fungi, and viruses; imprints and postoperative frozen sections can be studied using special stains (see the Infection section below), and the most informative areas can be submitted for fixation and permanent sections.

Historically, pulmonary complications have developed in one-quarter to half of allograft recipients and have frequently been fatal. Improvements in antimicrobial prophylaxis and therapy have dramatically decreased the incidence of viral pneumonias, although these can still develop through reactivation of latent infection and also through community exposure. A paramyxovirus, human metapneumovirus (hMPV), infects a significant proportion of transplant patients and can cause fatal pneumonia [161]. The exact nature and extent of its role in pneumonias previously classified as idiopathic are under study. Cytopathic tissue alterations diagnostic for hMPV have not been described, so antibodies for IHC need to be developed.

Interstitial pneumonia syndrome

Approximately 5–15% of allogeneic HCT recipients develop the interstitial pneumonia syndrome (IPS) in which there are multilobar infiltrates by X-ray or scan, symptoms and signs of pneumonia with evidence of abnormal physiology, and absence of active lower respiratory tract infection [162,163]. IPS is a generally severe and often fatal acute process, in contrast to the insidious chronic course of disorders such as usual interstitial pneumonia of Liebow. The histopathology of IPS is variable in both pattern and severity and may include diffuse alveolar damage with hyaline membranes, interstitial lymphocytic infiltration, increased alveolar macrophages, and pulmonary hemorrhage. These cases probably have a multifactorial etiology, which may include cytokine-induced damage [164], toxicity from therapy [165], cell-mediated injury, and occult infection [166,167]. The decreased incidence of IPS after reduced-intensity regimens strongly suggests that conditioning therapy can play a crucial role [168]. Alkylating agents such as cyclophosphamide and busulfan have long been implicated in pulmonary toxicity. There is now increasing evidence that high-dose melphalan [169] and also tyrosine kinase inhibitors such as dasatinib [170] may also cause lung damage. Acute GVHD is a risk factor for IPS [162,171]. However, IPS has not been firmly linked to an allogeneic reaction in humans, although there is evidence in mice that non-bacterial pneumonia is a combined effect of GVHD and radiation [172]. Pulmonary cytolytic thrombi (PCT) is an uncommon complication that presents radiographically as nodules or interstitial infiltrates [173]. Histologically, small- to medium-sized distal vessels are engorged by necrotic cytolytic cellular debris composed of monocytes that include an increased percentage of recipient origin relative to the percentage in the peripheral blood [174].

Pulmonary GVHD

The lung is a chronic GVHD target organ. Bronchiolar epithelium is evidently the major target tissue, although cellular damage is often difficult to identify in the epithelial monolayer. In contrast, there is no well-defined histologic pattern for acute pulmonary GVHD. Allo-reactivity may contribute to the non-specific features of cytokine-mediated damage and inflammation that characterize the IPS [165]. In a recent study, Xu et al. analyzed early histologic changes present in lung biopsies from patients with suspected GVHD and described three histologic patterns of pulmonary GVHD (acute lung injury, organizing pneumonia, and chronic interstitial pneumonia) [175]. All three histologic patterns are characterized by intrabronchiolar T cells, apoptosis, and perivenulitis, which help to distinguish GVHD from infections. The significant association of idiopathic bronchiolitis obliterans organizing pneumonia (BOOP) [also designated cryptogenic organizing pneumonia (COP)] with acute skin GVHD and also chronic GVHD of the gut and oral cavity in a large case–control study [176] indicates that this bronchiolocentric injury pattern with polypoid masses of granulation tissue obstructing the small airways (Figure 26.73) could represent a transition between acute

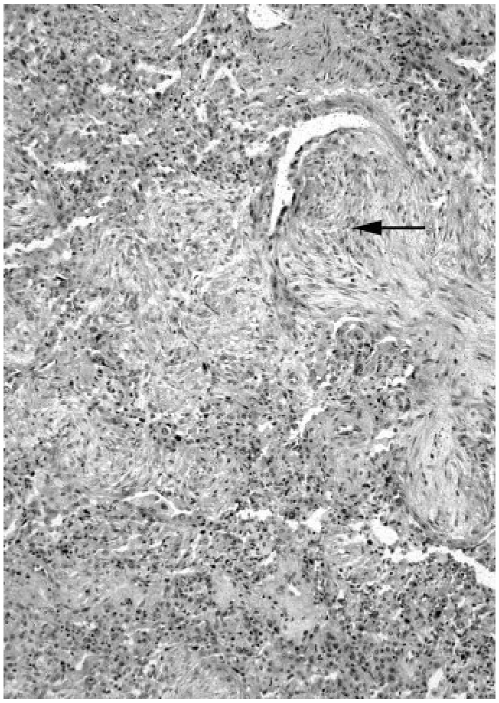


Figure 26.73 Lung, idiopathic bronchiolitis obliterans organizing pneumonia (BOOP) [or cryptogenic organizing pneumonia (COP)], day 120 following marrow allograft transplantation. The patient developed dyspnea and a cough following tapering of corticosteroid therapy for chronic GVHD. A chest X-ray showed multifocal alveolar opacities clinically suggestive of fungal or viral pneumonia. The biopsy demonstrated areas of consolidation in which bronchioles were blocked by granulation tissue (arrow) that also extended into nearby alveoli and was associated with interstitial and alveolar infiltration by mononuclear inflammatory cells. In HCT recipients, BOOP is associated with GVHD in the skin, oral mucosa, and gut [172].

and chronic phases. In the 49 patients with BOOP, day 108 was the median day of occurrence.

The diagnostic feature of pulmonary GVHD, constrictive bronchiolitis obliterans (CBO) (Figure 26.74) resembles lung allograft rejection where the small bronchioles contain intraluminal connective tissue with chronic inflammation that evolves into dense fibrotic scarring with luminal narrowing [167,177,178]. Secondary changes result in mucostasis, aggregates of foamy macrophages and late developing bronchiectasis. Stains for elastin such as the Verhoeff-Van Gieson and trichrome are helpful in demonstrating the scarred bronchioles. The end stage lung of patients dying of pulmonary GVHD may present a complex picture with loss of small airway and large airway disease including bronchiectasis and fibrosis secondary to repeated infections. It has become clear that the clinical bronchiolitis obliterans syndrome (BOS) encompasses several small airway disorders with differing stages of bronchiolar inflammation and fibrosis ranging from lymphocytic bronchiolitis (Figure 26.75) to complete fibrous effacement of the lumen (Figure 26.76). Two clinicopathologic studies of open lung biopsies in patients with suspected BOS and pulmonary chronic GVHD have demonstrated discrepant findings between the clinical 2005 NIH diagnostic criteria and the histology [179,180]. In the absence of signs of GVHD elsewhere, an open lung biopsy is recommended in the updated 2014 NIH document [81] (see Table 26.1).

Other sites involved in chronic GVHD

Several infrequent manifestations associated with chronic GVHD resemble autoimmune disorders, including vitiligo, myositis (Figure 26.77), serositis, arthritis, synovitis, myasthenia gravis, and bullous pemphigoid. Rackley et al. described a series of 12 patients, with a literature review of cardiac manifestations [181]. Striking findings included an obliterative coronary artery vasculopathy resembling that of cardiac allograft rejection (Figure 26.78) and myocarditis, associated with bradycardia and, in some instances, sudden death. A nephrotic syndrome is associated with membranous nephropathy and minimal change podocytopathy [182] are now regarded as manifestations of chronic

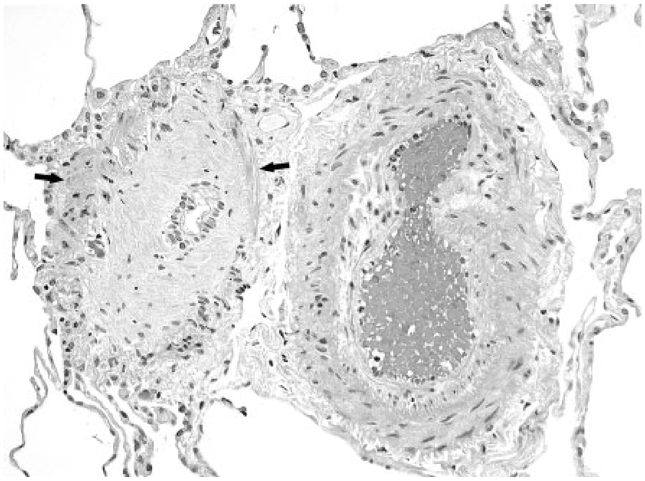


Figure 26.74 Bronchiolitis obliterans with constrictive subepithelial fibrosis at 5 years post allograft transplantation. The patient had developed obstructive pulmonary dysfunction. This biopsy confirmed that chronic pulmonary GVHD had developed following a history of skin and GI involvement. The arrows indicate smooth muscle bundles. There is also subintimal thickening of the accompanying artery.

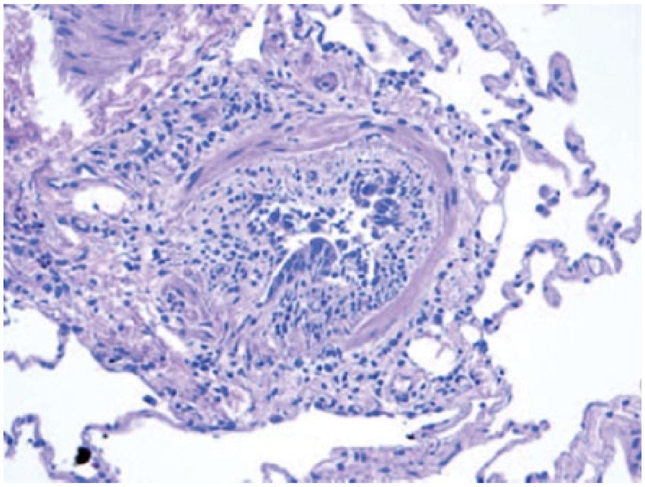


Figure 26.75 Bronchiolitis with evolving subepithelial fibrosis and lymphohistiocytic infiltration. There is marked epithelial damage. In this patient with chronic GVHD and obstructive pulmonary function abnormalities, the alterations are consistent with pulmonary GVHD. Similar changes could be seen in respiratory virus infection, which was absent in this biopsy. The ongoing inflammation and subtotal constrictive obliteration of this airway suggest that immunosuppressive therapy may be beneficial. (For a color version of this figure, please see the color plate section.)

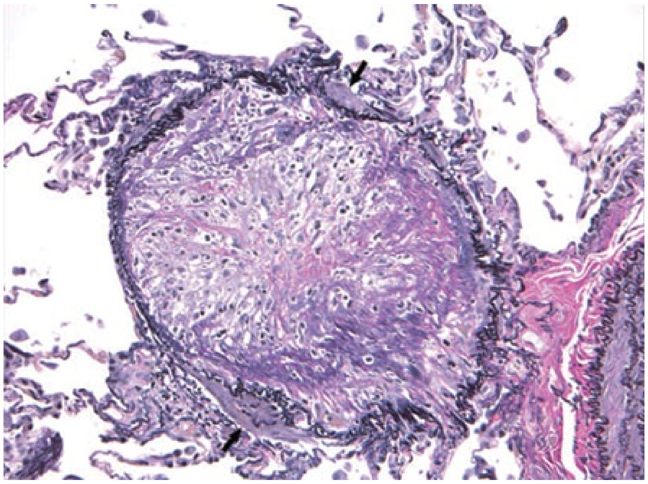


Figure 26.76 In a patient with a history similar to the preceding case (see Figure 26.75), the bronchiolitis obliterans of chronic pulmonary GVHD has progressed. This bronchiole is completely occluded by granulation tissue, and scattered mononuclear inflammatory cells are present. Without successful intervention with immunosuppressive therapy, this airway will finally probably be replaced by permanent scar tissue. The arrows indicate residual smooth muscle. The elastin fibers are black with this Verhoeff–van Gieson stain. (For a color version of this figure, please see the color plate section.)

GVHD (see Chapter 96 by Hingorani). Kamble et al. [183] and Grauer et al. [184] reviewed the literature on central nervous system lesions associated with chronic GVHD, including vasculitis neuropathy, demyelination, and immune mediated encephalitis. The NIH consensus on criteria for the diagnosis of chronic GVHD recommends that the diagnosis of chronic GVHD of the nervous system can be made only when other organs are affected by GVHD and neurologic differential diagnoses such as drug-induced toxicities or opportunistic infections are excluded.

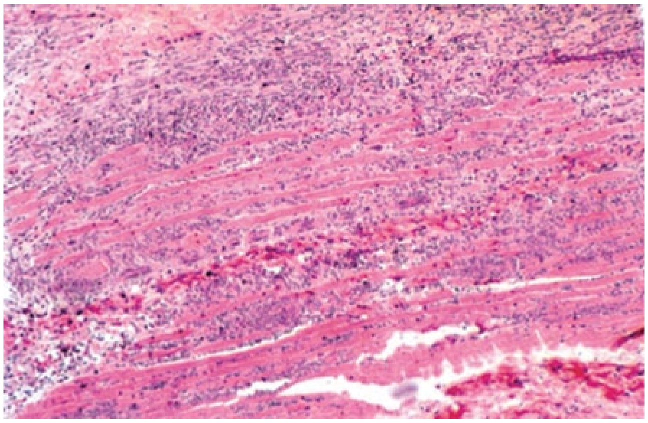


Figure 26.77 Polymyositis in deltoid muscle biopsy associated with chronic GVHD, day 1129. There is extensive endomysial chronic inflammation associated with myocyte destruction. The condition responded to immunosuppressive therapy. (For a color version of this figure, please see the color plate section.)

Infection

Infection remains a major cause of morbidity and mortality after HCT, which must be evaluated promptly and extensively in tissue and cytology preparations. The pathologist must be prepared to identify common pathogens and unexpected microorganisms, not just those suggested on the basis of non-specific clinical findings. *Helicobacter pylori* should be considered in all inflamed gastric biopsies (Figure 26.79). However, *Helicobacter* infection is

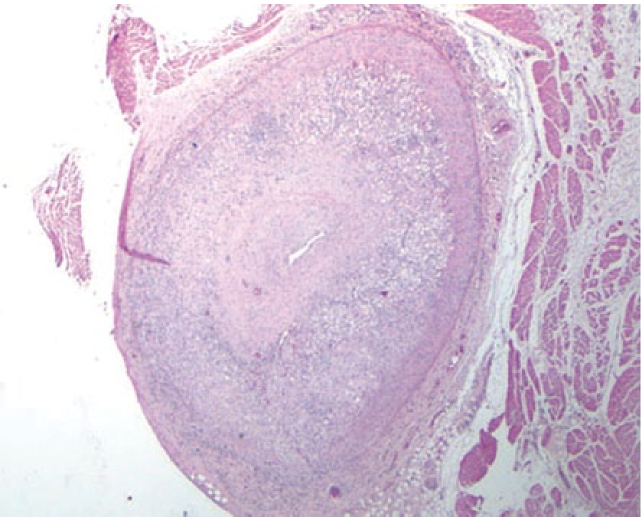


Figure 26.78 Coronary arteriopathy associated with chronic GVHD in a 15-year-old who died of myocardial infarction preceded by severe anginal symptoms. The lumen and muscular wall of all the major coronary arteries were subtotally to completely obliterated by a cellular infiltrate of lymphoid and monocytoid cells admixed with extracellular matrix. (For a color version of this figure, please see the color plate section.)

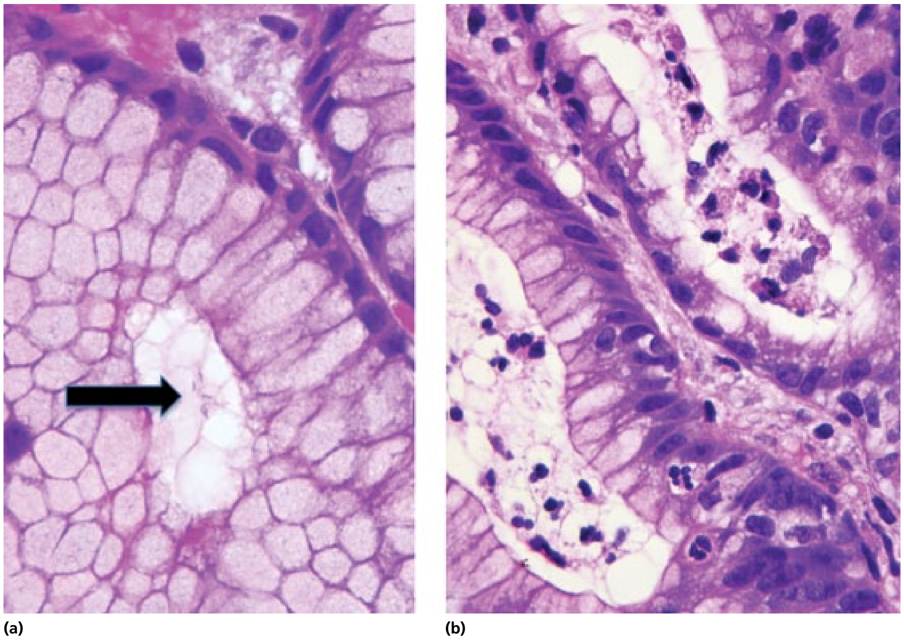


Figure 26.79 Gastric biopsy stained with H&E in a patient with chronic myeloid leukemia, day 37 post-HCT with persistent nausea and erythematous gastric lining by endoscopy non-responsive to steroids. (a) Gastric glands with a group of *Helicobacter pylori* organisms (black arrow) confirmed by immunohistochemistry. (b) Adjacent glands showing acute inflammation and a few *H. pylori* organisms. (For a color version of this figure, please see the color plate section.)

not common in post-transplant patients and specific stains are not routinely performed in post-transplant gastric biopsies at our institution. Although a nodular pulmonary infiltrate may indeed be the result of fungal infection, other treatable causes include nocardiosis, legionellosis, toxoplasmosis (Figure 26.80), and CMV (Figure 26.81), in addition to other viral pneumonias. It is obvious, but bears repeating, that identifying microorganisms requires looking specifically for them. This must be done quickly in immunocompromised patients if effective therapeutic drug levels are to be established. Since pneumonia can progress with devastating speed in HCT patients, lung tissue and/or lavaged cells should be immediately processed, stained, and evaluated. The modification of the original Grocott methenamine silver staining method by using heat has shortened the staining time from hours to less than 30 minutes, allowing rapid diagnosis of fungal and pneumocystis organisms. The pathologist does a disservice to the patient, and also to clinicians, by studying only H&E-, Gram-, and silver-stained sections and reporting that there is no evidence of infection. The preliminary stains include Wright–Giemsa, Papanicolaou, H&E, Gram, methenamine silver, PAS, modified Gimenez for *Legionella*, and Kinyoun for mycobacteria. Fite and Warthin–Starry stains and also a large number of IHC antibodies and in situ hybridization probes for organisms are used as needed. Control special stains are also needed to verify results.

Allograft recipients who received myeloablative conditioning and have GVHD are at significant risk for reactivation of latent viruses, especially members of the herpes virus group and adenovirus [185–187]. We now rarely see CMV or HSV in bronchoalveolar lavages or tissue biopsies because seropositive patients receive prophylactic antiviral therapy, and patients who develop antigenemia or PCR positivity are treated preemptively. However, treatment does not eradicate these viruses, so late infection may develop unnoticed after treatment is stopped (Figure 26.82). Late CMV pneumonia (Figure 26.81) occurring at a median of approximately 24 weeks post-transplant is increasing [188]. HCT recipients are at high risk for acquiring potentially fatal respiratory virus infections when the community prevalence of agents such as respiratory syncytial virus (RSV) (Figure 26.83) is high.

Occasionally, viral infection of a single organ will produce an ambiguous clinical presentation best resolved by prompt biopsy and rapid tissue evaluation. For example, severe abdominal pain may suggest impending bowel infarction but may actually represent acute herpes simplex virus (HSV), varicella-zoster virus (VZV) (Figure 26.84) or adenovirus hepatitis (Figure 26.85), which can be diagnosed within a few hours by histologic studies

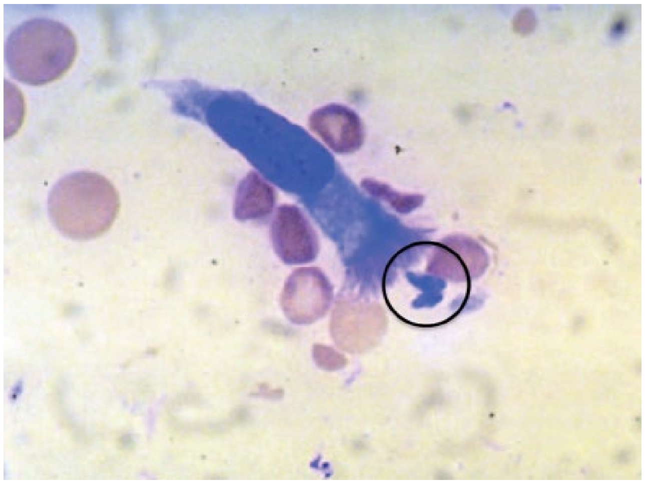


Figure 26.80 Two *Toxoplasma* tachyzoites (inside the circle) are present near the ciliated surface of a bronchial epithelial cell. Diagnosis of *Toxoplasma* pneumonia was established in less than 2 hours after bronchoalveolar lavage. Wright–Giemsa stain of cytospin preparation. (For a color version of this figure, please see the color plate section.)

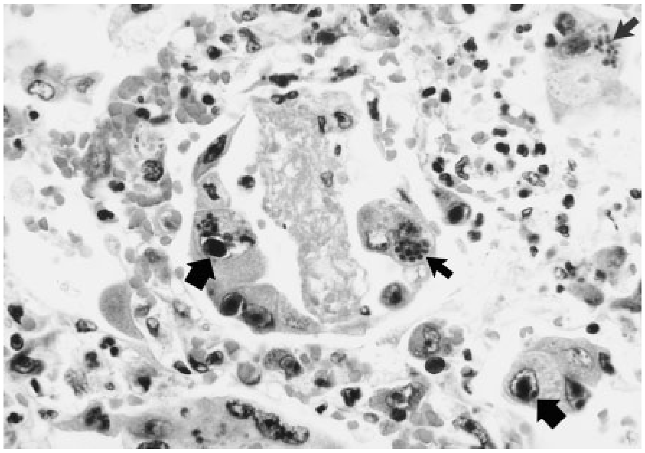


Figure 26.81 Lung, cytomegalovirus (CMV) pneumonia, day 68. Several large cells contain Cowdry type A nuclear inclusions (large arrows) and 6–12 smaller cytoplasmic inclusions (small arrows) diagnostic for CMV infection. (H&E.)

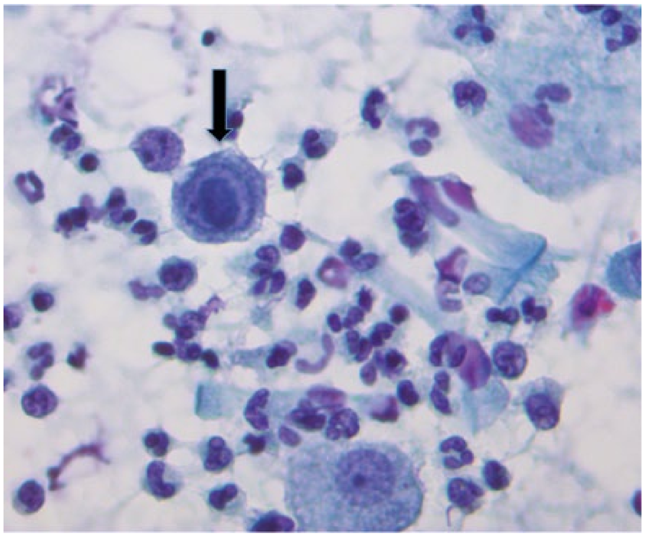


Figure 26.82 Cytomegalovirus cytopathic changes seen in a cytospin preparation of a bronchoalveolar lavage in a patient with B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma. Among a neutrophilic background is an infected alveolar macrophage (arrow) which demonstrates some cytoplasmic inclusion and a markedly enlarged nuclei with a single prominent enlarged central nucleoli. There is a relatively normal alveolar macrophage at the bottom middle of the picture for comparison. (For a color version of this figure, please see the color plate section.)

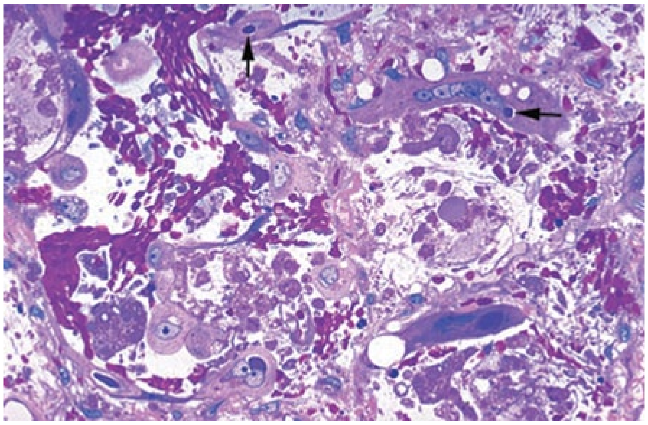


Figure 26.83 Respiratory syncytial virus (RSV) pneumonia in a lung biopsy, day 48. The presence of dark purple cytoplasmic inclusions in multinucleated epithelial cells (arrows) suggests the diagnosis but may be found with other viruses such as parainfluenza. During severe community outbreaks, infections may spread to hospitalized marrow recipients. (For a color version of this figure, please see the color plate section.)

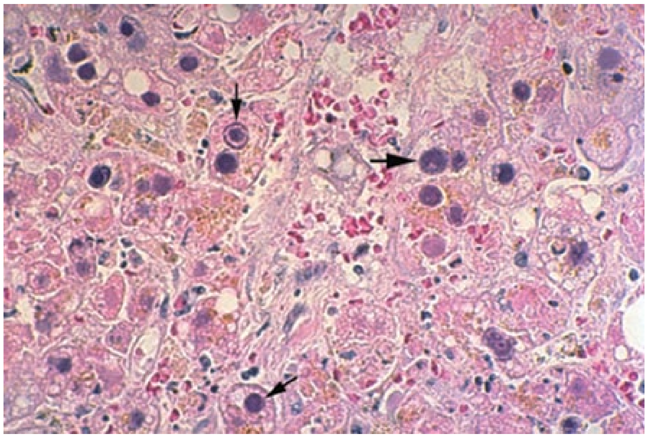


Figure 26.85 Overwhelming adenovirus hepatitis at autopsy 92 days after allogeneic BMT for AML. Dark basophilic nuclear inclusions produce "smudge cells" (large arrows) with blurred nuclear margins. Occasional cells with a halo around the inclusion (small arrow) suggest a combined infection with CMV, which was confirmed by isolation of both viruses. (For a color version of this figure, please see the color plate section.)

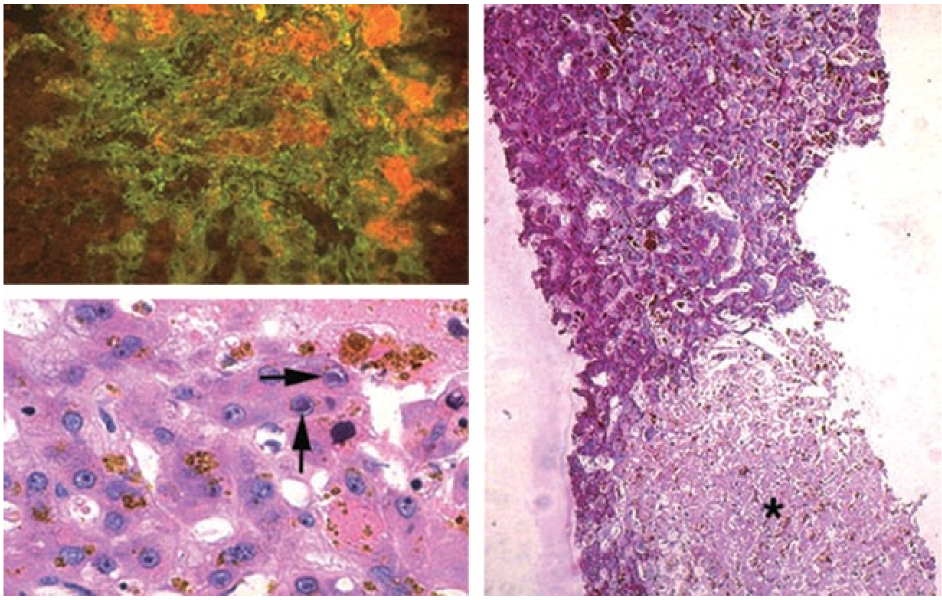


Figure 26.84 Varicella zoster virus (VZV) hepatitis in a percutaneous needle biopsy, day 79. On the right, a PAS stain demonstrates a pale necrotic area (*) bordered by cells containing nuclear inclusions (large arrows) visible by H&E stain on the lower left. Immunofluorescence confirmed the presence of VZV (upper left), and intravenous acyclovir was begun within hours of the biopsy. (For a color version of this figure, please see the color plate section.)

of a transjugular liver biopsy. Similarly, hematuria accompanied by costovertebral angle tenderness may be secondary to adenoviral nephritis (Figure 26.86), which can be diagnosed by renal biopsy [189]. Parvovirus infection should be ruled out in post-transplant patients with non-recovering or decreasing hematocrit/hemoglobin (Figure 26.20) [190].

The pathogenicity of human herpes virus type 6 (HHV6) and its impact on GVHD and other complications is complex [101,102, 191,192] (see Chapter 92). HHV6 can produce severe encephalitis, but viremia may not be necessarily associated with any clinical complications. The virus can be identified in tissues by PCR but results using in situ hybridization have been inconsistent. Identification of HHV6 in tissue sections by immunostaining with antibodies has been reported in a case of acute hepatitis [191].

Invasive mold infection develops in approximately 15% of patients undergoing either high-dose or reduced-intensity regimens. The most frequent cause is *Aspergillus*, followed by Zygomycetes and *Fusarium* species [193] (Figures 26.87–26.89) The histological demonstration of fungi in tissue is often crucial to the diagnosis and prompt treatment of invasive infection since fungemia is sporadic, and assay results for antigenemia are often inconclusive. Although not the most sensitive test, a bronchoalveolar lavage may show

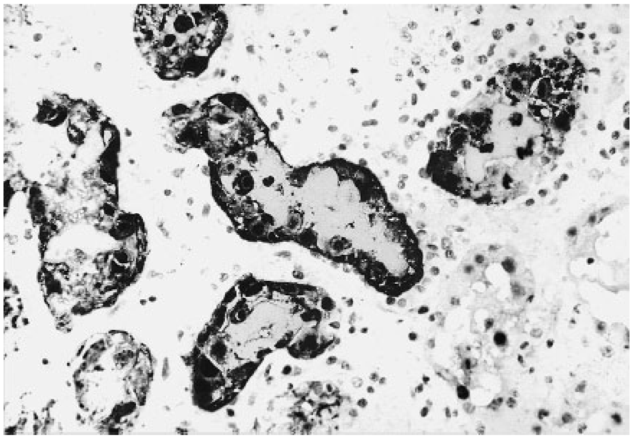


Figure 26.86 Kidney, adenovirus (ADV) nephritis, day 77. Tubules lined by degenerating epithelial cells are strongly reactive for ADV antigen by indirect immunoperoxidase staining using a monoclonal antibody to the adenovirus hexon protein (Chemicon International, Inc., Temecula, CA, USA). ADV species 11 was isolated in culture. Possible ADV nephritis should be considered in patients with hematuria and positive urine culture [189].

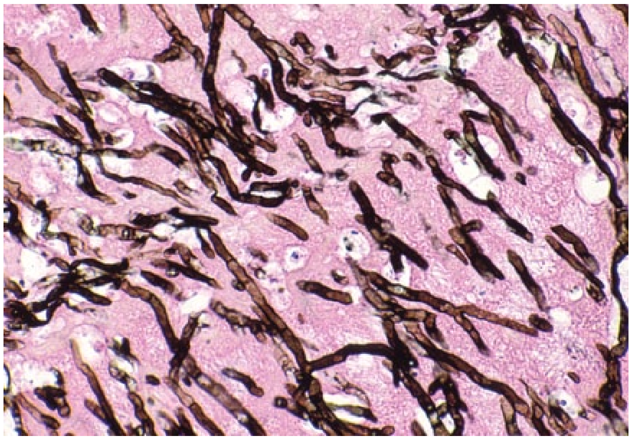


Figure 26.87 Mahan's methenamine silver stain of a lung biopsy showing a central necrotic cavity with infiltration by septate fungal 45° branching hyphae morphologically consistent with *Aspergillus*, which was subsequently confirmed with PCR. Source: reproduced with permission of Dr. Robert C. Hackman. (For a color version of this figure, please see the color plate section.)

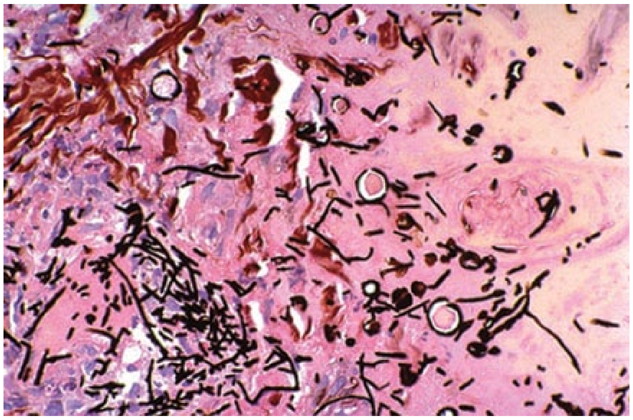


Figure 26.88 Mahan's methenamine silver stain showing that *Fusarium* hyphae invade the dermis in pre-transplant skin biopsy. (For a color version of this figure, please see the color plate section.)

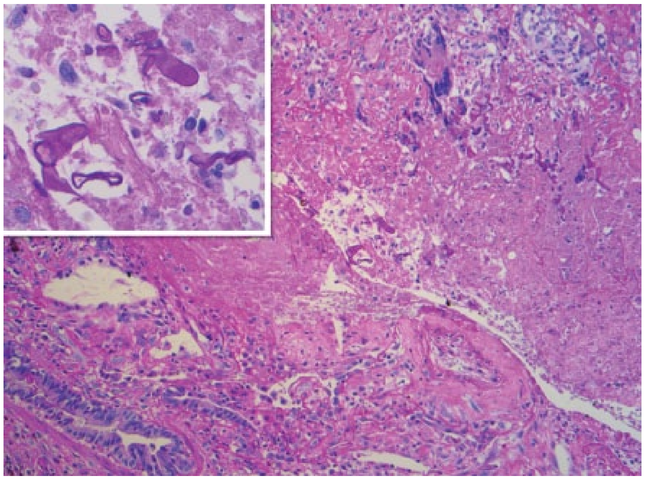
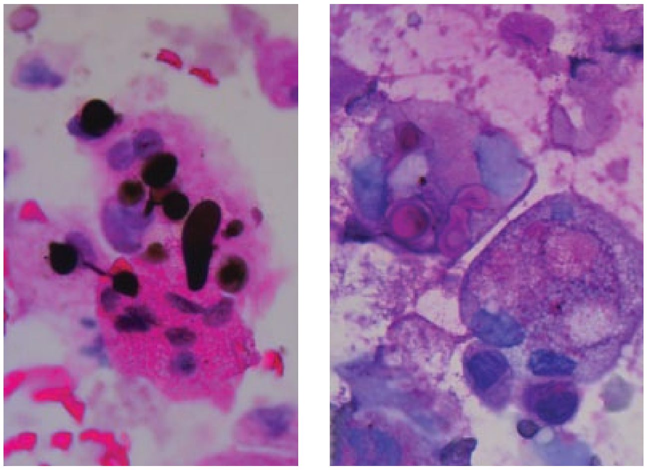


Figure 26.89 Lung biopsy specimen stained with PAS treated with diastase demonstrates Zygomycetes infection in a patient with sickle cell disease day 294 after HCT. There is extensive necrosis and giant cell reaction associated with the fungal organisms. Ribbon-like haphazardly arranged fungal hyphae are shown at high power in the inset. (For a color version of this figure, please see the color plate section.)



(a) (b)

Figure 26.90 (a) A cytospin preparation of a bronchoalveolar lavage stained with Mahan's methenamine silver showing intracellular fungal organisms in an alveolar macrophage, later shown to be *Candida albicans* by fungal culture. (b) Same specimen stained with PAS showing the same intracellular *C. albicans* organism including a budding yeast form. (For a color version of this figure, please see the color plate section.)

fungal infection and provide a comparatively rapid and specific result (Figure 26.90). Speciation using PCR has partially supplanted culture isolation, which is slow and insensitive. *Toxoplasma gondii* (Figure 26.80), *Pneumocystis jirovecii* (Figure 26.91), mycobacteria, *Nocardia* (Figure 26.92), and *Legionella* are also organisms that can be identified rapidly by histological and immunocytochemical staining and then specifically treated [194–196]. Although these organisms are unusual in most patient populations, their rapid identification can save lives. The incidence of toxoplasmosis over a 20-year period in Seattle was 0.3% [196], of nocardiosis over 25 years 0.2% [196], and of legionellosis over 12 years 0.6% [194].

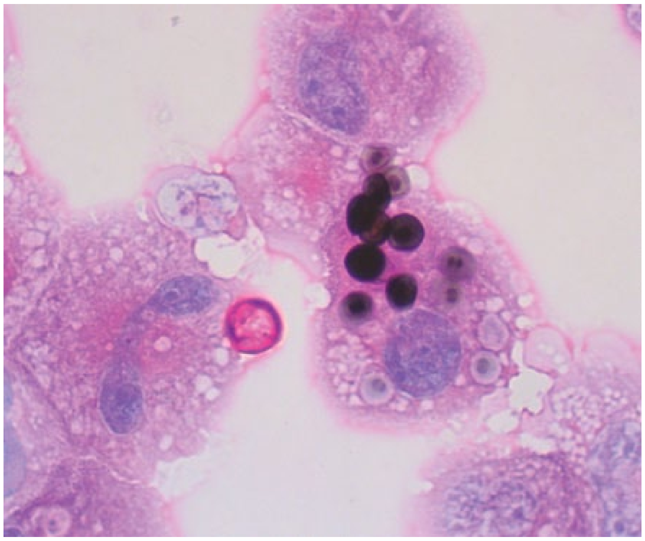


Figure 26.91 A cytospin preparation of a bronchoalveolar lavage stained with Mahan's methenamine silver showing intracellular pneumocystis organisms in an alveolar macrophage. (For a color version of this figure, please see the color plate section.)

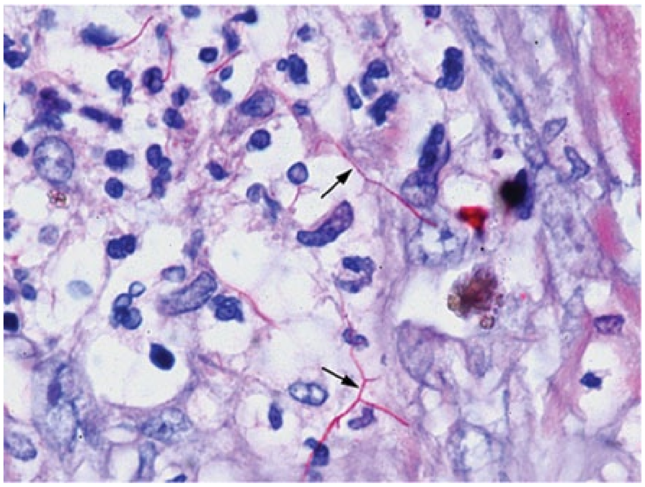


Figure 26.92 Fite stain of a lung biopsy showing thin filamentous branched *Nocardia* (arrow). Source: reproduced with permission of Dr. Robert C. Hackman. (For a color version of this figure, please see the color plate section.)

Additional comments

Since final submission of this chapter several new developments have occurred, which could not be incorporated in the main body of the text. These include the updated 2015 NIH clinical and histologic criteria which indicate the need for open lung biopsy only if clinical tests consistent with the bronchiolitis obliterans syndrome are not associated with any existent manifestations of active GVHD. Small airway disease related to chronic GVHD affects up to 10% of long term survivors and may have a continuum from lymphocytic bronchiolitis to constrictive bronchiolitis obliterans as the final common pathway. Unusual complications like myositis and bronchiolitis obliterans pneumonia are classified as lesions of uncertain significance. To improve interobserver reproducibility, recommendations for the final histologic assessment for GVHD have been simplified into three categories: "No GVHD," "Possible GVHD" and "Likely GVHD". The "likely" GVHD category combined the previous categories of "consistent with" and "diagnostic of" GVHD. The cord colitis syndrome story emphasized the lesson to use standardized histologic definitions of GVHD and that infection and GVHD are not mutually exclusive. Certain kidney lesions, membraneous nephropathy and minimal change disease are now regarded as manifestations of chronic GVHD.

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CHAPTER 27

Biostatistical Methods in Hematopoietic Cell Transplantation

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Introduction

With the tremendous strides made in the field of hematopoietic cell transplantation (HCT), advances in study design and biostatistical methodology are required to keep pace. Clinical trial design and analysis methodology continue to evolve, allowing HCT practitioners to conduct studies that lead to the most scientifically rigorous and defendable conclusions possible given the particular setting. This chapter, designed for a clinical audience, provides an overview of the biostatistical methods commonly used in HCT studies, both prospective and retrospective. We focus on the concepts, procedures, and interpretations of specific study designs and statistical analyses, from the phases of clinical trials through observational studies as applied to HCT. The principles for designing such studies and the fundamentals regarding study conduct are described, along with the subtleties, strengths, and potential pitfalls of various study designs and analytic approaches. It is assumed that the reader is acquainted with the usual concepts of a beginning course in statistics: probability, estimation of parameters, hypothesis testing, and confidence intervals; if not, suggested reading for an introduction to biostatistics includes books by van Belle et al. [1] and Zar [2].

Formulas and calculations are used minimally in this chapter, presentation instead favoring the underlying concepts and theories of biostatistical methodology. It is hoped that this approach will provide physicians, fellows, medical students, and healthcare professionals involved in HCT research with a basic understanding of, and guide to, appropriate approaches in the design, conduct, and statistical analysis of studies in HCT. The chapter is not meant to be exhaustive and detailed, as space constraints do not allow such an exposition and the authors appreciate that any planned research study would be developed and carried out in collaboration with a biostatistician. For those seeking a more detailed treatment of the history, rationale, and methodology underlying observational studies and clinical trials, the texts by Meinert and Tonascia [3], Pocock [4], Friedman et al. [5], Rosenbaum [6], Kelsey et al. [7], and Piantadosi [8] provide such elements. It is our intention to leave the reader with sufficient knowledge and guidance to continue to pursue topics of interest using the basic texts and articles referenced in this chapter.

Statistical methods in HCT research

Survival analysis

The term "survival analysis" is often used to describe a collection of statistical methods developed for the analysis of data in which the outcome under study is time until a prespecified event occurs (commonly referred to as time-to-event data). The occurrence of this event is typically referred to as a "failure," although the event can be considered a positive clinical outcome, such as engraftment or response to treatment. In HCT research, treatment "success" is often assessed by measuring the number of surviving patients following the intervention over some specified period of time, with the study objectives typically focused on the ability of the treatment to prevent or delay failure (e.g. disease relapse, death).

While most early phase I and II studies focused on shorter-term binary end-points such as toxicity or disease response, observational studies in HCT and phase III studies typically utilize longer-term events, such as time to relapse or survival time after transplant. The survivor function, *S(t)*, is defined as the probability that survival exceeds some specified time *t*, namely

$$S(t) = Prob(T > t) = 1 - Prob(T = t) = 1 - F(t)$$

where $F(t)$ is called the cumulative distribution function (CDF) of mortality. $S(t)$ is also defined as a function of the hazard of failure, where the hazard function at time *t* represents the instantaneous failure rate at *t* among patients who have survived to *t* without failure or, roughly speaking, as the probability of death within a small window among those who are at risk of failing within this window. In a study with a time-to-event outcome, each patient will contribute data consisting of (1) time from HCT (or study entry) to event (or last contact-evaluation date/study end date) and (2) event status (i.e. failure: yes/no). Any patient who does not experience failure by the time of analysis is said to be "censored," as all that is known for that patient is that the event did not occur as of the last contact date, although failure can occur at a point beyond the censored time. This form of censoring is referred to as "administrative" censoring, because the incomplete information on the patient is due to the administration of the study, rather than to any irregularities in the data collection or ability to follow the patient through the study period.

Kaplan–Meier survival estimate

If all patients have complete follow-up (i.e. there is no censoring), the survival estimate at time *t* is the simple proportion of patients alive at *t* divided by the number enrolled. If censoring occurs,

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however, one must appropriately take into account the observation time of the censored cases in order to obtain an unbiased estimate of survival. The Kaplan–Meier (KM) method [9] accomplishes this and is the most widely used approach for computing survival estimates. For each ordered failure time, the estimated survival probability is calculated using the product-limit method, with the following equation:

$$\begin{aligned}\hat{S}\left(t_{(j)}\right) &= \prod_{i=1}^{j} \hat{Pr}\left(T > t_{(i)} \middle| T \geq t_{(i)}\right) \\ &= \hat{S}\left(t_{(j-1)}\right) \times \hat{Pr}\left(T > t_{(j)} \middle| T \geq t_{(j)}\right)\end{aligned}$$

where T is the random variable for the subject's survival time, t is any specific value of interest for the variable T, and $S(t)$ is the survival function, $S(t) = Pr(T > t)$.

KM estimates of survival probabilities, when graphed, yield a "curve," also referred to as a "step function," that drops down a fraction of its value at each failure time and which, when a large enough sample is taken, approaches the true survival function for that population. Censored observations are commonly denoted by vertical tick marks on the curve. Figure 27.1 provides an example of a KM overall survival curve with censoring. Data for this example were derived from an unpublished study of survival times (in years), post-transplant, among patients with leukemia.

The plots of the survival probabilities correspond to each ordered failure time. KM plots start at a survival probability of 1, with all patients alive (no failures) at $t = 0$, and then drops down some fraction as we move from one ordered failure time to the next. Several statistical software packages are available to compute and plot survival probabilities over time, based on the number of patients at risk, number of failures, and number of censored persons at a given time.

Among HCT patients, the hazard of death near the time of HCT is much higher than the hazard at a time further away from HCT (the majority of deaths following HCT occur within the first 100–365 days post-transplant). As a result, the survivor function tends to plateau or semi-plateau at time points further from the time of transplant. Unfortunately, interest is often highest where the data are the least informative – at the end of the curve where few patients are still at risk, resulting in a variance in the estimate that is higher than at earlier time points. This uncertainty should be quantified by reporting either point-wise confidence intervals (CIs) or confidence bands. Standard errors can be computed for the KM curve at any time point, and the estimated survival curve graphed with a *plus* and *minus* distance (usually 95% CIs) around the curve at a few time points to reflect the range of the true survival probability.

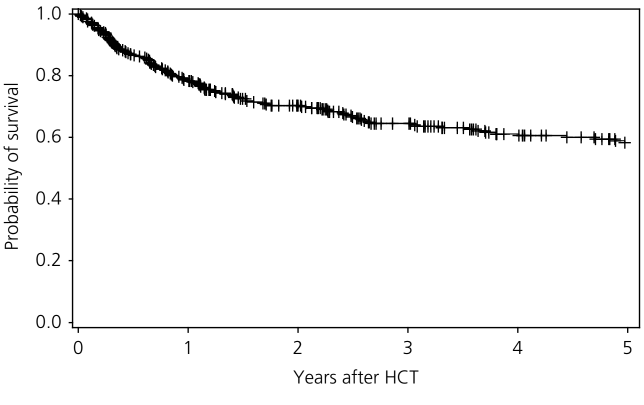

Figure 27.1 Kaplan–Meier (KM) overall survival curve.

Competing risks

We define a competing risk as the occurrence of an event that precludes the event (outcome) of interest from occurring. For example, if relapse is the event of interest, death without relapse [i.e. non-relapse mortality (NRM)] is a competing risk. The only end-points in HCT that do not have competing risks are overall survival (where failure is the occurrence of death) and failure for event-free survival (where failure is often defined as death or relapse, whichever occurs first). When estimating the probability of an event by a specified time that has competing risks, one must take care in how the competing-risk event is handled. Kalbfleisch and Prentice [10] recognized this as early as 1980, introducing what they denoted the cumulative incidence function (CIF). Despite this, it was not uncommon for investigators to use the complement of the KM estimate (1 – KM) inappropriately to estimate the probability of failure, where competing-risk failures are incorrectly censored. Because of this, Pepe and Mori [11] illustrated the flaw in the KM method for these purposes with examples focused on the field of HCT. Many other authors have written on this subject in the last two decades (e.g. [12–17]). Investigators are encouraged to review these published examples for instructional purposes. Nonetheless, one can still find recent instances where 1 – KM is used in error, disregarding the presence of competing-risk failures.

According to the definition of a competing risk, such failures are not capable of subsequent failure from the event of interest, but censored observations are implicitly assumed to be capable of failure at a time beyond the censored time. Therefore, 1 – KM results in an estimate that is larger than it should be. To illustrate better the differences in the cumulative probability of the cause-specific failure estimates produced by the two estimators (1 – KM and CIF), data from a study of relapse post-transplant, among patients with leukemia, are provided in Figure 27.2.

The graphs are identical for the majority of the first year post-transplant, given that there are very few competing events before $t = 0.96$ years; at $t = 0.96$ years the 1 – KM estimate is 0.18 and the CIF estimate is 0.17. The difference becomes more obvious after 3 years and increases with more competing events (e.g. death without relapse); at $t = 3.13$ years the 1 – KM estimate is 0.25 and the CIF estimate is 0.22.

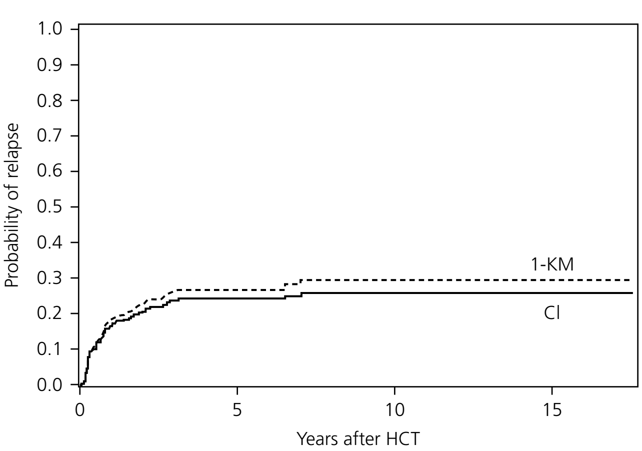

Figure 27.2 Cumulative probability of relapse produced by two estimators, 1-KM and CIF.

As stated earlier, this has been shown in several reports, including by Gooley et al. [14], which used the redistribute-to-the-right concept to demonstrate how 1 – KM fails. Briefly, their argument is as follows. With complete follow-up (i.e. all subjects are known to have failed or not failed by a specified point in time), the contribution of a single failure to the estimate of the probability of failure is simply $1/n$, where n is the number of patients being studied. If a patient is censored, however, and therefore capable of failure at a later time, this contribution of $1/n$ is uniformly redistributed among all patients who remain at risk beyond the censored time. As a result, subsequent failures contribute $1/n$ plus this redistributed amount to the estimate of the probability of failure. If a patient fails from a competing risk, 1 – KM censors this observation and the potential contribution of the censored observation is inappropriately redistributed among those who are at risk of failure beyond the competing-risk failure time. On the other hand, the cumulative incidence function correctly treats such a patient as a non-failure from the event of interest since such an observation has complete follow-up by this time, that is, it is known that this patient has not failed by this time and will not fail from the cause of interest beyond this time. Since 1 – KM does not distinguish between censored observations and competing-risk failures, it depends only on the hazard of failure from the cause of interest. The CIF makes such a distinction, however, and it therefore depends on the hazard of failure from the cause of interest in addition to the hazard due to the competing risk.

Testing of group differences for time-to-event end-points

Beyond estimation, one is ultimately interested in comparing outcomes between two or more groups. There are many ways to do this, but the most common method for time-to-event data that do not contain competing risks is the log-rank test [18], which is used to determine whether or not the KM curves (or survivor functions) from two or more groups are statistically equivalent. The null hypothesis is that there is no overall difference between the two survivor functions; the alternative hypothesis is that there is an overall difference between the two survival curves. The calculation of the log-rank statistic involves the formation of a 2 × 2 table at each failure time (one row each for groups A and B; one column each for lack of failure and failure), and within each table the number of observed failures is compared with the expected number under the null hypothesis of no difference. These comparisons are summed together across the times of failure, and a weighted version of this sum forms the log-rank test statistic. Under the null hypothesis, this test statistic follows a chi-squared distribution with one degree of freedom.

The log-rank test compares the survivor functions, but given the dependence of $S(t)$ on the hazard function, it equivalently compares the hazards between groups. In fact, the observed and expected numbers of failures that contribute to the log-rank test statistic are derived from estimates of the hazards of failure at the specified time. We also note that the log-rank test, as the name implies, is a rank-based test. Provided that the relative order of failures and censored times in group A versus group B remain the same, the actual values of the times will not change the log-rank test statistic.

Because high early mortality followed by a relative plateau is a common survival pattern following HCT, inferences based on the log-rank statistic are not well suited to studying long-term survival, potentially reporting a long-term difference between study arms even though no long-term survival benefit exists. An alternative approach is to conduct a separate analysis of long-term survival, modeling the population as a mix of long-term survivors (the cured fraction) and patients undergoing an earlier failure process (the uncured fraction). Ultimately, cure-rate models produce an estimate of the cure fraction, where a probability of long-term survival is postulated [19,20]; such modeling techniques are now implemented in S-*plus* [21].

If one is comparing outcomes for time-to-event end-points with competing risks, the log-rank test is still valid, but it is important to understand what are being compared in such cases [22,23]. As previously stated, the survival distribution is a function of the hazard of failure, and therefore comparison of the survival distributions is tantamount to comparing the hazards of failure. When the log-rank test is used for end-points with competing risks, what are being compared are what are called the cause-specific hazard rates of failure, where competing-risk failures are censored. It can be confusing when told that for estimation purposes competing-risk failures are not censored, yet for inferential purposes such failures can be censored. The distinction arises from the fact that the hazard is fundamentally a conditional quantity. The cause-specific hazard of a specific failure type at a given point in time is conditional upon patients being at risk of failure from this type at that time, regardless of whether patients are not at risk due to lack of follow-up or due to failing from a competing risk prior to this time. The cause-specific hazard depends only on the hazard of failure from the cause of interest, but the CIF depends on the hazards of failure from both the cause of interest and the competing risk. Several tests have been proposed for comparing cause-specific CIFs, but the most common method was proposed by Gray [24]. Similar to the survivor function, defined as a function of the hazard, the (cause-specific) cumulative incidence function is defined as a function of the hazard of failure from both causes (i.e. each cause-specific hazard). One can define a hazard-like quantity of this CIF in a fashion similar to the cause-specific hazard. Commonly referred to as the subdistribution hazard, this differs from the cause-specific hazard function in that it represents the probability of failure from the cause of interest in a narrow time window but among patients who not only have survived without any failure up to this time but in addition among those who have failed from a competing risk prior to this time. In other words, patients who fail from a competing risk are not dropped out of the "denominator" as they are in the log-rank test when used to compare cause-specific hazards. In this sense, the CIF hazard function can also be thought of as the hazard of an artificial time variable T' defined as $T' = t$ if a failure from the cause of interest occurred at time t and $T' = \infty$ if failure from the competing risk occurred.

As an example, consider the case where the true cause-specific hazard of relapse for each of groups A and B is 0.1, and the cause-specific hazard for NRM for groups A and B are 0.1 and 0.3, respectively. Suppose we draw a random sample for each group under these parameters, and we further assume that there is no censoring. We then wish to compare relapse between groups. Since the log-rank test compares the cause-specific hazards of relapse, one would expect the conclusion from such a comparison not to yield a statistically significant difference (as the cause-specific hazards are identical). Moreover, because the cause-specific hazard of NRM is higher in group B than it is in group A, there should be a larger number of NRM failures in group B at a given point in time. This will lead to a lower hazard-like function for relapse (the so-called subdistribution hazard that Gray's test compares) in group B, that is, the "denominator" for group B will be larger than the "denominator" for group A due to the larger number of NRM failures (recall that the subdistribution hazard for relapse is calculated among patients who have survived without relapse *or who have failed from the competing risk of NRM*; since more patients in group B will have failed from NRM, the denominator for group B will be larger). One would expect, therefore, that Gray's test would yield a larger difference in relapse than would the log-rank test. One particular random sample of 200 per group yields the relapse and NRM cumulative incidence curves shown in Figures 27.3 and 27.4), with the proportion of each type of failure listed. The log-rank test comparing the cause-specific hazards of relapse yields $p = 0.71$, and Gray's test comparing the subdistribution hazards of relapse yields $p = 0.03$. Both the log-rank and Gray's test yield $p < 0.0001$ for the risk of NRM.

In summary, both Gray's test and the log-rank test are valid. The conclusions from each test can differ since they do not test the same hypotheses (as demonstrated in the example above), although in our experience the two tests lead to the same qualitative conclusion most of the time. The log-rank test compares cause-specific hazards, quantities that are conditional upon patients being at risk of failure from the cause of interest at time *t*, whereas Gray's test compares hazard-like functions (commonly referred to as subdistribution hazards) that are conditional upon patients being at risk of failure at time *t* or failing from the competing risk prior to *t*. As demonstrated above, situations in which the two tests could lead to qualitatively different conclusions include the instance where the (cause-specific) hazards of failure from the event of interest are roughly the same between groups, yet the (cause-specific) hazard of failure from the competing risk is much larger in one group than the other. There are many excellent references that explore and explain the differences between these tests, for example [12,23,25]. In practice, one should consider applying each test for end-points with competing risks, as each test contains important information. In addition, a comparison of failure from the cause of interest and a comparison of failure from the competing risk should be conducted. Finally, interpretation is made easier if one is able to use an end-point without competing risks (e.g. relapse-free survival as opposed to relapse or NRM), although we acknowledge that this is not always feasible and/or of interest.

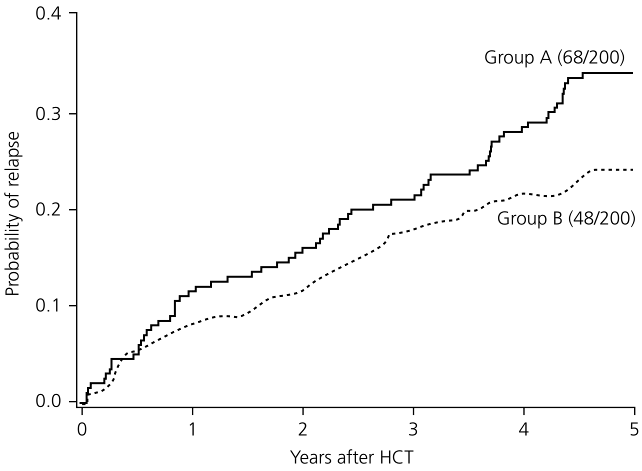

Figure 27.3 Cumulative incidence estimates of the probability of relapse.

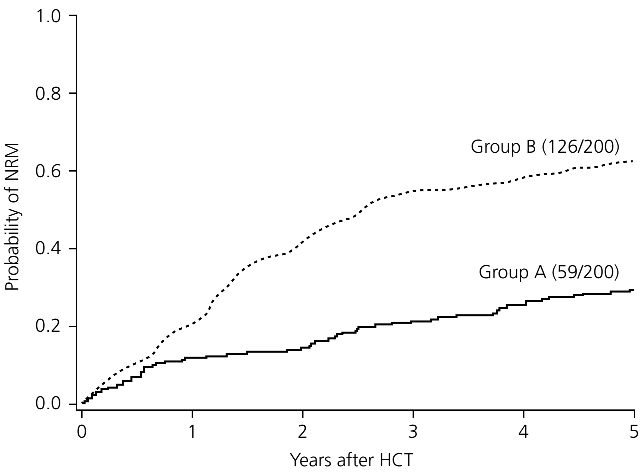

Figure 27.4 Cumulative incidence estimates of the probability of non-relapse mortality.

Regression

When comparing two groups, it is often the case that various factors known to impact outcome are unequally distributed. Therefore, the observed difference in outcome may be due more to the difference in the distribution of these factors than to a true difference. In addition, rather than treatment defining the two groups, patients may instead be categorized as those who do well (e.g. survive) and those who do not do well (i.e. die), and one is then interested in identifying factors that distinguish such groups. Each of these situations calls for regression techniques. No matter what type of regression, it is assumed that the outcome being modeled (referred to as the dependent variable) is related to one or more factors (referred to as the independent/explanatory variables or covariates) via some functional form. The outcome and the nature of the functional form determine which type of regression one uses. Two of the more common techniques in HCT are logistic regression and Cox regression (discussed below). Regardless of the type of regression, however, one uses observed data to estimate the value of the coefficients associated with each explanatory variable; one then tests the hypothesis that the average value of that coefficient is equal to zero. If this hypothesis is rejected, then this factor is said to be statistically significantly associated with the outcome; otherwise, it is concluded that the value of this factor does little to impact outcome.

Healthy approach to modeling

The purpose of modeling (multivariable regression analysis) is to assess the effects of multiple explanatory variables (e.g. age, disease status, related/unrelated donor) on the outcome variable (e.g. overall mortality, relapse). As stated above, regression modeling can be used to identify important prognostic factors and/or to assess the significance of a given factor after adjusting for other risk factors, and the type of regression used depends on the outcome being modeled. There are many issues that need to be considered when fitting a multivariable regression model, and we touch on some of them below.

Explanatory variables (or covariates) will be modeled as either continuous or categorical with two or more categories. The choice of continuous or categorical will be largely dependent on how best to interpret the impact of an explanatory variable given what is already known and supported by the literature. If continuous, one has many options for modeling such a variable. One choice is to model that variable as a continuous linear variable, where the underlying assumption is that the change in outcome associated with a specified change in the covariate is the same, regardless of the starting value of the variable. If the data do not support such a model, one can instead assume a non-linear association, of which there are many choices. On the other hand, if the interpretation/application of a continuous explanatory variable is complex or the covariate is better supported by a categorical rendering, a continuous variable can also be categorized, and a frequently used approach for this is to group the variable as above or below the median value for the sample population. Using such a cut point is convenient, but there may not be a scientific rationale for doing so. With this approach, the assumption being made is that patients with a value below the median have roughly the same outcome as do patients above the median. If such an assumption is not supported by the data or the literature, alternative cut points (including the possibility of using more than one) should be explored. There is often a biological reason for grouping a continuous variable, and in such cases one should use these groupings. If a continuous variable is categorized based solely on the data in an effort to maximize the difference in outcome between categories, one needs to recognize that these categories in future studies are not likely to show as strong an association.

Variable selection and modeling specifications

There are multiple approaches for choosing which variables to include in a regression model; perhaps the most popular is the forward selection stepwise procedure. In such an approach, the variable that improves the fit of the model most is included first, provided that the improvement meets some threshold level of significance usually prespecified by the biostatistician with input from the study team. With this variable in the model, the significance of the remaining variables is assessed, and the variable that leads to the largest improvement in model fit is added. This process is continued until no other variables improve the model to the degree set by the threshold significance level. When a variable enters the model, some approaches also allow for variables previously entered into the model to exit if the significance level of the previously entered covariate exceeds some threshold level. Threshold values for entry are typically set at a level higher than 0.05 (e.g. 0.10), but there are no definite rules for this. The goal of such an approach is to identify the most parsimonious model from the list of factors examined. Because some variables are known from clinical experience and/or the literature to be important, one should give careful consideration to including these factors regardless of their statistical significance; a model that excludes factors known to impact outcome, more than likely, will be questioned. That said, there are limits to the number of covariates that can be included in a model, however. Rules of thumb have been proposed for Cox regression such that roughly 10 events are required for each parameter that is estimated in the model [26]. For logistic regression, similar rules of thumb have been proposed, with 5–10 failures or successes (whether it is failures or successes, generally fit and stability are determined from taking the minimum between total number of failures or successes in the study). Particulars for each study will dictate how many factors can be included, but investigators should be aware that the number is limited.

Once one has settled on a final model, presentation should include the appropriate comparative summary measure (e.g. hazard ratio for Cox, odds ratio for logistic) along with CIs, number of patients/events per group and p-value for the estimate and overall model. For categorical variables, the reference group should be noted (i.e. the category that the other category or categories is being compared with via the hazard or odds ratio).

As already mentioned, when assessing the association of a variable with outcome one interprets the result as the correlation when all other variables in the model are held constant, and it is assumed that this association is roughly the same across all levels of the other covariates. The association, however, may not be roughly the same across levels of other factors, in which case one says there is a statistical interaction. For example, the impact of an HLA mismatch (relative to being matched) may be different in patients with less severe disease than among patients with more severe disease. Such interactions can be tested, and in some cases the clinical question and associated biology help to dictate which interactions should be examined.

Logistic regression

Logistic regression is used when the outcome is binary. One then models a function, called the logit, of the probability that the binary outcome occurs as a combination of various factors. Mathematically, this function is as follows:

$$\operatorname{logit}(p) = \log[p/(1-p)] = B_0 + B_1x_1 + B_2x_2 + \cdots + B_nx_n$$

where the x_i are the explanatory variables, the B_i are the coefficients that are estimated from the observed data, and p is the probability of the binary outcome. Along with estimates of the B_i, one also obtains estimates of the variance of the B_i, and these taken together provide the strength of evidence on how likely it is that each B_i is equal to zero. The quantity $\exp(B_i)$ is called the odds ratio. The odds of the outcome is defined as the probability of occurrence of the outcome divided by the probability that the outcome does not occur [i.e. $p/(1 - p)$], and the odds ratio is simply the odds for group A divided by the odds for group B if x_i is a dichotomous variable. Odds ratios that are greater than 1 mean that group A is more likely to have the outcome than group B, and odds ratios less than 1 mean that group A is less likely to have the outcome than group B. If x_i is continuous, then the odds ratio is interpreted as the change in the odds of the outcome as x_i increases by one unit.

When one includes more than one explanatory variable in the regression model, the interpretation of the odds ratio is that it represents the relative odds of the outcome for group A versus group B after considering levels of the x_k, with $k \neq i$. It is assumed that the difference between A and B is roughly the same across all levels of these x_k. This is commonly referred to as multivariate regression, although the more technically correct description is multivariable regression.

Cox regression

There are various ways to model time-to-event data, but the most commonly used approach is known as Cox, or proportional hazards, regression. Mathematically, one assumes that the hazard of failure is related to a group of covariates according to

$$H(t) = H_0(t)\exp\left(B_0 + B_1x_1 + B_2x_2 + \cdots + B_nx_n\right)$$

where the B_i and x_i are as described above, $H(t)$ is the hazard function, and $H_0(t)$ is what is known as the baseline hazard. Similarly to logistic regression, if x_i is categorical with two categories, then $\exp(B_i)$ is interpreted as the hazard ratio, also commonly called the relative risk. This is simply the hazard of group A divided by the hazard of group B. As with logistic regression, the hazard ratio for a particular factor represents the relative hazards of failure when all other factors in the model are held constant.

There are more restrictive assumptions involved in Cox regression than in logistic regression. In particular, the functional form of the model assumes that the hazards between "groups" that are being compared for a particular covariate are proportional. The hazard of failure can increase and/or decrease with time, but the assumption is that this increase and/or decrease occurs proportionally in the groups. If this assumption is violated, then one needs to consider modeling this non-proportionality using a time-dependent covariate (see below) or choosing a fixed point in time and using logistic regression. One feature that Cox regression allows is to model explanatory variables as what are called time-dependent covariates. This is useful when modeling variables that can change throughout time. For example, if one is interested in assessing the impact of chronic graft-versus-host disease (GVHD) on mortality/survival, one must take into account the timing of chronic GVHD given that the patient's chronic GVHD status is not known at HCT. Essentially, patients with GVHD are considered to be in the non-GVHD group until they develop GVHD, at which point they join the group with chronic GVHD. Unfortunately graphical methods do not exist for time-dependent covariates, so if one wishes to display, for example, overall survival among patients with and without GVHD, one must choose a time post-transplant, categorize surviving patients to that time as having GVHD or not, and then plot survival from that point forward. This approach is referred to as a landmark analysis.

As an example of fitting a Cox regression model, we show some results from an analysis of 1007 MDS patients [27], with the goal of identifying risk factors for overall mortality. There were 650 deaths by last contact, providing considerable flexibility in the number of covariates that could be reasonably included in a model. A total of 15 demographic variables were examined (for the purposes of this example, we did not consider variables involving treatment of the disease), some continuous and some categorical, with the categorical variables each containing between two and 11 distinct groups. Table 27.1 summarizes the results of a stepwise regression analysis. Note that for variables with more than two categories, all parameters needed to describe such a variable fully need to go in or out of the model together. Note that in this particular example, year of HCT, patient age, and platelet count were each modeled as a continuous linear variable. Examination of the underlying assumptions indicate that this is a reasonable assumption for age, for example, with hazard ratios by decade relative to 0–30 years being 1.30 (for 30–40 years), 1.72 (for 40–50 years), 1.79 (for 50–60 years), 2.23 (for 60–70 years), and 3.98 (for 70 years and above). Note also that results for groups with "small numbers" for various factors were not presented ("small" was arbitrarily defined in this example as less than 20).

Competing-risks regression

Similarly to the discussion above concerning the log-rank test versus Gray's test when comparing outcomes with competing risks, if one needs to adjust for potential confounding or examine risk factors for outcomes with competing risks there are methods that model the cumulative incidence function rather than the cause-specific hazard function (which is what Cox regression does in the presence of competing risks). One such method was proposed by Fine and Gray [28], where, as in Gray's test, the Fine–Gray method distinguishes patients who are still alive (without failure of any type) from those who have failed from a competing cause, allowing for the appropriate estimation of the effects of the covariates on the cumulative incidence function. Similarly to the distinction between the log-rank test and Gray's test, interpretation of regression modeling for end-points with competing risks needs to consider what is being modeled: the cause-specific hazard for Cox regression, and the cumulative incidence function for Fine–Gray regression.

Table 27.1 Results from fitting a stepwise Cox regression model for overall mortality among 1007 MDS patients

Factor	*N* events/*N* at risk	Hazard ratio	95% CI, *p*-value
FAB/WHO classification[a]			
RA/RARS/RCMD	251/468	1	–
RAEB	211/302	1.48	1.22–1.80, $p < 0.0001$
RAEBT	73/89	1.56	1.17–2.07, $p = 0.002$
tAML	91/107	1.25	0.91–1.72, $p = 0.17$
MDS	8/20	0.89	0.42–1.90, $p = 0.76$
Disease category			
MDS	435/728	1	–
tAML/MDS	215/279	1.57	1.17–2.07, $p < 0.0001$
Five-group cytogenetic risk[b]			
Good	253/451	1	–
Intermediate	112/187	1.06	0.84–1.34, $p = 0.61$
Poor	113/176	1.21	0.95–1.55, $p = 0.12$
Very poor	107/123	2.56	2.01–3.31, $p < 0.0001$
Donor type			
Matched sibling	306/470	1	–
Non-sibling relative	59/77	1.26	0.93–1.71, $p = 0.14$
URD matched	155/266	1.20	0.96–1.50, $p = 0.10$
URD mismatched	110/158	1.54	1.21–1.95, $p = 0.0004$
URD unknown	20/36	1.05	0.65–1.68, $p = 0.86$
Etiology[c]			
De novo	475/767	1	–
AA	32/54	1.40	0.94–2.08, $p = 0.10$
Lymphoma	47/56	1.12	0.79–1.59, $p = 0.53$
Solid tumors	38/57	1.54	1.08–2.20, $p = 0.02$
Autoimmune disorders	17/20	1.80	1.10–2.95, $p = 0.02$
Year of HCT[d]	–	0.96	0.94–0.97, $p < .00001$
Age at HCT[d]	–	1.20	1.12–1.28, $p < 0.0001$
Platelet count[d]	–	0.93	0.89–0.97, $p = 0.0003$

[a] 21 patients with unknown FAB/WHO classification.

[b] 13 patients in category "very good" not shown; 57 with unknown cytogenetics.

[c] 9 patients with myeloproliferative neoplasms, 8 with constitutional marrow failure syndromes, 5 with multiple myeloma, 4 with organ transplant, 2 with accidental radiation, and 17 with other forms of leukemia not shown; 8 with unknown etiology.

[d] Modeled as continuous linear variable; hazard ratio for year of HCT represents the increase in hazard of death associated with each increase in 1 year; hazard ratio for patient age represents the increase in hazard of death associated with each increase in age of one decade; hazard ratio for platelet count represents the increase in hazard of death associated with each increase in platelets of 50,000.

Multiple comparisons

The issue of multiple comparisons is concerned with the fact that the more often one looks for a difference, the more likely one is to find one that is due to chance alone. If one conducts 100 independent comparisons where there is no true difference between any of the groups being compared, one would expect roughly five of the 100 to lead to a statistically significant (at the significance level of 0.05) difference, i.e. a false positive. This is simply a product of how the Type I error rate is defined. Because of this, however, one must be careful about how one interprets results in which a large number of comparisons are made, as the chance of a false positive increases as more comparisons are made. As a simple example, suppose that one declares a coin to be biased if it comes up heads nine or more times in 10 flips. The probability of this occurring under the null hypothesis that the coin is fair (i.e. the probability of a head is 0.5) is, within rounding, 0.0107. This is, therefore, a relatively rare event under the null hypothesis, and if it were to occur, the null hypothesis should be rejected, that is, one would conclude that the coin is biased. If one were to flip 100 fair coins, the probability of any individual coin being declared fair (eight or fewer heads by our definition) is 1 – 0.0107. However, the probability that all 100 coins are declared fair is $(1 - 0.0107)^{100} = 0.34$. Therefore, with 100 fair coins it would be more likely to conclude incorrectly that at least one coin was biased than it would be to conclude correctly that all 100 coins were fair.

There is a large literature on how to adjust p-values for multiple comparisons [29–32]. One approach is to adjust each comparison (and the resulting p-value) so that the overall Type I error rate is 5% (doing so is called controlling the family-wise error rate). In other words, if there is no difference across any of the comparisons being made, then the probability of falsely concluding that at least one of the comparisons is consistent with a true difference is 5%. The simplest approach to control the family-wise error rate is called the Bonferroni correction, where one simply divides each individual p-value by the total number of comparisons. This can be overly conservative if there is even a modest degree of correlation between comparisons, however. For example, suppose one is comparing overall mortality, non-relapse mortality, grades 2–4 acute GVHD, grades 3–4 acute GVHD, chronic GVHD, relapse, relapse-free survival, time to neutrophil recovery, time to platelet recovery, and occurrence of Gram-negative infection between two groups. This totals to 10 comparisons, but clearly these outcomes are correlated and defining statistical significance as 0.05/10 = 0.005, while maintaining the overall Type 1 error rate of 5%, would also lead to an increase in the false-negative rate (i.e. falsely concluding that there is not a difference when there truly is). If two groups are being compared in a randomized phase III clinical trial, then one single end-point should be chosen (see the discussion later in this chapter).

Another approach to multiple comparisons is to control what is called the false discovery rate (FDR) [33]. This approach is particularly useful when a study is considered more exploratory as opposed to the goal being to define definitive differences. The FDR is defined as the expected proportion of false positives among all tests that are significant at the nominal significance level (typically taken to be 0.05). This approach is commonly taken in large-scale studies such as genomic studies, where one might be assessing the association of thousands of genes with outcome.

Clinical trial design in HCT research

In human subject research, confirmed randomized controlled clinical (intervention) trials provide the most compelling and generalizable evidence of an experimental treatment's effectiveness; observational (non-intervention) studies, although informative, are less compelling. Green and Byar [34] suggested that there is a "hierarchy of study designs," from the least convincing (anecdotal case reports) to the more convincing clinical trial (Table 27.2).

While the scientific question to be addressed by the clinical trial largely dictates the structure of the proposed study, prior to FDA approval, or expanded use of a new therapeutic intervention, a single- or multiple-drug regimen must proceed successfully through the phases of clinical trial investigation, including phase 0, a relatively recent trial classification from the FDA:

Phase 0: Evaluation of biological activity; active experimental therapies proceed to phase I.
Phase I: Evaluation of safety and declaration of maximum tolerated dose; safe/tolerable dose proceeds to phase II.
Phase II: Evaluation of effectiveness; experimental therapies that appear more effective than the standard therapy proceed to phase III.
Phase III: Comparison against the standard; therapies shown to be more effective than the standard therapy proceed to phase IV. Note: FDA approval is typically sought at the completion of a successful phase III study.
Phase IV: Evaluation of effects post-marketing; effect intended to hold in the expanded patient population.

Before a clinical trial can be conducted, many regulatory requirements must be satisfied; a comprehensive overview can be found in a book by Bankert and Amdur [35].

Table 27.2 Hierarchy of strength of evidence concerning efficacy of treatment

1. Confirmed randomized controlled clinical trial
2. Single randomized controlled clinical trial
3. Case series with historical controls
4. Retrospective case–control study
5. Analyses of existing databases
6. Series with literature controls
7. Case series without controls
8. Anecdotal case reports

Source: adapted from Green and Byar 1984 [34].

Phase 0 clinical trials

A "phase 0 clinical trial" is a term used to reference early phase I clinical trials. Phase 0 clinical trials are considered exploratory; designed to assess an agent's ability to "hit" its intended target and to provide investigators with a deeper understanding of the pharmacokinetics/pharmacodynamics of the agent or therapy. In phase 0 clinical trials, the agent is often given in small (sub-therapeutic) doses, typically single administration at minimal doses. Because the goal of such studies is to evaluate whether or not the proposed agent or therapy shows promise based on the agent's ability to reach the tumor, in most cases there is very little chance that a patient will benefit from participation. Phase 0 studies are intended to observe biologic effects, effects that could not be attributed to chance alone, thereby demonstrating sufficient potential to pursue further studies. While the use and application of phase 0 designs remain the subject of ongoing debate, discussions regarding the risk–benefit ratio and related ethics of phase 0 studies are often contentious; in 2006, the National Cancer Institute started its phase 0 program, based on FDA guidelines [36,37]. The details of the statistical considerations of such small sample calculations were described in Kummar et al. [36], but like most statistical methods, it is based simply on detecting an effect that would not reasonably be ascribed to chance alone. Theoretically, even a single patient can be sufficient where the observation could never have happened spontaneously; nevertheless, most phase 0 studies typically use 3–15 patients.

Phase I clinical trials

These trials are often first-in-human experiments and are concerned primarily with safety, not efficacy. By their nature, early-phase clinical trials tend to include higher risk patients within a certain age group, with particular clinical and disease characteristics, who typically have not responded well to the clinically indicated/available therapies. Such studies are relatively small, usually requiring no more than 40–50 (and often far fewer) evaluable patients. In many instances, the primary objective of a phase I trial is to determine an appropriate dose of the drug being tested, that is, how much drug can be given without causing undue rates of adverse events. This dose is often referred to as the maximum tolerated dose (MTD), and the MTD is defined as the dose that is associated with a specified true target toxicity [or dose-limiting toxicity (DLT)] rate. In oncology, it is commonly assumed that higher doses of drug lead to increased efficacy, but at the potential expense of added toxicity. Identifying the "correct dose" then becomes a balancing act between finding a dose that is high enough to provide sufficient efficacy, yet not so high that toxicity will outweigh the benefits in efficacy. The design of such a dose-finding study requires specification of this target toxicity rate, and also an unambiguous definition of what constitutes a DLT. Since the definition of the MTD involves the target toxicity rate, failure to specify this renders the dose-finding algorithm uninterpretable. In recent years, phase I designs have benefited from a higher degree of statistical rigor. We present here a few of the more commonly used methods for estimating the MTD. In all approaches, it is assumed that there is a range of dose levels to be (potentially) tested.

On occasion, phase I testing is divided into two steps known as phase Ia and phase Ib. Phase Ia studies normally are conducted as a short-term investigation to ensure safety, tolerability, and feasibility in a limited number of patients before embarking on the longer and more comprehensive phase Ib study. Additional patients are usually then treated during the expansion phase (phase Ib) of the ongoing phase I study. The objectives of the phase Ib study are to assess and characterize further the safety profile, pharmacokinetics, and pharmacodynamics of the investigational agent across multiple dose levels.

3 + 3 design

Probably the most commonly used (yet the least statistically rigorous) method, this approach works well if the target toxicity rate is on the order of 33%. The 3 + 3 design is not, however, a "one-size-fits-all" approach for dose-finding studies. This design dictates that patients are treated in cohorts of three. If no DLTs are observed in a cohort, the next group is treated at the next higher dose level. If two or more DLTs are observed, the next cohort is treated at the next lower dose level (and typically this dose level is never returned to). If one DLT occurs in a cohort, the next cohort is treated at the same level. If one additional DLT is observed (for a total of two of six), then this dose is identified as the MTD. If no additional DLTs are observed (for a total of one of six), the next cohort is treated at the next higher dose level. If two or more additional DLTs occur, then the next lower dose level is identified as the MTD.

There are many variations on this basic design, including identifying the MTD as the dose where one of six DLTs occur or just below the dose in which two of six DLTs occur. If this variation is used, then one should be using a target toxicity rate on the order of 15–20%.

Storer's two-stage design

One feature of this design is that it allows the possibility to move more rapidly through lower dose levels, thereby increasing the chance that fewer patients are treated at lower doses that are presumably less efficacious [38]. The design also more directly (relative to the 3 + 3 design) estimates the dose that is associated with the target DLT rate. In particular, in the first stage, single patients are treated at increasing dose levels until a DLT is observed. At this point, the second stage begins at the next lower dose level. In the second stage, a number of patients are treated in cohorts of a specified size with rules similar to those from the 3 + 3 design. The cohort size is chosen to reflect the target toxicity rate. For example, if the target is around 30%, a reasonable cohort size is three; if the target toxicity rate is 20%, a reasonable cohort size is five. This approach is continued until a specified number of patients have been treated in the second stage, at which point a dose–toxicity curve is fit to the observed data. From this fitted curve, the dose that is associated with the target toxicity rate is then taken to be the estimated MTD. The fitted dose–toxicity curve could theoretically take any form, but typically it is assumed to be a two-parameter logistic model.

Continual reassessment method

This is a Bayesian approach in which the design is adapted based on information gained as the trial progresses [39]. As with any dose-finding study, the design requires specification of a target DLT rate. A "prior" dose–toxicity distribution is assumed based on previous experience. Then a single patient or a cohort of patients is treated at the dose that, based on the investigator's best guess, is associated with the target toxicity rate. Based on the observed data, the dose–toxicity curve is then updated, and the next patient or group of patients is treated at the dose that is associated with the target DLT rate from the updated dose–toxicity distribution. This process is continued until either a specified number of patients are treated or until some level of precision is met. Some modifications of the continual reassessment method (CRM) have been proposed so that escalation cannot increase too rapidly and so that there is less dependence placed on the assumption of the prior distribution.

Bayesian design (considering toxicity and efficacy simultaneously)

Some dose-finding designs consider not only toxicity, but the dose modification is also based on efficacy [40]. This is only feasible if efficacy is a relatively short-term end-point, e.g. early response, acute GVHD, graft failure. This particular design seeks to balance the possibility of increased efficacy with higher dose levels with the downside of increased toxicity at the higher levels. A minimum acceptable efficacy rate is specified along with a maximum acceptable toxicity rate, and the design seeks to find a dose where the efficacy rate exceeds this level while at the same time having a DLT rate lower than the maximum acceptable rate. While such a design examines efficacy, it only does so to help identify the best dose to take forward. Once such a dose has been identified, it has not necessarily been proven to be potentially efficacious as is the goal in a phase II study (see below).

These are just a few of many possible phase I designs, leading to the question of which method one should use. Storer undertook an extensive simulation study to compare the operating characteristics of several different methods under a wide variety of assumed-true scenarios [41]. If one tailors the appropriate method to match the target toxicity rate, many of the methods that were examined led to acceptable operating characteristics. When designing a dose-finding phase I study, it is advisable to examine the operating characteristics of the design to ensure that one has a reasonably high probability of getting an acceptable answer.

Phase II clinical trials

Once one has completed a phase I trial and identified a dose to be taken forward, the next step is to conduct a phase II trial, where the primary objective is to examine potential efficacy. Such trials are typically larger than phase I trials but in the field of HCT rarely enroll more than 80–100 patients. As with phase I trials, there is a variety of phase II designs. We highlight here the salient features of some of the more common designs; more details in addition to other designs can be found elsewhere [42–48].

Single-arm trial: comparison with fixed outcome from historical controls

This is the most common and simplest phase II design. If one has a situation where there are reliable historical data that one hopes to improve upon with the treatment under study, then using traditional methods one can power a study to show a statistically significantly improved outcome over the fixed historical rate. The assumed-true outcome for the experimental treatment can come from preliminary data or from a desired level that, if not achieved, would generate little enthusiasm for continued study. The null hypothesis (assuming a binary outcome, which is common for phase II trials) is then set up as H_0: $p = p_0$ and the alternative hypothesis as H_1: $p = p_1$, where p is the true "success" rate, p_0 is the fixed rate that one wishes to improve upon, and p_1 is the assumed-true rate for the treatment under study. It is important to note that in such a study, the historical rate is treated as fixed, hence the statistical test is a one-sample test.

Frequently, single-arm studies are conducted in two stages so that if there is not sufficient evidence of potential efficacy in the first stage, the trial is terminated early. There are multiple specific approaches to this, the difference in approaches essentially being based on how one defines lack of sufficient evidence of efficacy. One of the most common approaches is known as the Simon optimal design [42]. This approach chooses the size of the first stage, the size of the second stage, the number of "successes" in the first stage required to proceed to the second stage, and the total number of successes (in stages one and two) required to deem the regimen under study as potentially efficacious for a specified significance level, power, response rate under the null hypothesis, and assumed-true response rate under the alternative hypothesis. These parameters are chosen so that the expected sample size (under the significance level and power) is minimized.

As an example, suppose one is testing a regimen intended to decrease the probability of acute GVHD, where historical data indicate a probability of 70% among patients consistent with the eligibility criteria for the trial being designed. If the assumed-true GVHD rate is 50% for the experimental treatment, then with 90% power and a 5% significance level, the Simon optimal design dictates that if 16 or more cases of GVHD occur in the first 24 patients, the trial should be terminated owing to lack of efficacy. If 15 or fewer cases occur, then an additional 39 patients are to be enrolled for a total of 63. If 38 or fewer cases of GVHD occur among the 63 patients, then the drug is regarded as being potentially efficacious. The expected sample size of such a design if the true GVHD rate is 70% is 34.7 patients. Note that the design specifies that the observed sample size is either 24 or 63, and the expected sample size is therefore simply a combination of 24 and 63. In this example, the probability of 16 or more cases of GVHD (the trigger for early termination) among 24 patient under the null hypothesis 0.725, so the expected sample size is $(0.725 \times 24) + [(1 - 0.725) \times 63] = 34.7$.

Randomized phase II clinical trials

In recent years, there has been increasing debate regarding the most appropriate use of randomized designs for phase II studies in HCT. Because efficacy end-points in a single-arm phase II trial can be impacted by differences in patient selection, disease/disease status, type/extent of prior treatment(s), and changes in supportive care, outcomes can vary between the experimental and the historical/reference patient groups. The introduction of these potential biases makes definitive conclusions regarding improvement in outcome problematic. Findings from simulation studies performed by Tang et al. showed that a historical estimate of success that is misspecified by as little as 5% can triple the false-positive rate in an uncontrolled trial [49,50]. Although a case can be made for randomization in place of a single-arm phase II trial, more often than not the expense – both human and financial – associated with the formal comparison of outcomes between different treatment groups can make the pursuit prohibitive.

In general, there is agreement that one of the best uses of the randomized phase II design is in the context of evaluating multiple treatment regimens (≥2), where the primary objective is to select the "better performer." This type of design is referred to as the "phase II selection design" and is not powered to test for differences between treatment arms, rather a comparison of performance [51]. Under the selection design, patients are randomized to one of two or more treatments; each treatment must show some prespecified benefit at the end of the first stage (e.g. percentage decrease in relapse/progression rate) to proceed to the second stage. The time required to enroll, treat, and assess patients for the outcome of interest is intended to be short, both by evaluating after a relatively small number of patients and also by utilizing end-points that are reached early and where the minimal clinically relevant difference is relatively large [51]. Because the emphasis is on selecting the best performer from a group of promising experimental regimens, standard of care or control arms are not typically included.

Phase III clinical trials

If a treatment is found to be potentially efficacious in a phase II trial, then generally the experimental therapy is tested within a phase III trial against either a placebo or the standard treatment for the indication for which the treatment is being given. Typically, the comparison of treatment arms is done based on a single primary end-point, and a difference, or lack thereof, in this end-point is what dictates whether or not the two arms are deemed as being truly different. A single primary end-point is chosen to avoid the issues of multiple comparisons as detailed above. A phase III trial requires a very high level of detail in the written protocol, from the points of view of both planning and implementation and also the planned statistical analysis of the observed data. A well-designed phase III clinical trial is necessary to provide scientific proof of efficacy of the treatment under investigation. In fact, if FDA approval for a drug or approval for a particular indication is being sought, typically two positive phase III trials are required. There are several important components that contribute to a phase III clinical trial, outlined below.

Randomization

The primary rationale for randomization in a phase III trial is the desire to balance the treatment arms with respect to factors known (or unknown) to influence the outcome so that an unbiased comparison between arms can be made. Ideally, one hopes that, on average, the only attribute that separates the arms is the treatment, and therefore any differences observed in outcome can be ascribed to the treatment, where the observed difference is assessed relative to differences expected due to chance alone.

Randomization should only occur after it has been determined that a patient is eligible for study and has consented. It is also desirable to randomize as close to possible to the administration of the treatment arms to avoid patients dropping out without receiving the treatment to which they were randomized. The actual randomization process can be done in any number of ways. It is relatively common to conduct the randomization in "blocks" of a certain size (typically a multiple of the number of arms), such as 4–8 patients, so that at the end of enrollment of each block the number of patients in each arm is the same. Block sizes are generally varied randomly and the size is not specified in the protocol. Another approach to ensure that the number of patients randomized to each arm is roughly the same is adaptive randomization [52]. If imbalance begins to occur between arms, subsequent patients are randomized to the arm with fewer patients with a probability higher than 0.5. This is continued until some specified level of balance is achieved.

Although randomization is conducted to minimize the chance of bias (i.e. one arm having a higher proportion of high-risk patients than the other arm), considerable variation in prognosis can lead to a non-negligible chance of this still occurring. Because of this, the randomization is often stratified, where patients are divided into groups, or strata, based on factors that are known to have a major influence on the outcome. Some examples of common strata for HCT trials include age, disease status, and type of donor. Randomization is then typically conducted within each stratum. One must be careful in such instances not to stratify for too many factors, as the number of strata increases rapidly with additional factors, and some strata are then likely to have relatively few patients enrolled. This can lead to an imbalance in the number of patients randomized to each arm. Typically, stratification is limited to 2–4 factors if possible, although this number certainly depends on the particular factors, the number of patients expected within the various strata, and on the total number of patients to be randomized.

If a patient is randomized, regardless of whether that patient receives the treatment to which they were randomized, they need to be analyzed based on their randomized treatment. This is known as the intent-to-treat principle. The primary analysis in a phase III clinical trial is done on an intent-to-treat basis, as patients who go on to receive a treatment to which they were not randomized could have done so for reasons that can have an influence on the primary end-point. The notion of intent-to-treat is relevant only to randomized trials, although the term is frequently used (inappropriately) is the non-randomized setting. For further discussion on the intent-to-treat principle, see [5,53].

Sample size

One of the most important features of a phase III clinical trial is specification of the sample size. The number of patients to be enrolled needs to be large enough that if meaningful differences are observed, it can be concluded with high probability that such a difference is real and not due to chance alone. On the other hand, phase III trials often are constrained by the number of patients available to be enrolled, and therefore a sample size that is larger than necessary also is not desirable (admittedly, this is a problem that rarely occurs in HCT). The methods used to calculate a sample size are dependent on the primary end-point. The primary end-point can almost always be categorized as being associated with one of three types of outcome: binary, continuous, or time-to-event. In order to calculate the required sample size, one must specify the assumed-true probability of success, value, or rate in each arm. Depending on the outcome, some specification of the variance is also required (depending on the type of outcome, this may or may not need to be explicitly stated). One can either estimate the power to observe a statistically significant difference given the assumed-true parameters, or one can estimate the number of patients required to have a specified power to observe a statistically significant difference. The power (or sample size) is then essentially a function of the assumed-true difference in the outcome divided by the standard error of the difference, with factors included that represent the sample size (or power) and the significance level.

If the outcome is time-to-event, then sample sizes are not as straightforward as for binary or continuous outcomes, as there are many more assumptions that go into the calculation. For time-to-event outcomes, the required sample size decreases as the failure rate increases when the relative difference between groups is the same. For example, if the failure rates are 90% and 45% (twice as high in one group) relative to 30% and 15%, the former case will require fewer patients to attain the same level of power relative to the latter case. In other words, power for time-to-event end-points increases as the number of events increases. Formulas for power exist for such end-points, but they require fairly restrictive assumptions [54]. One particularly flexible method to estimate power for time-to-event end-points is to fit a "piecewise exponential model" to the observed data for the standard or control arm, in addition to the experimental arm, that lead to the phase III trial. If the hazard of failure is constant, then the time-to-event distribution follows an exponential model. One can therefore break the time axis into mutually exclusive and exhaustive windows, within each of which it is assumed that the hazard of failure is constant. In most cases, specification of as few as 5–10 windows can lead to assumed-true time-to-event distributions that closely fit the observed data. From these distributions, one can then randomly generate observed outcomes for a specified number of patients, thereby generating simulated outcomes from a simulated clinical trial. The outcomes for the two groups can then be compared with the appropriate test statistic that is specified for the phase III trial (e.g. the log-rank test), thereby generating a p-value for each simulated trial. This can be done over and over many times, and the proportion of trials in which the p-value is less than the significance level (typically 0.05) represents an estimate of the power of the trial given the specified sample size, significance level, and assumed-true time-to-event distributions.

As an example, consider the data shown in Figure 27.5, where the step function (in black) represents the observed progression-free survival (PFS) for a "standard" therapy group (i.e. the KM estimate). Superimposed on this observed outcome is a piecewise exponential model, where it is assumed that the hazard of relapse or death is constant within each of several windows (but different across these windows). As can be seen, this assumed-true curve nicely fits the observed data. One can then assume that the true hazard of relapse or death for the experimental arm is a constant factor less than that for the standard arm (i.e. that the hazards are proportional), leading to the assumed-true PFS distributions shown for two potential hazard ratios (where the hazard ratio represents the hazard of failure in the standard arm relative to the experimental arm).

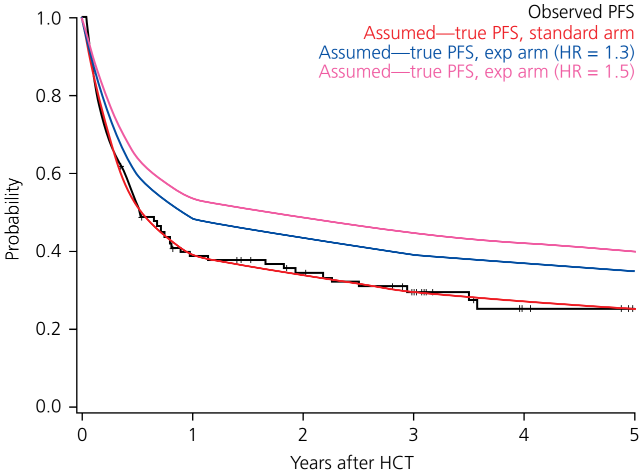

Figure 27.5 Piecewise exponential progression-free survival fit to observed data (assumed-true PFS, standard arm) with assumed-true PFS for two hypothetical experimental arms. (For a color version of this figure, please see the color plate section.)

Once these assumed-true distributions are specified, one can randomly draw PFS times for a specified number of (simulated) patients for each group, thereby generating a simulated clinical trial and its corresponding *p*-value. Since power for time-to-event end-points depends on the number of events, in order to generate PFS times randomly for a specified number of patients one must assume an enrollment period and a minimum follow-up time. Suppose we assume a 3-year enrollment period in the current example with a minimum of 2 years of follow-up. Randomly generating 5000 simulated trials based on the above with 200 patients per group yield an estimated power of 90% (and an average of 255 events) for the case where the hazard ratio HR = 1.5 and 57% power (and an average of 265 events) for the case where HR = 1.3.

Interim analyses

Most phase III trials examine the outcome between groups at least once at some point before all patients have been accrued. If one group is performing dramatically better than the other, then it is not ethical to deny future patients the treatment that is clearly better. On the other hand, if it is extremely unlikely that completion of the trial will lead to a statistically significant difference given the results thus far observed, continuation of the trial may not be worth the effort and expense since the answer (with respect to the primary end-point) is already almost certainly known. The examination of the data before the trial is complete is called an interim analysis. Care must be taken with how interim analyses are conducted, however, owing to the issue of multiple comparisons discussed above. In other words, the more often one examines the data, the more likely one is to see a difference due to chance alone. Moreover, multiple looks at the data lead to a larger significance level at the end of the completion of the trial than what was used to design the study. In order to maintain the overall level of significance at the level specified (usually 0.05), the interim looks require a lower level of significance to stop the trial early. There are many ways to specify the significance levels of the interim looks [5], but all are consistent with the notion that the more interim looks, the lower is the significance level of each. Some approaches also utilize lower significance levels when the interim looks are earlier in the trial and higher significance levels (but still below 0.05) closer to the end of the trial.

Adaptive designs

Recently, adaptive designs for phase III trials have become more popular. In such designs, patients are not necessarily randomized 50:50 as they enter the trial; rather, a particular patient is more likely to receive the treatment that has, on average, had the better outcome up to that point. Such designs are Bayesian, and some feel that they are more ethical. They are not, however, without controversy. Several references contain excellent discussion on the issues, both favorable and unfavorable, of adaptive designs [55–57].

Interpreting the results of phase III trials

As noted above, phase III trials generally should have a single primary end-point, upon which the sample size of the trial and conclusions, for the most part, are based. All trials have secondary end-points that are specified in the protocol, but if the treatment groups are not statistically significantly different for the primary end-point, differences in the secondary end-points at the nominal significance level of 0.05 need to be viewed with caution, again due to multiple comparisons. It is also common to see subgroup analyses conducted at the end of a phase III trial. Such analyses should be specified in the protocol prior to the start of the study, and the significance of such analyses must take into account the multiple tests that have been conducted.

Observational study designs

Although clinical trials are the most scientifically valid method of addressing a clinical hypothesis, they require substantial patient and financial resources, particularly phase III clinical trials, and such resources simply do not exist to answer all questions of interest. Moreover, it is often the case that it would be unethical or not possible to test a particular hypothesis in a randomized clinical trial. For example, suppose one wishes to examine if patients mismatched at HLA-DRB1 at the allele level have more GVHD and mortality than those matched at HLA-DRB1 at the allele level among patients who are matched at HLA-A, -B, and -C at the allele level) and at HLA-DR at the antigen level with their unrelated donor. It would not be ethical to randomize patients to be matched or mismatched with their donor. In fact, all advances in the requirement for HLA matching have been attained from observational studies. Researchers should recognize, however, that the statistical analysis of observational studies can often be more difficult than prospective clinical trials because measuring confounding factors can be difficult, costly, or impossible, and some such factors may not even be recognized. In addition to such confounders, observational studies that examine survival end-points can be misleading if follow-up is not standardized. For example, if standard follow-up is planned for 5 years, and passive follow-up occurs subsequently, the survival estimates will be unreliable, as passive follow-up gathers more information on the more-at-risk patients. Despite these limitations, observational studies can prove to be useful alternatives to clinical trials when a prospective trial is not possible. Observational studies can also be used as a prelude to and motivation for conducting a randomized phase III trial. We discuss below three types of observational studies.

Retrospective cohort study

This is the most common type of observational study performed in the field of HCT. Part of its popularity is due to the existence of large amounts of data from transplants that have already been conducted, either within single institutions or from registry data through organizations such as the Center for International Blood and Marrow Transplant Research (CIBMTR) and the European Group for Blood and Marrow Transplantation (EBMT). Similarly to a clinical trial, an observational study is aimed at answering a particular question among a specified group of patients. Many of the statistical methods used to analyze data from a clinical trial are also appropriate in a retrospective study, although regression analysis is the bread-and-butter tool in such studies. Since patients have not been randomized or accrued based on some list of eligibility criteria, there is a much larger chance of selection bias and unequal distribution of risk factors in retrospective studies. Regression analysis is therefore commonly used to account for these imbalances. The objective of a retrospective cohort study can be broadly categorized as one of three goals: one might be interested in comparing outcomes between groups A and B; one might be interested in testing the impact of a particular variable on outcome; or one might be interested in identifying risk factors for a particular outcome. Note that the last two objectives could be done in the context of a randomized clinical trial, but this would be done after the trial has been completed and would not have been the primary objective (or likely even a secondary objective) of the clinical trial. Therefore, even though the data may have come from a randomized trial, the conclusions from the retrospective study would not carry the same scientific validity as those from the clinical trial.

Given that this type of study is retrospective, patients have already been treated and outcomes observed, and one has no control over factors that were measured as the data have already been collected. If the objective of the study is to compare outcome between two groups or to examine the association of a particular factor with outcome, the appropriate regression technique is employed (based on the type of outcome) and every effort is made to control for factors that may influence outcome so that the observed difference between groups can be attributed to the groups alone or so that the association of the factor examined is not explained by other factors. If the goal is to identify risk factors for an outcome, stepwise regression techniques are typically used. As discussed above, care must be taken in the number of factors that one includes in the regression model, and one also needs to consider the effect of multiple comparisons.

When comparing the outcome between two groups in this sort of study, investigators often wish to impose balance by matching for certain factors, leading to a so-called matched-cohort design. In our experience, we feel that this is often driven by the misplaced desire to obtain two groups that are roughly similar, that is, to mimic a randomized clinical trial. However, if all data for "eligible" patients are available, we prefer to use all patients and then use regression techniques to control for potential imbalances. This will increase the power to see a difference (since the sample size is maximized) and obviates the need for some sort of selection process in the matching of patients. We also frequently see a matched-cohort design misinterpreted as being a matched case–control study (see below). In a cohort study, patients are not identified based on whether or not they experience some outcome.

Retrospective case–control studies

When the outcome of interest is relatively rare, a retrospective case–control study may be the best design for identifying potential risk factors or comparing two groups. In a case–control study, cases are defined as subjects who fail from the outcome of interest. One or more controls are then chosen for each case, where the controls did not fail from the outcome of interest. The controls are typically matched to the appropriate case for certain factors known to influence outcome. Care must be taken not to overmatch cases and controls on more than a few key factors, owing to the increased difficulty of identifying appropriate matched controls. If failure for the outcome under study can occur reasonably late after HCT, controls should be chosen such that the follow-up time is at least as long as the failure time for the relevant case (this is called a nested case–control design).

Since only a subset of controls (i.e. non-failures) is examined, failure rates cannot be directly estimated in a case–control study. Instead, an odds ratio (OR) is calculated using conditional logistic regression to quantify the difference in outcome between groups that are being compared or to assess risk factors that are being examined for association with failure for the outcome being studied. Like the retrospective cohort study design, case–control studies are subject to uncontrolled variation and potential biases. Matching for various factors helps to reduce this, but the results can certainly be confounded by other factors. As such, adjustment for such factors is often essential in order to obtain a more precise estimate of the true effect being examined.

Prospective cohort study

Of the three types of observational discussed, this is likely the least common. In a prospective cohort study, one identifies a population to study and this group is followed forward in time for the outcome of interest. What makes this type of study different from a clinical trial is that there is no intervention given to the patients being followed. This type of study can be used to estimate the incidence of and risk factors for a particular outcome, and it allows the consistent, accurate collection of data that might not otherwise be available. As with the other types of observational studies discussed, adjustment for factors that might affect the outcome is usually necessary. Because such a study is prospective, long follow-up is often required to observe a sufficient number of events relevant to the outcome under study.

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CHAPTER 28

Outcomes Research in Hematopoietic Cell Transplantation

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Introduction

The field of hematopoietic cell transplantation (HCT) has grown dramatically since the first successful allogeneic transplant procedure was performed in 1968. However, as the subspecialty has matured, questions about costs and cost-effectiveness, quality of life (QOL), patient preferences, medical decision-making, and aggregation of different data sources to guide treatment decisions have become relevant. These questions are best addressed by "outcomes" research, a field of study focused on obtaining the best results, broadly defined, given the available medical knowledge and limited healthcare resources. A closely related discipline, health services research, is concerned with social and political determinants of outcome such as access to healthcare and quality of care [1].

There are several features of HCT that make outcomes studies especially relevant: (i) HCT involves high treatment-related risks compared with other medical interventions; (ii) significant practice variation exists; (iii) costs are high; and (iv) the long-term results, considering both disease-free survival (DFS) and QOL, have much room for improvement. On the other hand, several characteristics of HCT make outcomes studies challenging. For example, HCT patients are often not represented in large, administrative databases that collect population-based clinical, outcome, and resource utilization data. The most active transplant centers perform only a few hundred procedures per year, and smaller centers may do fewer than 10. Thus, the overall impact of HCT on the health of the general population and healthcare finances is still relatively small. Also, HCT is changing rapidly, and adequate information on long-term results lags the innovation in the field.

This chapter presents a brief history of outcomes research in American medicine to help frame the research topics and methods. The remainder of the chapter is organized around several specific questions in HCT that outcomes research is well suited to answer. Representative HCT studies are used whenever possible to illustrate the principles discussed. Readers are referred to Chapter 27 (Biostatistical Methods in HCT), Chapter 33 (Assessment of Quality of Life in HCT Recipients) and Chapter 37 (Bone Marrow and Peripheral Blood Cell Donors and Donor Registries) for detailed information on related research methods and data sources.

Definition

It is difficult to state a precise definition of "outcomes research." At some level, all results can be considered outcomes; thus, most scientific investigation is concerned with measurement and interpretation of outcomes. However, phase I, II and III clinical studies addressing efficacy questions are generally excluded from the definition of "outcomes research" when the primary end-points are toxicity, disease control, and survival. Similarly excluded are clinical epidemiology studies that describe an institutional experience with a disease or treatment. However, when the research question begins to consider how well a treatment works outside a clinical trial or institutional setting ("effectiveness"), subjective end-points (e.g. QOL, patient preferences), non-biologic influences on outcomes (e.g. access, quality of care, physician–patient communication, medical decision-making), healthcare policy (e.g. economic evaluation), or aggregation of data from multiple sources (e.g. decision analysis, meta-analysis, use of administrative databases or registry data), then the title "outcomes research" is appropriate. A conceptual framework that distinguishes outcomes research from other types of clinical research is presented in Figure 28.1 [1].

Major questions for outcomes research in HCT include the following:

- What are the costs of HCT and how can they be reduced? (Resource utilization, cost minimization.)
- Are the clinical benefits of HCT worth the monetary cost? (Cost–benefit, cost-effectiveness, cost–utility analysis.)
- What is the patient's experience with HCT? (Qualitative research, QOL, development of patient-reported measures.)
- How can one combine information from several different data sources? (Registry studies, decision analysis, meta-analysis, evidence-based medicine.)
- How can the practice of HCT be improved through health services research? (Access, quality of care, practice variation.)

History

The ultimate goal of outcomes research is to improve the practice of medicine through the provision of data about the effectiveness, costs, risks, and benefits of treatment options, incorporating both individual and societal level considerations. In 2012, the term "comparative effectiveness" was promoted to encompass information on the effectiveness, risks, and benefits of treatments. Hundreds of millions of dollars in research funding are being disbursed through the Patient-Centered Outcomes Research Institute (PCORI), a private, non-profit oversight committee created by the Affordable Care

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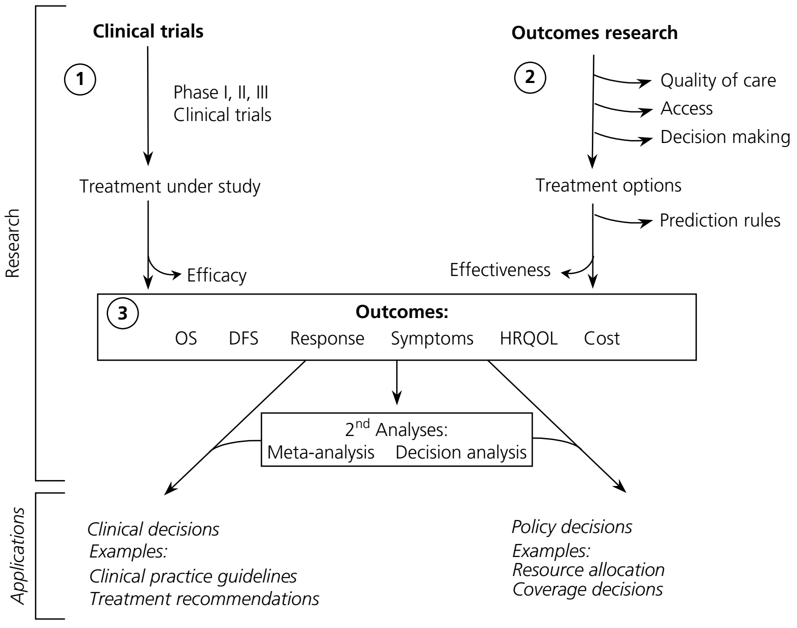


Figure 28.1 Conceptual framework. Interaction is shown between research topics, end-points, analytic techniques, and applications in defining outcomes research. In **1** are depicted the classical clinical trials and analytic techniques that are not outcomes research. Item **2** shows the study topics, end-points, and analytic techniques that are considered to be outcomes research. Outcomes depicted in **3** may or may not constitute outcomes research, depending on the context. For example, overall survival as measured in a phase III trial is not an outcomes study (efficacy), whereas it is if observed in a large community cohort (effectiveness). Symptoms have both efficacy and outcomes influences. Applications are indicated in *italics* and may emanate from either clinical trials or outcomes research. DFS, disease-free survival; HRQOL, health-related quality-of-life; OS, overall survival. Source: Lee et al. 2000 [1]. Reproduced with permission of Oxford University Press.

Act legislation. But approaches to achieve this goal in the United States have been ongoing for a century, influenced by the social and political environment.

The "father" of the American outcomes movement was a surgeon named Ernst Codman. As early as 1914 he argued that the quality of hospitals could be judged only if procedure success rates were made available on a routine basis [2]. He advocated standardized measures of outcomes so that different institutions could be compared on a level playing field. In 1966, Avides Donabedian reintroduced the term "outcomes" when he developed his concept of quality assessment and its three components: *structure*, *process*, and *outcome*. He broadened the end-points of interest: "Although some outcomes are generally unmistakable and easy to measure (death, for example), other outcomes, not so clearly defined, can be difficult to measure. These include patient attitudes and satisfactions, social restoration, and physical disability, and rehabilitation" [3].

In the 1970s and 1980s, the looming crisis of healthcare costs pushed outcomes research into the political spotlight. Archie Cochrane, after whom the Cochrane evidence-based database is named, warned that the medical system would become bankrupt if expensive technologies were routinely applied without evidence of benefit [4]. In 1973, Wennberg and Gittelsohn documented surprising geographic variations in resource utilization, expenditures, and rates of hospitalization and procedures without differences in outcomes [5]. This observation focused attention on practice variation and the possible cost savings that could be realized by eliminating unnecessary procedures.

Several national databases were established to study practice variation in the United States. These included the National Cancer Data Base (focusing on surgical practice) and the linkage of Medicare billing and Surveillance, Epidemiology and End Results (SEER) data (providing resource utilization and cancer-specific information on patients common to these databases). In addition, the Federal government established the Agency for Health Care Research and Quality (AHRQ) to support outcomes research. Although this organization has been renamed and refocused several times, it is probably most famous for developing the Patient Outcomes Assessment Research Teams (PORTs) in the 1990s. The PORT program was designed to determine the relative effectiveness of treatments for common diseases, with an undertone of trying to standardize clinical practice.

The 1990s were a period of national economic growth in the United States, and concerns about healthcare financing for specific procedures faded into the background behind debate about the overall structure of healthcare coverage. Managed care and health maintenance organizations thrived, and physicians practiced in a more constrained setting with new concerns about financial risk. The incentive to save money may have replaced the original goal of outcomes research, which is to spend money wisely to improve overall health. As it became clear that the Clinton administration's major restructuring of healthcare delivery and financing would fail, outcomes research faded from the political spotlight and returned to its origins, focusing on more applied questions. Treatment guidelines proliferated in the late 1990s, but these efforts grew out of a desire to standardize physician practice and improve patient outcomes rather than to contain costs.

In 2000–2001 the technology bubble popped and in 2007–2008 the housing and credit debacles plunged the country into financial crisis. President Obama signed the American Recovery and Reinvestment Act of 2009, which included \$1.1 billion dollars for comparative effectiveness research, to be overseen by the Federal Coordinating Council. However, the Patient Protection and Affordable Care Act of 2010 superseded the previous funding and structure, and created PCORI. In 2012, the US Supreme Court upheld the constitutionality of the Affordable Care Act by a 5–4 margin.

It is difficult to tell what the future holds for outcomes/comparative effectiveness research, especially in HCT. HCT is a highly specialized practice that for many will fall outside healthcare policy and economic considerations. Nevertheless, there are many aspects of HCT that may make outcomes research more relevant. Attention to long-term outcomes, patient decision-making, and guidance based on what is known about short-term outcomes is especially critical for the field.

Specific questions for the field of HCT

What are the costs of HCT, and how can they be reduced?

On a per patient basis, the costs of HCT are high relative to other available medical interventions, ranging from approximately $70,000 for an uncomplicated autologous procedure to $200,000 for an allogeneic, myeloablative procedure using an unrelated donor [6,7]. According to AHRQ, HCT is one of the 10 medical procedures with the fastest rate of cost increases due to both increasing hospital days and costs of hospitalization [8]. The investment in infrastructure is immense, requiring support of the transplant centers and national resources such as the National Marrow Donor Program and the public cord blood banks. In the United States, private and public payers have tried to limit access to some HCT procedures, but are often forced by state law, threat of patient lawsuits, or public opinion to acquiesce and provide coverage for the procedures.

Well-established research methods are available for quantifying monetary costs [9–11]. “Direct medical” cost refers to the monetary value of goods and services provided. These costs are usually captured through administrative billing systems or other itemized methods of determining resource utilization. Notably, countries with nationalized healthcare systems often have poorly developed administrative cost databases, making cost research in these countries difficult or impossible. The distinction between “charges” and “costs” is important in the United States. Charges are the amount billed to the patient or insurance company. Costs are usually lower and reflect the actual resources needed to provide a service. Because healthcare organizations offset one expense against another and usually expect some amount of profit, charges are not directly linked to the resources needed to provide a service.

Outcomes studies favor the use of costs since they reflect the actual resources expended. When costs are not available directly, conversion between charges and costs may be determined by “ratios of costs to charges” (RCCs), a fraction recalculated on an annual basis. Institutions often aggregate logical groups such as clinical departments when setting their RCCs. Fixed costs (such as physical space, personnel) and variable costs (supplies) are totaled and divided by the amount billed by the department during the same period of time.

Direct *non-medical* costs are expenditures related to healthcare, but not directly used for goods and services (e.g. transportation to the hospital, hotel charges for family members accompanying patients). These costs have proven much more difficult to quantify since they must be captured directly from patients through cost diaries or receipts. *Indirect* non-medical costs are even harder to quantify and include time off work and the loss of future earnings. Which costs to include in the analysis depends on the desired perspective. The “societal” perspective includes all costs to the system regardless of who pays. Other perspectives can be imagined, such as the hospital, insurance company, or patient, and would include only costs borne by that payer.

One important feature of costs is that they vary by year because of inflation, so costs in different years must be “inflation adjusted” to allow comparability of studies performed at different times (see http://stats.bls.gov). “Discounting” is distinct from inflation adjustment, and is normally set at 3% per annum. Discounting reflects the fact that costs and benefits in the future are valued less than those that are immediately available, and allows conversion of future dollars or future improvements in health to their current value.

Once the relevant costs have been captured, they can be analyzed in a variety of ways. Studies may simply report the costs of an intervention or look for patterns of costs, predictors of costs, or ways to decrease costs. Totals, breakdowns by specific categories, trends over time, and association with clinical characteristics or treatments have all been reported in HCT. For example, several studies have evaluated the costs associated with specific complications or patient characteristics [12,13]. Cost-minimization studies compare the costs of treatment approaches that result in similar clinical patient outcomes. In these cases, adoption of the least costly approach saves money and does not compromise patient outcomes [14–16].

Are the clinical benefits of HCT worth the monetary costs?

Deciding whether the clinical benefits of HCT are worth the monetary costs may seem to conflict with a physician’s duty to their patient. However, in a society where healthcare dollars are constrained, spending money for one person’s procedure ultimately means that another person may not receive some necessary treatment. As discussed below, the various forms of economic analysis (cost–benefit, cost-effectiveness and cost–utility) differ primarily in how they quantify clinical benefits. However, all are trying to provide information that may be used by policy makers to allocate resources and maximize the health and welfare of the entire population.

Cost–benefit analysis requires that clinical benefits be converted into monetary values to determine the net financial impact of an intervention. This is sometimes straightforward (inexpensive prophylactic antibiotics may prevent costly infections later) but is often quite complicated and fraught with unpalatable value judgments. For example, what is the economic value of a life extended or saved? Attempts to use income as a surrogate lead to the uncomfortable conclusion that the lives of high-wage earners are more valuable than those of homemakers or retired people [9]. As a consequence, cost-benefit analysis is rarely performed in healthcare; it is much more common in business and environmental applications.

Cost-effectiveness analysis avoids such value judgments by calculating a “cost/effectiveness ratio” expressed as dollars per unit of clinical benefit [10]. To facilitate comparison across interventions, clinical benefit is usually measured in years of life gained (life-years, or LYs), but may be any clinically recognized unit of benefit [e.g. cases of chronic graft-versus-host disease (GVHD) prevented, days of hospitalization, lives saved]. Cost/effectiveness ratios are by definition comparisons of one treatment approach versus another (which may be “no treatment”) since they are calculated as

$$\text{(cost of treatment X minus cost of treatment Y)} / \text{(benefit of X minus benefit of Y)}$$

Because cost-effectiveness analyses are intended for policy makers, it is important to specify the perspective (e.g. government program, hospital, health plan) and time horizon (e.g. 1 year, 100 years) of the analysis in order to reflect which costs and benefits were included. The strategy that is most cost-effective for a health maintenance organization may not be the one that is most cost-effective from the viewpoint of a hospital, the patient, or society.

Many people use the terms “cost-effectiveness analysis” and “cost–utility analysis” interchangeably. However, a *cost–utility analysis* specifically incorporates QOL considerations and usually has a denominator of quality-adjusted life-years (QALYs) where survival time is adjusted for the QOL associated with that survival. These adjustment factors are called “patient utilities” and usually range from 0 (equivalent to being dead) to 1.0 (the year of life is fully valued). A utility less than 0 represents a health state worse than death. Utilities fulfill the mathematical condition of linearity so that 1 year of perfect health is considered equal in value to 2 years of life with a utility of 0.5. Some quoted patient utilities for health states are 0.98 for suffering the side effects of beta-blockers [17], 0.8 when 1 year after autologous transplantation for non-Hodgkin lymphoma (NHL) [18], and 0.5 following a stroke [19]. Interestingly, the Affordable Care Act specifically prohibits inclusion of utilities that would value the lives of elderly, disabled or terminally ill people less than those of young and healthy people. Empiric data suggest that age is not necessarily associated with worse QOL, and older people often report better QOL than younger people. Conversely, failure to account for worse QOL might undervalue interventions that prevent disability or terminal illness.

What makes cost-effectiveness and cost–utility analyses powerful is that diverse interventions may be compared and selected for their ability to provide maximum health benefit for money spent. For example, if healthcare dollars are limited, these methods allow some rational basis for recommending whether society should routinely provide coverage for a patient with acute myeloid leukemia (AML) in third complete remission scheduled for an unrelated donor HCT, or instead cover patients with congestive heart failure and severe diabetes who need heart transplants. Similarly, one can compare the healthcare gain of one high-cost treatment procedure, such as an autologous HCT for relapsed NHL, with the preventive strategy of providing statin therapy to several middle-aged men. Based on the cost/effectiveness ratio for hemodialysis, a procedure covered separately by the Federally funded Medicare program and thus available to all people, an acceptable cost/effectiveness ratio of <$50,000 per QALY has been proposed. A ratio of >$100,000 is questionable because that money applied elsewhere may buy better health for the population. A ratio between $50,000 and $100,000 per QALY is in the gray zone. Some countries, such as Australia and Canada, require information on cost-effectiveness prior to drug approval. Table 28.1 shows examples of published cost-effectiveness and cost–utility studies in HCT and some comparisons in other fields [20–28]. Online resources such as https://research.tufts-nemc.org/cear4/ contain comprehensive lists of cost analyses and patient utilities [29]. League tables help to put cost/effectiveness ratios into perspective.

Table 28.1 Examples of cost-effectiveness and cost–utility studies in hematopoietic cell transplantation (HCT) and medicine

Ref.	Year of publication	Treatment	Alternative	Cost/effectiveness ratio[a]
[20]	1989	Allogeneic BMT for AML	Conventional chemotherapy	$10,000/LY
[21]	1992	Autologous BMT for HD in 2nd CR	Conventional chemotherapy	$26,000/LY
[22]	1997	Autologous BMT for relapsed NHL	Conventional chemotherapy	$9200/LY
[28]	1998	Unrelated donor BMT for stable phase CML	IFN-α	$51,800/QALY
[23]	1999	Second allogeneic transplantation after relapse of acute leukemia	Conventional chemotherapy	$52,000/LY
[24]	2001	Autologous PBSCT for MM	Conventional chemotherapy	$23,300/LY*
[25]	1989	Smoking cessation program	No intervention	$1300/LY
[26]	1987	Hemodialysis	No dialysis	$50,000/LY
[27]	1990	Captopril for hypertension	No therapy	$72,000/LY

AML, acute myeloid leukemia; BMT, bone marrow transplantation; CML, chronic myelogenous leukemia; CR, complete remission; HD, Hodgkin disease; IFN, interferon; LY, life-year; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; PBSCT, peripheral blood stem-cell transplantation; QALY, quality-adjusted life-year.

[a] £0.64 = $1.00.

It would be naïve to believe that costs do not affect access to HCT, although sometimes policies can be surprising. Gratwohl et al. found that countries with low or medium gross national incomes are performing the same number of HCTs for chronic myelogenous leukemia (CML), whereas the transplantation rate has fallen substantially in high-income countries. It is less costly overall for poorer countries to perform an up-front HCT than to pay for lifetime drug therapy or several years of drug therapy followed by eventual HCT [30].

What is the patient's experience with HCT?

Beyond the traditional biological end-points of survival and relapse reported in HCT studies, outcomes research tries to measure and put into perspective other factors that determine whether an intervention is ultimately judged a success or failure. Many of these factors are subjective (not directly observable) and are referred to as "constructs." For example, measurement of health-related quality of life (HRQOL, the QOL related to health, disease, and medical treatment) and patient utilities are considered part of outcomes research. Measuring these end-points relies heavily on methods developed for survey research, the collection of data directly from patients.

Qualitative methods

The goal of qualitative studies is to capture the breadth of possible patient attitudes or experiences. One forum is a focus group, in which 8–10 people are led by a moderator and discuss particular topics. Focus groups usually last about 2 hours, and participants may be paid a nominal amount for participation. They are often audio- or videotaped, with an additional researcher taking notes. The interactive nature of the communication process allows topics to be probed and ideas developed under the influence of group dynamics, which may lead to unexpected insights about the topic under discussion. Focus groups are often used for formative research to explore patient attitudes and opinions prior to launching a formal study.

Qualitative information may also be collected through interviews or open-ended survey questions. In contrast to the majority of studies in which generalizability is critical, the goal in truly qualitative studies is not to obtain a representative sample. In fact, "purposive" or targeted sampling can be performed to ensure representation of the spectrum of possible patient experiences. For example, if 80% of the population have a typical experience, 5% have a less typical experience, and the remaining 15% all have unique experiences, the goal would be to interview 17 people, one from the majority, one from the minority, and all 15 who had unique experiences. Usually, transcriptions are made of the interviews, and qualitative coding software is used to mark the transcripts for easier analysis. Transcripts are reviewed by a limited number of individuals who code them for themes, aggregate the concepts into broader groups if possible, and report on the range of patient experiences.

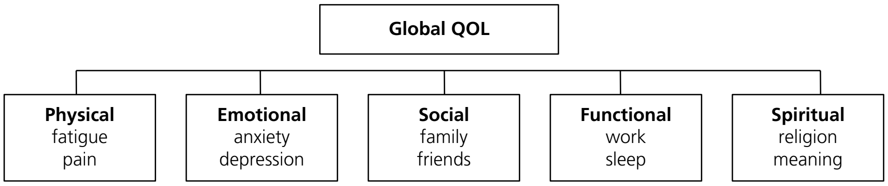
Qualitative methods have been used in several studies to evaluate aspects of recovery following HCT [31–34]. These studies revealed several themes that are not particularly well covered in standardized instruments, for example, strategies that patients use to compensate for limitations, multiple losses in all aspects of their lives and the greater appreciation for life brought about by the HCT experience.

Quality of life

Quality of life (QOL) is composed of diverse determinants, including physical abilities, symptoms, social well-being, psychoemotional status, and spiritual/existential experiences (for details regarding QOL after HCT see Chapter 33). QOL studies in HCT have generally sought to: (i) describe the long-term QOL, adaptation, and recuperation of patients, (ii) find predictors of better or worse QOL, and (iii) compare populations treated with different procedures. There are numerous, multi-dimensional, validated instruments available for measuring QOL in HCT. When scored according to psychometrically tested methods, they provide standardized QOL information that may be compared with other populations. One commonly used instrument is the Medical Outcomes Study Short Form 36 (SF-36), a generic multidimensional 36-item instrument that has been used on thousands of patients and healthy people to measure physical and mental health status [35]. Other cancer-specific instruments include the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (QLQ) C30 (30 items) [36–38] and the Functional Assessment of Chronic Illness Therapies (FACT) (27 items) [39]. Both cancer-specific instruments also offer HCT-specific modules that can be added to the core form to capture issues specific to HCT. There is a fee to use the SF-36 whereas the EORTC and FACT instruments are free for academic studies if the trial is registered. The instruments are available in multiple languages, and respondents must also be able to complete the survey in a language with which they are comfortable. It is a common mistake to assume that interpreters can administer English instruments to a non-English speaker. Instead, the validated version in the subject's native language should be used since interpreters may unintentionally change the meanings of items and responses. Figure 28.2 shows an example of the physical functioning scales from the core instruments. Of note, comparative studies have shown that despite similarly named subscales, instruments are actually measuring different constructs and it is difficult to compare studies unless they use identical instruments [40].

Since 2004, the Patient Reported Outcomes Measurement Information System (PROMIS) team has been working to create item banks for common domains of health and well-being (www.nihpromis.org). These items have not been used in HCT research yet.

Biostatistical methods for analyzing QOL data can be complicated because they must address issues of longitudinal data analysis, informatively missing data and other problems exacerbated by the nature of self-reported QOL data [41–44]. Repeated measures analysis and mixed models allow differences between populations and over time to be studied. Developing methods to place QOL differences into their clinical context is an active area of research. Although validated scales are psychometrically sound and allow comparison of treatment groups by statistical testing, the results of QOL studies are not intuitive to patients and physicians [45,46]. For example, a survival difference of 10% is easily interpretable, but many find it harder to interpret a QOL difference of 50 versus 35 on a given scale and place such an observation in its clinical context. There is no intuitive feeling for what a person with a score of 50 feels like compared with someone with a score of 35. To address this limitation, the concept of a "clinically meaningful difference" was introduced, defined as the difference in QOL that would prompt adoption of the intervention or a change in practice. It is the difference in a scale that is "noticeable" or "meaningful" to patients. There are two approaches to its determination: anchor-based and distribution-based. Anchor-based methods rely on patient-reported differences to determine what is clinically meaningful. For example, patients are asked a global change question such as, "Overall, is your QOL a lot better, a little better, somewhat better, somewhat worse, a little worse or a lot worse." This overall category is then compared with their QOL scores [47–49]. However, this method uses patient-perceived differences in QOL as the gold standard, raising the question of why we cannot just ask patients directly about changes in their QOL. The second approach is based on the statistical distribution of QOL



SF-36 (physical)
Does your health now limit you in…

- Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports
- Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf
- Lifting or carrying groceries
- Climbing several flights of stairs
- Climbing one flight of stairs
- Bending, kneeling, or stooping
- Walking more than a mile
- Walking several blocks
- Walking one block
- Bathing or dressing yourself

EORTC (physical)

- Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?
- Do you have any trouble taking a long walk?
- Do you have any trouble taking a short walk outside of the house?
- Do you have to stay in bed or in a chair for most of the day?
- Do you need help with eating, dressing, washing yourself or using the toilet?

FACT (physical)

- I have a lack of energy
- I have nausea
- Because of my physical condition, I have trouble meeting the needs of my family
- I have pain
- I am bothered by side effects of treatment
- I feel ill
- I am forced to spend time in bed

Figure 28.2 Dimensions of quality of life and examples of the physical domain from validated questionnaires.

scores. Generally, a difference of half a standard deviation is considered to be clinically meaningful.

After HCT, patients generally rate their global QOL fairly highly despite many specific physical and emotional difficulties. One possible explanation for this paradox is called "response shift," the theory that perceptions of current QOL are more dependent on one's perspective than on one's actual health, and that this perspective changes with illness [50]. For example, consider two previously healthy patients with identical diseases, physical ailments, and social support systems. Upon diagnosis, the first patient believes he is afflicted with a terrible disease and embraces HCT as a life-saving procedure. Afterwards he compares himself with others who died, and rates his overall QOL fairly highly as a result of his response shift. The second patient considered himself healthy before the transplant despite his disease and reluctantly undergoes transplantation. After HCT, he pines for his previously healthy self and is very dissatisfied with his QOL. This patient did not have a response shift.

Results and conclusions of specific QOL studies are discussed in more depth in Chapter 33.

How are patient-reported measures developed?

Instrument (or survey) development is considered a facet of outcomes research because it establishes the validity of clinical tools to measure subjective end-points. In order for an instrument to be useful, it needs to reflect what it purports to measure (validity), be an accurate measure (reliability), separate people into clinically meaningful groups (discrimination), and detect important changes (sensitivity). Instrument development from scratch is a demanding process. First, a list of relevant concepts should be created from prior literature, focus groups, or other means of formative research. Then, a draft scale is created. Attention should be given to the wording of specific questions and response items to allow sufficient variability to capture the range of clinical conditions. A pilot study is then conducted with cognitive interviewing to ensure that patients understand the questions and are selecting response options consistent with their intent. Finally, a larger study is performed to document validity, reliability, and sensitivity. Many survey developers neglect the final step of confirming sensitivity to change. This is an important feature since many instruments are intended to describe the experiences of population subgroups, compare one population with another, or show changes over time.

Reliability refers to whether a measure is consistently reflecting the true status of a subject. Internal reliability is usually reported as a Cronbach's alpha (α) with acceptable values >0.7. Cronbach's alpha measures whether items are correlated with each other and measure the same underlying construct. Stability of measurements is reported as "test–retest reliability," the correlation between two measurements separated in time when an individual's status has not changed. The range is from 0 to 1.0, with higher values reflecting greater stability and values >0.5 generally considered acceptable. If test–retest reliability is <0.5 and the subject's clinical situation has not changed, the scale is probably susceptible to influences unrelated to the clinical status of the person.

Validity refers to whether an instrument is truly reflecting what it is supposed to measure, and is usually expressed as correlation coefficients or effect sizes. Content validity refers to how well the scale measures the different aspects of the construct. Convergent validity is demonstrated when the scale correlates highly with other scales measuring similar constructs, whereas discriminant validity means that there is little correlation with scales measuring unrelated concepts. Discrimination refers to the ability of the scale to separate people into clinically meaningful groups, whereas sensitivity to change means that as a person's clinical situation changes, they should score differently on the instrument. For example, Lee et al. developed a chronic GVHD symptom scale [51]. This scale was designed to be self-administered and brief (5 minutes) and to follow patients with chronic GVHD over time to detect improvement or worsening in their symptoms.

How can one combine information from several different data sources?

Registry studies

Several large transplant registries were established to provide national and international information on the outcomes of HCT. These include the Center for International Blood and Marrow Transplant Research (CIBMTR) (http://www.cibmtr.org), the National Marrow Donor Program (NMDP) (http://bethematch.org), European Group for Blood and Marrow Transplantation (EBMT) (http://www.ebmt.org), and Eurocord (http://eurocord.org). These registries collect, computerize, and make available data for analyses. Although they suffer from limitations common to registries, including incomplete capture of all procedures, problems with data standardization, validation issues, and difficulty obtaining detailed clinical information including long-term follow up, they also provide the only means for determining the effectiveness of HCT as practiced outside clinical trials and single institutions. Many important research questions can only be answered by registry studies because large patient numbers are required. Rarer diseases and clinical situations for which no single institution has adequate experience are also best approached by registry studies.

Large administrative databases have not been useful for HCT research in the past, with two exceptions. The Nationwide In-patient Sample provides a representative sample of inpatient hospitalization lengths of stay, costs, and hospital codes for more than 33 US states. This database has been used for some economic studies [52]. Majhail et al. used a private payer database to document the cost of autologous and allogeneic transplantation [7]. In 2006, the C. W. Bill Young Cell Transplantation Program mandated development of a Stem Cell Therapeutics Outcomes Database (SCTOD) in the United States to collect outcomes data on all procedures using allogeneic hematopoietic cells. This important resource is already providing a rich research database of population-based data on HCT use.

Decision analysis

There is rarely a definitive clinical trial or report that provides all the data necessary to settle a clinical question. Many people believe that they can weigh complicated decisions fairly, but research shows that this ad hoc approach is subject to serious cognitive biases and frequently results in suboptimal decisions. Decision analysis uses computer modeling to recommend the optimal treatment choice based on what is known about the probabilities and consequences of different treatment options [53]. Advanced programming capabilities allow the probabilities of different outcomes to vary depending upon a patient's characteristics, time from diagnosis, or prior clinical course, assuming such data are available.

The results of decision analysis provide a population-based approach to recommend an optimal treatment choice [54]. Comparisons of LYs or QALYs obtained from a decision analysis are not amenable to statistical testing in the classic sense, since p-values and confidence intervals (CIs) reflect the uncertainty in measurements and the likelihood of chance findings. In decision analysis, it is up to the reader to compare the gains in LYs or QALYs and determine if one treatment is optimal. Obviously, such comparison is easier when the survival benefit associated with one option is several years and the other offers several weeks. Published league tables can help put gains in life expectancy into perspective [55]. In practical terms, a decision analysis may not distinguish between one individual surviving an extra 10 years and five individuals surviving an extra 2 years, although patients may view these outcomes differently. Sometimes decision analysis can identify key pieces of information that *should* influence treatment decisions and, conversely, point out which considerations *should not* affect a rational decision. Several assumptions may be tested at the same time to see if any combination of values would change conclusions.

Many decision analyses have been performed in HCT. For example, an analysis of allogeneic bone marrow transplantation (BMT) versus periodic blood transfusion for patients with sickle cell anemia and elevated cerebral blood velocities suggested that either treatment approach was reasonable [56]. An analysis of immediate versus delayed HCT in patients with myelodysplastic syndrome (MDS) suggested that patients with intermediate-2 or high-risk MDS should undergo up-front HCT. In contrast, the population with lower risk MDS maximized their LYs with delayed HCT [57]. An analysis of allogeneic HCT versus chemotherapy for patients with AML in first remission suggested that HCT was beneficial for older patients with a related donor and younger patients with unfavorable cytogenetics [58].

Meta-analysis

Meta-analysis allows the results of several clinical studies to be aggregated, increasing the power of the analysis and enhancing confidence in the results [59–62]. This statistical technique is particularly useful in detecting treatment differences if negative studies are due to small sample size and lack of power. *Study-level* meta-analyses use individual studies as the unit of analysis. Results are displayed as point estimates and CIs for each study with some indicator (size of a dot, width of a box) to reflect relative study size. A diamond is often used to depict the aggregate estimate. Both randomized controlled trials and observational studies are often included in meta-analyses. *Patient-level* meta-analyses retrieve and analyze data on individual patients, although the source of each patient is incorporated into the analysis. For example, the Stem Cell Trialists' Collaborative Group aggregated patient-level data from nine randomized trials in human leukocyte antigen (HLA)-identical sibling HCT. The study concluded that peripheral blood is associated with decreased relapse rates and better overall and disease-free survival in patients with late-stage disease, but with the disadvantage of more extensive chronic GVHD [63].

All meta-analyses depend upon data available in the literature or otherwise attainable. Hence publication bias may significantly affect results. A funnel plot is a graphic that can help determine if a meta-analysis is likely to suffer from publication bias. Effect size is plotted against study size. If all studies are published and available regardless of their conclusions, then the plot should result in a funnel shape with the apex centered on the true value. This pattern occurs because the ranges of effect sizes are wider for the smaller studies due to statistical factors, whereas larger studies should provide results closer to the actual truth.

Evidence-based medicine

Critical reviews of the literature summarize the available evidence for or against certain practices, and they are often translated into practice guidelines. The methodology for evidence-based reviews is very well established and rigorous, although the specific terminology used to reflect the levels of evidence and grades of recommendation have evolved. A typical grading system for critical reviews is shown in Table 28.2, and is based on the type, frequency, and consistency of evidence [64].

Table 28.2 Levels of evidence and grades of recommendation

Level	Types of evidence
1++	High-quality meta-analyses, systematic reviews of RCTs, or RCTs with a very low risk of bias
1+	Well-conducted meta-analyses, systematic reviews of RCTs, or RCTs with a low risk of bias
1–	Meta-analyses, systematic reviews of RCTs, or RCTs with a high risk of bias
2++	High-quality systematic reviews of case–control or cohort studies; high-quality case–control or cohort studies with a very low risk of confounding, bias, or chance and a high probability that the relationship is causal
2+	Well-conducted case–control or cohort studies with a low risk of confounding, bias, or chance and a moderate probability that the relationship is causal
2–	Case–control or cohort studies with a high risk of confounding, bias, or chance and a significant risk that the relationship is not causal
3	Non-analytic studies, for example, case reports or case series
4	Expert opinion
Grade	**Grade of recommendation**
A	At least 1 meta-analysis, systematic review, or RCT rated as 1++, directly applicable to the target population or a systematic review of RCTs or a body of evidence consisting principally of studies rated as 1+, directly applicable to the target population and demonstrating overall consistency of results
B	A body of evidence including studies rated as 2++, directly applicable to the target population, and demonstrating overall consistency of results or extrapolated evidence from studies rated as 1++ or 1+
C	A body of evidence including studies rated as 2+, directly applicable to the target population, and demonstrating overall consistency of results or extrapolated evidence from studies rated as 2++
D	Evidence level 3 or 4 or extrapolated evidence from studies rated as 2+

RCT, randomized controlled trial.
Source: Jones et al. 2005 [64]. Reproduced with permission of Elsevier.

One practical limitation of evidence-based reviews compared with consensus statements or clinical reviews is that the published evidence is strictly interpreted. Lack of randomized controlled trials in some areas can result in the frustrating conclusion that "evidence is insufficient to recommend for or against" a particular practice, even when widespread use seems to support its effectiveness [65]. However, the results of the Women's Health Initiative, showing that routine hormone replacement after menopause can cause more harm than good [66], and lack of efficacy seen in trials of autologous HCT for high-risk or advanced breast cancer [67–69], reinforce the idea that randomized trials provide the highest level of evidence for a practice.

Many organizations have produced evidence-based or consensus practice guidelines. A non-exhaustive list relevant to transplantation includes the American Society for Blood and Marrow Transplantation (www.asbmt.org), Cochrane Collaboration, Cancer Care of Ontario, National Comprehensive Cancer Network (NCCN) (http://www.nccn.org), and Physician Data Query (PDQ) (http://www.cancer.gov/cancertopics/pdq). However, relatively little research has evaluated the influence of practice guidelines on clinical practice and patient outcomes, and what has been published suggests less improvement than hoped for [70].

How can the practice of HCT be improved through health services research?

Access

Studies seeking to understand the non-medical barriers to appropriate healthcare are termed "access" studies. They generally focus on socioeconomic, political, cultural, and other non-biological factors. For example, internationally there is a strong association between transplant rate and team density, gross national product per capita, and healthcare expenditures per capita suggesting better access in some countries [71]. In the United States, ethnic and racial minorities have long been under-represented in HCT statistics for unclear reasons, and survival varies by racial group even after controlling for disease and transplant characteristics [72]. Neither GVHD nor relapse appears to explain the higher mortality seen in certain racial groups [73].

Unfortunately, one of the best data sources for access studies is not relevant for HCT. The linked SEER–Medicare database provides cancer-specific information and inpatient and outpatient billing data on approximately 14% of the US population, but is limited to patients aged 65 years or older [74]. The size and comprehensiveness of this database along with the fact that it is population-based have made it a tremendous resource for studies elsewhere in medicine. For example, researchers have found that African Americans are less likely than white Americans to receive screening examinations for cancer, to be diagnosed with cancer in its early stages, and to receive adjuvant therapy and aggressive care [75–81]. African Americans are also less likely to undergo some invasive procedures than white Americans, such as renal transplantation, even after correcting for clinical characteristics [82] and patient preferences [83]. However, once access to treatment is controlled, survival and DFS are similar between African American and white American patients, suggesting that the biological responses to treatment are comparable [84,85].

Quality of care

HCT has been relatively spared from scrutiny about quality of care. Significant practice variation has been tolerated and, in fact, encouraged, as a means of testing different approaches that could eventually improve the field of transplantation. The relatively low volume of procedures per center and the inevitable case mix differences have made it difficult to provide reliable standardized center statistics. Nevertheless, as part of its Health Resources and Services Administration (HRSA) contract, the NMDP has been reporting center-specific disease activity and risk-adjusted survival information for patients undergoing unrelated donor transplantation. Starting in 2008, the CIBMTR began collecting data on all allogeneic procedures performed in the United States as mandated by the SCTOD. They are also required to publish individual center outcome statistics, adjusted for the case-mix severity of the center's population (http://bloodcell.transplant.hrsa.gov/RESEARCH/index.html).

There is every reason to believe that greater attention to institutional and programmatic factors contributing to patient outcome may identify ways to improve care and decrease costs [86]. Volume–outcome and experience–outcome relationships have been documented in solid organ transplantation and complicated surgical procedures [86–89]. A time-series study of costs during autologous HCT for NHL suggested that technological advances and learning curve effects (institutional familiarity with a procedure tends to improve outcomes) probably both contribute to falling costs, although the study was not designed to compare clinical outcomes [90]. Loberiza et al. found that lower 100-day mortality for allogeneic recipients was associated with a higher patient-per-physician ratio and centers where physicians answer calls after hours [91].

From a regulatory perspective, accreditation by the Foundation for the Accreditation of Cellular Therapy (FACT) ensures compliance with standards for clinical care and cell processing facilities. Programs are required to show evidence of documentation, standard operating procedures, and quality management programs. Increasing Federal oversight of outcomes reporting through the SCTOD will provide both a tool for quality monitoring and improvement and also a wealth of research data that can address health services questions [92]. In Europe, accreditation by the Joint Accreditation Committee of ISCT-Europe and EBMT (JACIE) has been associated with better outcomes [93].

Practice variation

The term "practice variation" refers to systematic heterogeneity in clinical practice unjustified by medical considerations. Undesirable practice variation results from external influences on physicians (e.g. training, practice environment, reimbursement structure) [94], patient characteristics (e.g. race, ethnicity, gender, and age, if these features should not influence treatment choice), and lack of definitive evidence for the effectiveness of various procedures [5]. In some contexts, practice variation is considered a measurement of poor quality care. Within HCT, wide practice variation has been noted in infectious prophylaxis and compliance with published guidelines [95], supportive care [96], and medical decision-making [97]. By studying outcomes associated with current heterogeneous approaches, it is hoped that we can narrow variation towards more effective practices.

Conclusion

Outcomes and health services research seeks to answer questions that are relevant as a procedure matures beyond the experimental phase – What are the costs of providing these services? Is society getting its money's worth? What do patients experience with the procedure? How do you pull together disparate sources of data (now that they are available)? Is the procedure equally available to all people and of the highest possible quality? Answers to these questions are moving targets as HCT evolves. Nevertheless, for people afflicted with diseases treated by HCT and societies trying to control healthcare spending, decisions have to be made today. Outcomes research tries to provide the necessary data so that personal and societal decisions can be based on the best information available.

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CHAPTER 29

The Evaluation and Counseling of Candidates for Hematopoietic Cell Transplantation

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Introduction

One must begin by acknowledging that there is a lack of clinical trial data regarding the appropriate work-up and counseling of transplant patients. Much of the dogma that is currently in place is based on experience and opinion rather than clinical trial results. Patients who are under consideration for hematopoietic cell transplantation (HCT) require a careful clinical evaluation and in-depth counseling by experienced physicians, nurses, and a knowledgeable social worker at the respective transplant center. Most transplant candidates are referred by practicing hematologists or oncologists to the tertiary center where the transplant procedure will be performed. Information regarding the prior course, including initial diagnostic studies, previous drug and radiation treatments, and responses to these interventions, and also psychosocial aspects of the transplant candidate, are of the utmost importance.

Written information for patients

Prior to the time of the initial meeting with the medical and paramedical staff at the transplant center, patients should have received at least some written educational material regarding the rationale, principle, potential complications, and projected treatment results of HCT. An increasing number of patients are obtaining information from the Internet and may be fairly knowledgeable and informed (or misinformed) based on the quality of the material that they are able to acquire.

Many transplant centers have developed their own center-specific written information, together with educational videotapes or compact discs, which are mailed to future patients and their families in advance of their first clinic visit. Another excellent source of general information is the Blood and Marrow Transplant Information Network. The Network maintains a website (www.bmtinfonet.org) (for further details, see Chapter 35).

It may be extremely helpful for transplant candidates to communicate with former transplant recipients who can share their experience with the future patient. Such contacts are much more valuable than the visit to so-called Internet "chat rooms" that the present authors consider to be "goldmines for misinformation."

The patient's first visit to the transplant center

For many patients, their first visit to the transplant center is an initial consult to determine if transplant is indicated. This is followed by an explanation of the potential risks and benefits of HCT for an individual patient. The physician should form an impression as to whether the examination and the medical data identify the patient as a suitable transplant candidate. Conversely, the patient should be provided with well-organized general and also individualized information about the intended treatment course, potential complications, expected transplant outcome, and alternative procedures. With most patients, the communication should occur in lay language. Technical terms should be explained and abbreviations be avoided whenever possible. Simple illustrations can be particularly valuable, and a glossary may prove to be very helpful. Conversely, some patients are already very well informed, and time at the counseling meeting may be spent more effectively on discussion of specific issues.

Until they become ill, most patients have never heard words such as "allogeneic," "autologous," "haplotype," "graft-versus-host disease" (GVHD), and so on. The concept that a small number of donor stem cells can restore the entire bone marrow with all its production of blood cells needs to be explained. A well-informed patient is much more likely to participate actively in his or her future care.

Finally, we need to be most careful with the terminology that we introduce to our patients. A word such as "mini-transplant" implies to most listeners that this must be a minor procedure and nothing can go wrong. Consider that, even at the most experienced centers, the day 100 non-relapse mortality (NRM) after a reduced-intensity allogeneic HCT is at least 5%, and at 2 years it reaches approximately 20%. What will be your response to the spouse, parent, or child of a dying patient when the question is asked how a "mini"-procedure could directly or indirectly lead to a patient's demise?

Advice on counseling new patients

Each physician and clinical investigator should develop his or her own approach to the education of patients and the counseling process. As Dr. Karl Blume has previously written, a simple four-question

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answer system is very useful in the initial counseling of patients (why, how, when, and what?):

1. *Why* is HCT being done?, i.e. the rationale for the procedure and discussion of alternative therapies.
2. *How* is the procedure performed?, including the explanation of side effects from the preparatory regimen, the transplant process itself (including the impact on an allogeneic donor), the description of the reason for and clinical presentation of GVHD, the efforts for the prevention of GVHD, and the potential risks for other early or late side effects and relapse.
3. *When* should the procedure be performed?, for example, very soon in the case of relapsed acute leukemia, or electively for patients with multiple myeloma who may have several months or sometimes years until the preparation for HCT is begun.
4. *What* is the projected treatment outcome for the individual patient who is being evaluated? It is advisable to quote a range of percentages for disease-free survival (DFS) and not a precise figure, which would be arbitrary at best.

Patients may elect to tape the conversations in order to review the information presented to them at their leisure. The physician should also encourage the patient to take notes and to write down questions that may come up between meetings or at a later time. It is also our practice to send a copy of the initial consult letter to the patient, which can be reviewed at a follow-up meeting. An atmosphere of trust between patients and their families and the transplant team is of the greatest importance for the long weeks before, during, and after the transplant procedure. The more carefully patients are prepared for the difficult phases of HCT and the more they understand the rationale for diagnostic and therapeutic procedures, the significance of expected (and sometimes unexpected) events, and the risks of the entire medical approach, the easier will be the task of the healthcare team to guide these often frightened individuals through one of the physically and emotionally most challenging times of their lives. Issues such as the potential use of life support need to be addressed before the preparation for HCT is begun. Some 10% of HCT recipients require admission to an intensive care unit, most often during the first 2 months after HCT, and only a few of such unfortunate patients recover [1,2]. Despite improvements in the care of critically ill patients, recent reports continue to indicate a mortality rate of 87% in patients who require mechanical ventilation following HCT [3].

It may take years for physicians to gain the necessary experience, medical knowledge, and sound clinical judgment to counsel their patients "perfectly." Personally, we have found this to be an area of continuous process improvement, and it has become clearer over time that consultations are not only tailored to specific diseases, but also to expectations and baseline knowledge of individual patients. It obviously requires a high level of comfort to present a medical procedure that is offered with curative intent but is also associated with significant procedure-related morbidity and mortality. Even under optimal conditions, autologous HCT is associated with an approximately 5% early fatality rate, and still 10–25% of recipients of allogeneic HCT succumb sooner or later to non-relapse causes of death, including GVHD or GVHD-associated complications such as overwhelming infections, sinusoidal obstruction syndrome, prolonged immunodeficiency, or secondary malignancies.

The patient and the family members need to hear from the counseling physician that the intended treatment planned to provide a cure of the life-threatening disease may – in the case of one of the potentially severe complications – lead to the patient's early demise. Still, much of what is explained during the pre-transplant meetings is frequently forgotten when devastating complications occur later on. The special situations of desperate patients and their families explain why often little information from the counseling meeting is retained. Therefore, patients should be provided with written copies of the initial consultation if possible. A randomized trial performed in cancer patients demonstrated that an audio tape enhanced patient satisfaction and improved recall of diagnostic information [4].

End-of-life issues need to be addressed early. Many patients may hesitate to bring this issue up but are actually relieved to talk about it openly when encouraged. This complex problem is particularly important in families with complex relationships (in couples who are separated but are not divorced and in couples who are not married; sometimes, there are children, siblings, parents, or step-parents who want to make decisions later on but are not entitled to do so by the patient). Therefore, it is important to address these issues at the beginning of a patient's course rather than at the end, when frequently they may be so incapacitated that they are unable to participate. Retrospective analyses have shown that overall survival is better in those for whom advanced care planning discussions have taken place [5].

Table 29.1 lists issues and topics that should be addressed during the counseling meetings with transplant candidates. This list is only a guideline and should be reduced or expanded as appropriate by each transplant physician and for each patient under evaluation.

The problem of permanent infertility

For many young transplant candidates, the high probability of permanent infertility after high-dose therapy and HCT represents a major problem. Modern technologies such as cryopreservation of sperm and fertilized eggs can be used to overcome this serious issue in only a limited number of patients. Unfortunately, the ejaculates of many younger men are aspermic or hypospermic due to the effects of prior combination chemotherapy used for remission induction treatment. In the case of many younger women, the procedures to harvest eggs for in vitro fertilization fail. Despite successful fertilization, the number of those in whom a viable post-transplant pregnancy occurs is still extremely low.

A small number of patients, men and women, have spontaneously recovered reproductive function, with healthy children being born during the years after HCT [6]. These patients were usually children or adolescents at the time of HCT. It cannot be predicted at the time of HCT which patient will be able to regain fertility. However, it should be noted that patients conditioned with high-dose cyclophosphamide as therapy for aplastic anemia often are able to have children during the years following HCT. For more on the psychologic and physiologic aspects of infertility, sexuality, quality of life, and delayed long-term effects after HCT, the reader is referred to Chapters 32, 33, 34, and 104.

The recent introduction of reduced-intensity regimens into the field of HCT may result in a higher number of patients from both genders who retain or recover fertility. Since such regimens have so far included mainly older individuals, the information regarding this important issue is still lacking; however, cautious optimism seems to be justified.

Table 29.1 Issues and topics that should be addressed during counseling meetings with transplant candidates and their families

- I. Rationale (why is HCT offered to you?)
- II. Principle (how is HCT performed?)
  - A. Autologous versus allogeneic HCT
  - B. Preparatory regimen
    - 1. Choice of a high-dose or reduced-intensity regimen (with or without irradiation)
    - 2. Risks associated with high-dose therapy
      - a. Nausea, emesis, diarrhea
      - b. Fluid retention
      - c. Venocclusive disease/sinusoidal obstruction syndrome
      - d. Pancytopenia, immunosuppression, and associated infectious complications
      - e. Mucositis and pain in the oral cavity and throat
      - f. Hemorrhagic cystitis after cyclophosphamide
      - g. Cardiomyopathy after cyclophosphamide
      - h. Dermatitis after etoposide
      - i. Pneumonitis after nitrosoureas
      - j. Need for transfusion support
      - k. Temporary or permanent alopecia, especially after busulfan
      - l. Infertility
      - m. Cataracts
      - n. Second or secondary malignancies
  - C. Hematopoietic cell grafting
    - 1. Marrow versus peripheral blood cell graft
    - 2. Risks associated with infusion of a hematopoietic cell graft
      - a. Allergic reactions
      - b. Discomfort from cryoprotectant
      - c. Graft failure and graft rejection
  - D. Risks for GVHD
    - 1. Symptoms and prognosis of acute GVHD
    - 2. Need for drugs to prevent/treat acute GVHD
    - 3. Side effects of immunosuppressive drugs
    - 4. Symptoms and prognosis of chronic GVHD
    - 5. Drugs to prevent/treat chronic GVHD
    - 6. Immunodeficiency resulting from GVHD including bacterial, fungal, viral, and other infections
  - E. Risk of relapse after HCT
  - F. Risk of mortality after HCT
    - 1. Financial affairs, a will
    - 2. Durable power of attorney for health care
    - 3. Use of life support
- III. Timing of transplant (when should the procedure be performed?)
    - 1. Very soon, e.g. progressive acute leukemia
    - 2. Within weeks or few months, e.g. acute leukemia in remission
    - 3. Elective HCT, e.g. follicular small cleaved lymphoma in early stages
- IV. Projected result (what can the candidate expect from HCT?)
    - 1. Underlying disease and remission status
    - 2. Compatibility of donor–recipient pair
    - 3. Performance status of candidate
    - 4. Comorbid conditions
    - 5. Prior therapies
    - 6. Nutritional status
    - 7. Age of HCT candidate
    - 8. Previous infections
    - 9. Transfusion history
    - 10. Other individual aspects
- V. Other important issues
    - 1. Banking of sperm, in vitro fertilized eggs
    - 2. Habits such as nicotine, alcohol, or drug addiction
    - 3. Need for active participation by patient and caregiver during entire course
    - 4. Rationale and need for clinical trials including randomization
    - 5. Duration of stay in the area of the transplant center
    - 6. Return to home and work
    - 7. Sexual activities
    - 8. Time to full recovery
    - 9. Quality of life issues

The choice of transplant procedure

Most patients arriving at a transplant center have been told by their referring physician that they are in need of a transplant procedure. Many of them are surprised when they hear that there are many different types of HCT: autologous HCT, allogeneic HCT, and occasionally syngeneic HCT. The counseling physician has to use their best judgment and knowledge, together with the experience at the respective transplant center, to advise the patient concerning the type of procedure that has the greatest likelihood of success for the candidate's particular condition.

Which outcomes data should one quote?

The single most important issue for the patient is the expected overall survival. Information described below indicates that the remission status and patient age are the most predictive factors – with age losing some of its prognostic power with the current use of reduced-intensity regimens. Ideally, each transplant center would have a rich database from which one could generate information pertaining to each disease, remission status, age, and so on. In the absence of such a powerful tool, which data should be quoted by the counseling physician?

Recent studies involving 163 HCT centers in the United States indicate a significant "center effect," that is, 100-day mortality after allogeneic HCT was lower in centers with a higher patient-per-physician ratio and in those centers where physicians answered calls after office hours [7]. The data from a less experienced center are likely to be less favorable. The patient counseled at that center should either be quoted center-specific results (if available) or the relatively representative data from the large national and international bone marrow transplant registries.

What does the patient hear and remember?

The patient comes to the transplant center with the expectation of being cured. Much of the information provided during the counseling meetings is viewed and interpreted by the patient in a more favorable light than is actually realistic. An important analysis has previously been reported from the Dana Farber Cancer Institute indicating significant discrepancies between patient and physician estimates for the success of HCT [8]. In this prospective study, 313 autologous or allogeneic HCT recipients and their physicians responded to baseline and follow-up questionnaires concerning their expectations for cure with or without HCT and for treatment-related mortality. Information on actual treatment-related mortality and disease-free 1-year survival was subsequently available for 263 patients. The data are given in Table 29.2. Patients and their physicians had the most concordant and accurate expectations when the outcome of HCT was likely to be favorable. However, patients with more advanced diseases failed to recognize the higher risk associated with their situations. This important study illustrates the urgent need to improve further the communication with transplant candidates.

The second counseling meeting

After the first visit to the transplant center, the patient should be allowed adequate time (several days) to consider the often overwhelming nature and amount of information. In a second meeting, the transplant physicians should assure themselves that the patient understands the whole impact of the proposed treatment, both in general and in its details. An adequate amount of time should be set aside to address all issues and questions concerning the planned treatment and its potential complications.

During the second meeting with the patient, the issue of advanced care planning, including the need for arrangements of financial affairs, a will, Durable Power of Attorney of Health Care, and the

Table 29.2 Discrepancies between patient and physician estimates for the success of HCT [8]

		Estimate				
Type of Transplant	**No. of Patients**[a]	**Cure without stem cell transplantation (%)**		**Cure with stem cell transplantation (%)**		**Actual percentage with Disease-Free Survival (95% confidence interval)**[b]
		Patient	**Physician**	**Patient**	**Physician**	
Autologous[c]	94	20	7	70	32	44 (30–58)
Allogeneic	169					
Disease status						
Early	78	20	8	80	62	52 (40–64)
Intermediate	71	13	7	73	42	32 (21–44)
Advanced	20	19	7	80	31	10 (0–23)

[a] Five patients did not provide an estimate of cure without stem cell transplantation and 3 did not provide an estimate of cure with stem cell transplantation.

[b] At 2 years.

[c] Ninety patients with intermediate and 4 patients with advanced disease.

possible use of life support (if indicated), are to be tactfully but distinctly presented and discussed. A study on 155 HCT recipients indicated that 69% reported having designated a healthcare proxy, 61% had prepared an estate will, and 63% had discussed their wishes regarding life support with family and friends. In contrast, only 16% had communicated their choice of life support to their clinicians [9].

When to advise against HCT

No firm rules for a minimal success rate can be established that would be generally applicable. The patient and the entire healthcare team need to keep in mind that a long-term 10% DFS rate implies that nine of 10 patients sooner or later succumb to either relapse or NRM. For example, most patients with active central nervous system (CNS) leukemia have already received CNS therapy including irradiation prior to arrival at the transplant center. They will require further CNS treatment (irradiation and intrathecal drug instillations), which puts them at a high risk for leukoencephalopathy, a devastating, debilitating complication. Although not all such patients end up with extensive intellectual deficits, such long-term complications need to be considered carefully, especially because even less extensive neurologic manifestations can severely affect the future quality of life of the transplant recipient.

Patients with acute leukemia whose disease has recurred several times represent a similar dilemma. The chances that their underlying disease will persist or recur after HCT are exceedingly high. Unless innovative treatment concepts to overcome resistance are pursued in the form of exploratory research trials, these patients should be advised against HCT.

Of course, the patient is expecting that everything possible is going to be done, and many transplant physicians forget – while counseling such desperate patients – that the long-term transplant outcome data, for example for patients with acute leukemia in relapse, are extremely disappointing [10]. Finally, subjecting a volunteer unrelated donor to the physical and emotional stress of marrow donation for a patient with refractory disease and a very poor prognosis requires serious consideration. How often in our decision-making process before HCT do we think about the donor, and how often do we think about this volunteer once the graft is infused? Honestly, rarely. A negative outcome of a transplant effort affects many donors for months or even years. They may suffer feelings of loss, failure and – fairly often – guilt, especially if the patient dies of GVHD. Needless to say, the same issues apply to all related donors; for details concerning HCT donors, see Chapter 37.

How to counsel patients seeking multiple opinions

Receiving the diagnosis of cancer is one of the most devastating events in the life of any human being. It is certainly understandable that patients are very concerned about their future health and wish to obtain the best treatment that could restore their natural life expectancy. Therefore, many patients seek second or even more opinions, especially when it comes to risky procedures such as HCT. We all have met these "traveling" patients who arrive at the transplant center with stacks of computer printouts to obtain another opinion. They are clearly in need of very careful guidance. These consultations often take several hours.

We need to explain that the HCT procedures performed at most centers differ only in one or the other detail. In principle, we employ the same concepts, be it high-dose or reduced-intensity regimens, followed by infusion of hematopoietic cells. It is the experience and the care provided at a particular transplant center that make the difference. Moreover, many academic centers may offer innovative approaches that are tested in the setting of clinical research trials and are not available at other institutions.

It is the task of the counseling physician to guide the concerned and often confused patient to a most important decision while objectively explaining the pros and cons of one versus the other method, for example, T-cell depletion of the graft at the Memorial Sloan Kettering Cancer Center in New York versus post-transplant immunosuppression of the host at the Fred Hutchinson Cancer Research Center or the Stanford Hospital and Clinics. In this situation, the patient–physician relationship returns to its basic meaning. There is no room for dogma. Ultimately, most patients will decide in favor of the center at which they feel most comfortable and where they understand the rationale for one or the other approach.

The new foreign patient

Many patients from other countries are unfamiliar with the American insistence on discussing all pertinent issues openly with them and their families. They may be more accustomed to having unpleasant facts glossed over. Also, there may be many different dietary preferences and regulations, making it advisable to involve a dietitian early on. Rules on body invasion (endoscopy, biopsy, and autopsy) are important considerations requiring particular explanations and great sensitivity. No detailed rules can be provided in this chapter, as the expectations and needs vary with patients from different countries of origin. The issue of preferred language should be addressed prior to the arrival of any patient, and appropriate interpretative services are a vital component of patient education and informed consent. Family members should not be expected to serve as an interpreter for the patient as this could lead to intrapersonal conflicts relating to full disclosure of information. For example, some family members may decide that they do not want to burden their loved ones with negative information. Furthermore, it is difficult for family members to provide emotional support and serve as an interpreter at the same time.

The issue of clinical trials

All progress made during the past 50 years in the field of HCT is based on well-designed preclinical experiments and carefully conducted prospective clinical trials. Almost every transplant center is engaged in one or, more commonly, several clinical trials. Without patient participation, such important investigations are never possible. Therefore, all patients should be informed about clinical research trials as early as possible and be encouraged to consider enrollment in studies as part of their HCT procedure. The concept that a patient simultaneously serves as a research subject and the physician accepts also the role and responsibility of a clinical investigator should be explained during the first and subsequent counseling meetings. The rationale for clinical research studies should be addressed.

Most patients and some referring physicians do not have a clear understanding of the rationale of and need for randomized trials. These clinical research efforts are made to define how a new treatment compares with a previously utilized approach. It should be explained to the patient that the "new arm" might be better than, the same as, or worse than the "old arm." If the answer were known, the trial would be superfluous.

During either the first or second meeting, the patient should receive a copy of the written informed consent for his or her perusal. Non-English-speaking patients regularly need assistance from interpreters, and – depending on the region of the transplant center – informed consent documents are routinely provided in other languages. The consenting process itself should be completed only after all questions have been satisfactorily answered and should meet both institutional and Federal standards. Investigators with a conflict of interest need to provide pertinent information to their patients.

Conditions affecting treatment outcomes

A number of pre-transplant factors and conditions strongly influence the treatment outcome and thus impact the advice to patients regarding their candidacy for HCT.

Disease and remission status

This literature will provide the most up-to-date information on the treatment outcomes of autologous or allogeneic HCT for the many different diseases during the various remission stages. The extent of the underlying disease and its sensitivity to chemotherapy strongly influence the treatment result for recipients of autologous and allogeneic hematopoietic transplants. The reader will find multiple examples of the effect of the pre-transplant remission status on long-term DFS, for example, in acute leukemia, non-Hodgkin lymphoma, and most other conditions.

In order to evaluate a patient's candidacy for HCT, the most recent clinical information regarding the extent of the underlying disorder must be reviewed or must be obtained newly if the previous information is no longer considered to be accurate. For example, a patient with acute leukemia referred during first complete remission should undergo another bone marrow examination if the previous study was performed more than 1 month earlier. Appropriate diagnostic procedures (biopsies and cytogenetic and molecular methods) must be selected to assess the extent of current disease activity. For some conditions, for example, the myelodysplastic syndromes, recommendations concerning the timing of HCT have been proposed [11]. These decision analyses are based on a widely used International Prognostic Scoring System, which includes cytogenetic findings, number of blasts, and blood cell counts.

Patients with acute bone marrow injury following exposure to toxic agents may require a new assessment of their marrow cellularity and function to detect whether there are signs of spontaneous recovery obviating the need for the transplant procedure.

Candidates referred for autologous HCT who have received extensive and prolonged therapy with drugs (especially alkylating agents) and irradiation require detailed cytologic, cytogenetic and – if feasible – molecular analyses of their bone marrow to rule out any clonal abnormalities that would predispose them to later myelodysplastic or leukemic conditions [12,13].

Patients with solid tumors, regardless of their assumed stage, should be restaged to rule out new metastatic disease to the brain, liver, or elsewhere, conditions that are considered strong prognostic indicators for early relapse after HCT.

Compatibility of the donor–recipient pair

Many patients referred to a transplant center for allogeneic HCT have been previously tissue-typed, and an intended donor has been identified at that time. The transplant center should repeat the histocompatibility testing at its own laboratory to confirm the accuracy of the typing data. After all, the transplant team assumes responsibility for the future care of the transplant candidate; hence the expense of repeated laboratory procedures is fully justified.

Tissue-typing information influences the candidacy for allogeneic HCT and, therefore, the discussion with future patients during the counseling meeting. Granulocyte colony-stimulating factor mobilized peripheral blood stem cells (PBSCs) with unrelated donors have now become the most commonly used graft source for patients undergoing allogeneic HCT [14].

The field of HCT from unrelated donors has made remarkable strides during the past two decades, in particular, through the improved understanding of the major histocompatibility complex (see Chapter 10) and the introduction of molecular human leukocyte antigen (HLA) typing methods for donor selection [15–18]. The transplant center should assume the primary direction and the responsibility in the search for the unrelated donor. The success rate of HCT from unrelated donors is improving continuously and is comparable to results obtained with HLA-identical sibling donors based on large retrospective analyses [19–25]. Despite these improvements, the preferred source for allogeneic HCT remains an HLA-identical sibling over a younger HLA-matched unrelated donor [26].

A randomized trial comparing PBSCs with bone marrow from unrelated donors has been completed [27]. There were no significant differences in overall survival or relapse rates, but there was a higher incidence of chronic GVHD in patients who received peripheral blood stem cells from an unrelated matched donor. A similar trial performed with related donors showed an improved DFS in recipients who received PBSCs compared with bone marrow stem cells [28]. Long-term follow-up of the patients randomized to receive PBSCs indicated no significant differences between the two groups with regard to incidence of chronic GVHD or duration of immunosuppressive therapy, thus indicating that the optimal stem-cell source may be modified by whether the donor is unrelated or related. With regard to safety concerns of the donor, long-term results demonstrated the safety of PBSC mobilization in 3928 donors with no evidence of increased incidence of leukemia [29]. More details are provided in Chapter 41.

The utilization of alternative donor sources such as cord blood [30] and haploidentical donors [31] has increased in recent years. Thanks to these alternative donor sources, more patients now are able to undergo stem-cell HCT successfully. Several questions remain regarding the utility of these donor sources in various hematological malignancies. The best choice for an alternative donor source may depend on specific patient or disease parameters. A randomized trial comparing haploidentical donor stem cells with cord blood stem cells in patients with leukemia or lymphoma and no matched related or unrelated donor is currently accruing patients through the auspices of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN).

Performance status

The invasive nature of high-dose cancer therapy, with or without irradiation, always causes some extent of regimen-related toxicity, sometimes resulting in little or, at other times, extensive morbidity and NRM. Therefore, the transplant candidate must be in good-to-excellent condition when preparation for HCT is begun. The performance score system introduced more than 60 years ago by Karnofsky et al. is still a useful tool for the clinician in the assessment of patients [32]. It has been generally accepted that a patient's performance score should be 70% or above to qualify for HCT. Most cooperative groups follow this requirement for patient enrollment, although some groups have introduced their own scoring system; for example, the Eastern Cooperative Oncology Group uses a 0–3 score, with 0 being asymptomatic and without any evidence of disease.

The Karnofsky scoring system was adapted for HCT recipients by Sullivan and Siadak [33] (Table 29.3). This grading system can also be used to describe the performance status following HCT. By definition, the best score early after HCT is 70% (on day 100 following allogeneic HCT).

The previous need to be a young, vigorous patient even to be considered for HCT has changed considerably over the past 10 years. Reduced-intensity conditioning regimens have been introduced and explored in rapidly growing clinical trials [34–41]. This novel approach relies heavily on the graft-versus-malignancy effect derived from donor T cells. Because of the less toxic side effects of the low-dose preparatory regimens compared with the previously used high-dose combinations, the extensive morbidity of the first few weeks following HCT is not encountered in this setting, and it is possible to perform these kinds of procedures on an outpatient basis. With reduced-intensity regimens, it is possible to treat older patients and others who would previously have been excluded because of serious comorbid conditions. Therefore, age alone is no longer considered as a contraindication for allogeneic HCT [42]. Increasing evidence points to a greater emphasis on underlying comorbidities or physiologic age rather than actual age [43]. This approach has been greeted with a great deal of enthusiasm, partially explaining the poorly chosen term "mini-transplant." However, we should realize that these HCT recipients still face all the serious clinical transplant-related problems of allografting for malignancy, namely GVHD, infections, and relapse. The only major aspect that has changed is the lack of toxicity previously observed during the first post-transplant month. Retrospective studies in a variety of hematologic malignancies have consistently shown no major differences in overall survival between high-dose and reduced-dose conditioning intensity [44–51]. However, the cause of death is different as patients who receive high-dose conditioning are more likely to die from toxicity of conditioning, whereas patients who receive reduced-intensity regimens are more likely to die due to relapse [52,53]. A prospective randomized trial comparing high-dose with reduced-dose conditioning intensity in patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) who are in morphologic remission is currently accruing patients and is operating under the auspices of the BMT CTN.

Table 29.3 Karnofsky performance status scale [32]

Index	Specific criteria	General
100	Normal, no complaints, no evidence of disease	Able to carry on normal activity; no special care needed
90	Able to carry on normal activity, minor signs or symptoms of disease	
80	Normal activity with effort, some signs or symptoms of disease	
70	Care for self, unable to carry on normal activity or to do work	Unable to work, able to live at home and care for most personal needs, varying amount of assistance needed
60	Requires occasional assistance from others but able to care for most needs	
50	Requires considerable assistance from others and frequent medical care	
40	Disabled, requires special care and assistance	Unable to care for self, requires institutional or hospital care or equivalent, disease may be rapidly progressing
30	Severely disabled, hospitalization indicated, but death not imminent	
20	Very sick, hospitalization necessary, active supportive treatment necessary	
10	Moribund	
0	Dead	

Prior and maintenance therapies for the underlying diseases

Every drug and every exposure to radiation administered since the time of the original diagnosis of the underlying malignancy have long-lasting cumulative effects on the patient. For example, the treatment with standard doses of radiation in the form of a mantle field for Hodgkin disease given a decade earlier may pose a significant risk factor if such a patient becomes a transplant candidate and is considered for total body irradiation (TBI) or for treatment with high doses of nitrosourea drugs. The cumulative effect of anthracyclines, which are widely employed in the standard therapy of leukemias and lymphomas, is well known and may limit the further tolerance of cardiotoxic drugs that are under consideration for use in preparatory regimens. Fortunately, newer agents may have less untoward effects, as was demonstrated for imatinib when administered to patients with chronic myelogenous leukemia (CML) prior to allogeneic HCT [54], and this drug even seems useful in the prevention of post-HCT relapse of patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL) or high-risk CML [55]. Also, rituximab, which is widely employed in the pre-HCT therapy of patients with all variants of

Table 29.4 HCT-specific comorbidity index score [87]

Comorbidities	Definition	Score[a]
Migraine/headache		0
Osteoporosis		0
Osteoarthritis		0
Hypertension		0
Gastrointestinal	Including inflammatory bowel disease	0
Mild pulmonary	DLCo and/or FEV_1 >80% or dyspnea on moderate activity	0
Mild renal	Serum creatinine 1.2–2 mg/dL	0
Endocrine		0
Bleeding		0
Coagulopathy	Deep venous thrombosis or pulmonary embolism	0
Asthma		0
Arrhythmia		1
Myocardial	Coronary artery disease, congestive heart failure, history of medically documented myocardial infarction, EF ≤50%	1
Mild hepatic	Chronic hepatitis, bilirubin >ULN – 1.5 × ULN, or AST/ALT > ULN – 2.5 × ULN	1
Cerebrovascular accident	History of transient ischemic attack or cerebrovascular accident	1
Morbid obesity		1
Diabetes	Requiring treatment	1
Depression/anxiety		1
Infection	Requiring continuation of treatment after day 0	1
Rheumatologic	Systemic lupus erythematosus, rheumatoid arthritis, polymyositis, mixed connective tissue diseases, polymyalgia rheumatica	2
Moderate pulmonary	DLCo and/or FEV_1 66–80% or dyspnea on slight activity	2
Peptic ulcer	Patients who have required treatment	2
Moderate–severe renal	Serum creatinine >2 mg/dL, on dialysis, or prior renal transplantation	2
Valvular heart disease	Except mitral valve prolapse	3
Prior solid tumor	Requiring treatment with chemotherapy	3
Moderate–severe hepatic	Liver cirrhosis, bilirubin >1.5 × ULN, or AST/ALT >2.5 ×ULN	3
Severe pulmonary	DLCo and/or FEV_1 ≤65% or dyspnea at rest or requiring oxygen	3

[a] Total score is the sum of all comorbidities present at time of HCT.

B-cell lymphoma, can be given as a consolidation measure after autologous HCT [55]. Finally, the concept of "total therapy" for multiple myeloma revolves around extensive combination therapy, which is continued at reduced intensity after autografting [56,57]. Randomized studies evaluating agents such as FLT-3 inhibitors in AML and oral hypomethylating agents in MDS are planned as future strategies to reduce the risk of relapse post-transplant. How these agents affect the overall toxicity in HCT is currently unknown.

Comorbid conditions

The need for a careful history and a physical examination, and also objective tests that allow for a determination of the patient's organ functions, must be stressed. In addition, regimen-related toxicity remains an important issue for all candidates for autologous HCT who will be exposed to maximum tolerated doses of chemotherapeutic agents (with or without radiotherapy).

A pre-HCT specific comorbidity index (HCT-CI) has been developed and validated [43,58]. In these retrospective analyses, patients with a low score (0), intermediate score (1–2), and high score (≥3) had a 2-year cumulative incidence of NRM of 7, 19–21, and 27–37%, respectively. The components of the HCT-CI are summarized in Table 29.4. General eligibility criteria for adult candidates for allogeneic or autologous HCT are described in Table 29.5. Below, some of the most important aspects of compromised organ function are discussed.

Table 29.5 General eligibility criteria for adult candidates for allogeneic or autologous HCT

Criterion	Allogeneic HCT	Autologous HCT
Age	No strict age cut-off	No strict age cut-off
Karnofsky performance score	70–100 for high dose ≥50 for reduced dose	70–100
Left ventricular ejection fraction	>35%	≥40% BEAM, ≥50% cyclophosphamide
Pulmonary function test; forced vital capacity	≥60%	≥60%
Diffusion capacity (DLCO)	≥35%	≥50%
Kidney function	≤2 mg/dL	≤2 mg/dL, unless for myeloma
Liver function	No cirrhosis	No cirrhosis
Mental health	Adequate psychosocial and financial support	Adequate psychosocial and financial support

Cardiac evaluation

Patients over the age of 50 years or those with a history of extensive exposure to anthracycline drugs may be at risk for future cardiac complications, especially if high doses of cyclophosphamide are part of the planned conditioning regimens [59–61]. In our experience and that of other centers, life-threatening cardiotoxicity after HCT is rare, occurring in less than 2% of all transplant recipients [62]. An ejection fraction of less than 50% as measured by two-dimensional echocardiography and a prior history of congestive heart failure are significant independent correlates of clinical cardiotoxicity [63]. Many cooperative cancer research groups and also some insurance companies insist on the use of radionuclide tests of ventricular function as a requirement for the enrollment of patients to clinical transplant trials or for the authorization of financial coverage, respectively. There is little reason to insist that all transplant candidates be evaluated with multigated acquisition scans [64]. Considerable financial resources can be saved by relying on two-dimensional echocardiography (if indicated with a stress echocardiogram). Additional advantages of a two-dimensional echocardiogram include the ability to evaluate for evidence of valvular disorders, and this may be a preferred test in patients with a history of valvular disorders or an abnormal cardiac examination. Retrospective studies performed in MDS and AML indicate that there are some patients in whom cardiac evaluation preinduction chemotherapy is not warranted [65]. Studies evaluating the necessity of radiographic cardiac evaluations in the pre-stem-cell transplant population are lacking.

Respiratory evaluation

Pulmonary function tests (PFTs) such as the forced expiratory volume, forced vital capacity, and diffusion capacity have gained a firm place in the pre-transplant evaluation of HCT candidates [66–70]. A decrease in these parameters before HCT predicts for post-transplant respiratory impairment, especially if chronic GVHD occurs, in both children and adults [70]. The data regarding the utility of PFTs are not as clear in recipients of autologous grafts [71]. Recent data have indicated that PFTs alone should not be used to exclude patients from a stem-cell transplant but should be considered as part of an overall assessment of comorbidities [72].

Hepatic evaluation

Liver function tests are routinely obtained before and after marrow HCT in an attempt to predict or detect hepatic compromise. An elevation of transaminases at the time of admission for HCT was significantly associated with post-transplant hepatic sinusoidal obstruction syndrome (SOS). Other risk factors associated with this complication included vancomycin therapy during cytoreductive therapy, acyclovir therapy before HCT, administration of gemtuzumab ozogamicin, and radiation therapy to the abdomen at any time before HCT. The incidence of SOS after HCT varies greatly among transplant centers, ranging from less than 2% to over 50% [73,74]. Possibly, patient selection and donor–recipient matching, and also tight control of the patient's fluid status, may play an important role in the occurrence or prevention of this syndrome. The incorporation of ursodeoxycholic acid as a prophylactic strategy post-transplant has reduced the incidence of hepatic SOS [75].

Renal evaluation

Testing of renal function is a standard requirement at all HCT centers. Serum creatinine of under 1.5 mg/dL (for adult patients) and a creatinine clearance over 60 mL/min are considered adequate as eligibility criteria for transplant candidates. Considering the potential nephrotoxicity of several drugs that are frequently used after HCT, such as calcineurin inhibitors, vancomycin, foscarnet, and other agents, adequate kidney function is an important component of the pre-transplant assessment. However, there are some patients who have successfully undergone allogeneic HCT while dialysis-dependent at our center. However, in patients with multiple myeloma who are treated with high-dose melphalan and autologous HCT, even highly abnormal kidney function may still permit a successful transplant outcome [76], and there are some patients with renal failure due to multiple myeloma who have improvement in renal function following HCT [77]. Although dialysis dependence is not an absolute contraindication to HCT, the development of renal failure following HCT is associated with an increased risk of mortality [78].

Nutritional status

A nutritional assessment by an experienced dietitian, including an exploration of the patient's dietary habits, should be part of the evaluation process of each transplant candidate; for details, see Chapter 99.

Extreme nutritional conditions such as cachexia or morbid obesity require special considerations and counseling. Malnourished patients may require pre-transplant enteral or parenteral nutrition to improve their general condition, and patients suffering from excessive obesity may be advised to reduce their body weight under the guidance of a dietitian – provided that the underlying disease permits the delay of the transplant procedure.

It has been demonstrated in a large clinical study of 2238 patients at the Fred Hutchinson Cancer Research Center that body weight impacts on NRM after allogeneic HCT [79]. This analysis indicated that patients whose actual body weight (ABW) ranged between 95% and 145% of ideal body weight (IBW) experienced comparable NRM, whereas patients whose weight was less than 95% or above 145% had decreased survival. The treatment outcome was poorest in patients whose ABW was less than 85% of ideal body weight (IBW). A similar analysis was made at Stanford University School of Medicine in 473 consecutive adult patients with hematologic malignancies who received high-dose combination therapy followed by autologous HCT. Again, those patients who were malnourished or those who were extremely obese fared the worst [80].

The dosing of drugs in the preparatory regimen is frequently adjusted to IBW, which may be considerably different from the patient's ABW. In the case of very obese patients, this dosing policy would result in serious underdosing with all its inherent consequences, such as insufficient immunosuppression and decrease in tumor cell kill. To overcome this problem, some transplant centers have made provisions in their clinical protocols by using a formula to calculate an adjusted IBW, namely, IBW plus 50% of the difference between ABW and IBW. For example, if a patient's IBW is 80 kg and the ABW is 130 kg, then the adjusted IBW would be 105 kg. Similar calculations apply if the patient's body surface is used to determine drug doses.

For several drugs (busulfan, cyclophosphamide, and others), pharmacodynamic monitoring is more meaningful for dose decisions than is body weight (see Chapter 19).

Comorbidity assessment

The assessment of pre-transplant patient-specific factors to determine post-transplant outcomes is an area of ongoing research. Several different risk assessment systems have been published. The three most commonly used systems are the European Society for Blood and Marrow Transplantation (EBMT) risk assessment score [81], the pretransplant assessment of mortality (PAM) score [82], and the hematopoietic cell transplantation-specific comorbidity index (HCT-CI) [58]. There have been few studies that compared these risk assessment models. One study compared the HCT-CI with the PAM risk score and found that the HCT-CI was the best predictor of NRM [83]. In addition, there have been studies validating the HCT-CI in external cohorts [84]. One major issue in the application of these systems is the inter-rater reliability, which can be fairly high [85]. The components of the HCT-CI are summarized in Table 29.4. Ultimately, prognostic models in the future will involve the conflation of patient-specific and disease-related risk factors [86,87].

Age of the transplant candidate

Age of allogeneic HCT candidates

During the early years of allogeneic HCT, successful outcome was limited mostly to children, adolescents, and younger adults. In 1986, two groups reported encouraging results after high-dose therapy and allogeneic marrow HCT in older adults, aged 32–54 and 45–68 years, respectively [88,89]. The issue of age and eligibility for HCT is particularly relevant for diseases such as MDS, where there is a clear association between increasing age and development of the disease. Retrospective reviews have indicated that patients aged up to 65 years are able to tolerate high-dose conditioning with the use of related donors [90]. Additionally, post-transplant outcomes do not appear to be related to age or the intensity of the conditioning regimen but rather the disease stage at time of HCT [91].

The arrival of less toxic, reduced-intensity regimens has allowed the extension of allogeneic HCT to patients in the age group 60–75 years [34,38,92,93]. A retrospective analysis from the Center for International Blood and Marrow Transplant Research (CIBMTR) in patients with MDS or AML aged 40 years or more indicated that age alone did not have a significant impact on overall outcomes following reduced-intensity HCT [42]. A similar study in our center in 372 patients aged 60–75 years with a variety of hematologic malignancies showed no impact of age on overall survival, NRM, progression-free survival (PFS), or the incidence of acute GVHD [94]. The 5-year overall survival and PFS were 35% and 32%, respectively. This further highlights the concept that consideration for stem-cell transplant should be based on overall measures of comorbidities rather than specific singular factors.

Age of autologous HCT candidates

Several transplant centers have analyzed the effect of age on the outcome of autologous HCT [95–99]. Although there appears to be an increased risk of NRM during the early phase after HCT in patients over the age of 50 years, it does not seem to be warranted to exclude older patients from autologous HCT. The majority of the data regarding age and post-transplant outcomes is derived from patients with a diagnosis of multiple myeloma [99–101]. A retrospective analysis in patients who underwent autologous HCT for lymphoma indicated that age alone should not serve as a contraindication [102] .

Ethnicity, race, and socioeconomic issues

Decisions regarding the beneficial role of HCT must take into account the overall circumstances of an individual patient. These also include psychosocial aspects. The living environment certainly plays a role in whether patients have a successful outcome with HCT, in addition to other socioeconomic factors such as access to good dental and medical care prior to HCT. In a retrospective analysis of 6207 individuals who underwent high-dose conditioning followed by unrelated donor HCT, overall survival was worse in patients from a lower socioeconomic status. This decreased overall survival was primarily attributable to a higher treatment-related mortality, thus implying that non-disease-related factors are important determinants of post-transplant outcome and that these non-disease-related factors are at least in part attributable to socioeconomic status [103].

There are multiple issues that contribute to the availability of HCT based on race and ethnicity. Socioeconomic factors certainly play a role in both the autologous and allogeneic setting. However, there appears to be greater disparity in the allogeneic setting potentially attributable to donor availability [104]. Numerous studies have dealt with the effect of race on the outcome of autologous or allogeneic HCT [103,105–108]. With respect to autologous HCT, race and ethnicity were not significantly associated with survival of 1366 patients [105]. Analyzing the outcome of 2221 recipients of allogeneic grafts from histocompatible siblings indicated that patients of African American background had a significantly greater mortality than white patients [106]. HCT was more associated with severe acute GVHD, and NRM was higher among African American than white patients, presumably as a result of increased HLA mismatching or genetic polymorphisms that affect drug delivery. The increasing use of cord blood stem cells will likely impact this disparity in a positive manner. Mortality among other racial and ethnic groups was not significantly different from that of white patients. In another study on 1752 recipients of grafts from histocompatible siblings, it was found that white Americans, African Americans, and Irish cohorts were at significantly higher risk for acute GVHD than Japanese or Scandinavian cohorts, indicating that there may be more HLA disparity in white Americans, African Americans and Irish cohorts compared with Japanese and Scandinavian cohorts [107]. Despite the increased risk of acute GVHD, there was no difference in overall survival. The increasing use of cord blood cells as a stem-cell source will likely address some of the racial disparities regarding access to HCT [109], but socioeconomic factors will likely continue to play a role in post-transplant outcomes.

Previous infections

Beginning with the time of their initial diagnosis, patients enter a phase in their treatment when they become more susceptible to infections than healthy individuals. Their conditions are even more compromised by antineoplastic treatments with chemotherapy and/or irradiation or, in the case of marrow failure, exposure to immunosuppressive drugs such as cyclosporine, antithymocyte globulin, or glucocorticosteroids. Many of the patients referred to transplant centers have a history of prior infections with bacteria, fungi, or viruses [110]. Modern medicine has provided us with powerful antimicrobial drugs, which is one of the factors that led to improved survival following HCT [111,112]. Preemptive strategies with ganciclovir to prevent cytomegalovirus (CMV) disease have dramatically reduced CMV infection as a cause of post-transplant mortality [113]. Randomized phase III trials have failed to show a significant reduction in overall outcomes in patients who receive preemptive therapy with the new azole compounds as compared with fluconazole [114,115]. A meta-analysis indicated that the routine use of polyvalent intravenous immunoglobulin (IVIG) post-transplant does not decrease infectious complications following HCT [116]. Additionally, the use of polyvalent IVIG was associated with an increased risk of hepatitis SOS/venocclusive disease (VOD).

The evaluation of transplant candidates, in particular those who are scheduled to undergo allogeneic HCT, should include a meticulous search for any signs of subclinical infectious sources. Such studies include standard X-rays and, if clinically indicated, computed tomography (CT) and magnetic resonance imaging (MRI) scans [117]. A prospective study has shown that the underlying disease and leukocyte count are associated with the ability to isolate fungal organisms in respiratory samples obtained via bronchoscopy [118]. In addition, a dental examination is recommended and corrective dental procedures should be performed prior to transplant if possible given the underlying disease and donor availability.

Infections with HIV, hepatitis B, or hepatitis C are not contraindications for either autologous or allogeneic HCT [119]. Certainly, patients with these underlying infections are at increased risk of reactivation following transplant. Concerning specific infections, the reader is referred to Chapters 85–93.

Transfusion history

For those patients who have been transfused, a detailed transfusion history should be obtained, namely, number of and response to red blood cell and platelet transfusions. In addition, patients should be questioned about the use of granulocyte transfusions as they may lead to increased HLA sensitization. It is also important to monitor the number of transfusions in order to assess cumulative iron load. Elevated serum ferritin levels are strongly associated with lower overall survival and DFS in patients transplanted for AML or MDS [120]. Ferritin is a poor marker for iron overload given its role as an acute phase reactant. For that reason, some have questioned the legitimacy of using ferritin as a marker for iron overload in patients undergoing HCT. Indeed, a recent analysis indicated that patients who have iron overload as measured by MRI of the liver are not at increased risk of mortality following HCT [121]. Additional retrospective studies showed an association between transfusion dependence and increased comorbidities prior to HCT [122], which may explain the increased mortality observed post-transplant. Recent studies indicate that many long-term transplant survivors have secondary hemosiderosis and may need iron mobilization therapy [123].

Psychosocial assessment

It is the goal of the psychosocial assessment to get to know candidates' personalities and related issues in order to maximize patients' participation in their own care and to lay the foundation for a successful return to their personal and professional life after HCT. It is furthermore the goal to minimize problem areas that would interfere in any way with the transplant procedure and the recovery phase. Pretransplant family conflicts are among other factors associated with impaired medical and psychosocial recovery [124]. A patient's psychosocial history and condition have the potential to enhance or detract from the transplant procedure. The assessment, including an evaluation of previous response to pain control, should be made by a qualified professional at the time the patient's medical candidacy is being evaluated. In addition, appropriate insurance funding for HCT is part of the evaluation process. The purpose of the psychosocial assessment is summarized in Table 29.6.

Substance abuse is associated with a poor outcome of HCT [125], and uncontrolled drug, nicotine, and alcohol dependences are exclusion criteria. Several studies have demonstrated an association between NRM and smoking [126,127]. A history of any substance dependence indicates the need for a formal psychiatric evaluation prior to acceptance for HCT. Since there is an association between poor pre-transplant executive functioning and risk for post-transplant delirium, steps should be taken to ensure that patients with lower executive functioning have appropriate monitoring and intervention at the first sign of impairment [128]. Adherence to post-transplant guidelines has been assessed in a prospective survey [129]. Non-adherence was associated with poor knowledge and concerns about medical costs. Therefore, it is important that patients be appropriately informed regarding the importance of medical compliance and that healthcare costs are adequately addressed during the initial and ongoing visits.

Several of the issues mentioned with psychosocial assessment overlap with what has been stated in earlier paragraphs describing the patient–physician interaction. It is desirable that some of the psychosocial issues be assessed by more than one person, i.e. the attending physician and the medical social worker. The impression of both examiners should be presented in a formal meeting in order to create an impression of the patient's candidacy for HCT. During such a meeting, all medical and social issues should be presented to an institutional group of transplant physicians, social workers, research nurses, dietitians, and so on, and the candidacy for HCT of the referred patient, including enrollment in institutional or national clinical trials, be determined.

Table 29.6 Goals of the psychosocial assessment of adult HCT candidates

Purpose of psychosocial assessment:

1. Documentation of psychosocial stability
2. Identification of patient and family strengths and weaknesses in coping mechanisms
3. Assistance with preplanning for the HCT procedure, e.g. arrangement for childcare, transportation, state and Federal program linkages
4. Coordination of any corrective action, if necessary, for the success of the transplant procedure, e.g. referral for psychiatric evaluation, compliance contract, drug testing

Individual areas of patient assessment should include:

1. Previous methods of dealing with treatment issues and crises
2. Past and present drug, alcohol, and tobacco usage
3. Past and present psychiatric history
4. Understanding of diagnosis, treatment plan, and prognosis
5. Compliance with previous therapy needs
6. Motivation
7. Unique cultural, religious, literacy, and language needs
8. Ability to use information given
9. Previous activity and interest levels

Other important areas for assessment:

1. Emotional support for the patient during all phases of HCT
2. Caregiver availability, i.e. family members or friends
3. Employment issues
4. Advanced care directives, e.g. durable power of attorney for health care, a will
5. Stability of the environment to which the patient is expected to return
6. Family adjustment to diagnosis, therapy, and prognosis
7. Ability to relocate geographically for HCT
8. Other stressors, e.g. parents, children, insurance, transportation

Special considerations for pediatric transplant candidates

The matter of consent

Obviously, many of the medical and psychosocial concerns of the adult HCT candidate are pertinent to children, but because the patient is a child, the physician must also attend to the concerns of the patient's guardian(s). Foremost among these concerns is the matter of consent.

Whereas for adult HCT candidates the impact of HCT on a spouse and other family members must be considered, ultimately the patient, informed of the risks and benefits of HCT, consents to treatment. Children and adolescents are not legally able to consent. The primary caregiver, usually the parents, on behalf of the child must weigh the options and decide the best course. Providing parents with information and direction and helping them reach an appropriate decision for their child is the role of the physician. It also important to involve the adolescent in the decision-making process as well. At our institution, we use simplified assent forms for young children (aged 7–13 years) and regular consent forms for teenagers (aged 14–17 years). In both circumstances, we also obtain consent from the parent or legal guardian. This process allows for some feeling of control from the adolescent and the young child and also keeps them involved in the decision-making process.

Initial visit by the patient and parents

The patient, be it an infant, young child, or adolescent, is the physician's primary concern. Although the parent or legal guardian signs the informed consent, the physician must be attuned to the patient's capacity to understand the transplant process. Young children, precocious in the understanding of their disease and its implications, require an explanation of HCT rationale suitable to their level of comprehension. Alternatively, adolescents who do not choose to be involved in decisions regarding their treatment should be allowed to participate up to their level of comfort. The physician must grasp the child's emotional maturity and provide an appropriate level of treatment discussion. Parents may be helpful in advising the physician of the patient's desires, although some parents, irrespective of the child's wants, seek to protect the patient from knowledge of the serious risks and consequences of treatment.

Children with malignant disease

Whether a child is a candidate for an allogeneic or autologous HCT depends upon the disease diagnosis and status. Depending upon the circumstances, the initial family conference must clarify the rationale for HCT. It happens that parents arrive at this meeting with preformed impressions and fears, which have arisen from conversations with other parents or with family and friends. Often what parents have heard is erroneous or exaggerated, and these notions must be corrected.

The indications for HCT for children with malignant disease continue to evolve just as they do for adults. Where opinions differ regarding the necessity for HCT, it is important to discuss the alternative treatment with the parents. In centers where physicians limit their practice to HCT patients, the obligation regarding counseling families differs from centers where the physician is responsible for managing both the standard chemotherapy and HCT. In the former, a primary physician has determined that HCT should now be considered an option and refers the patient. Although the transplant physician must decide based upon his or her own understanding whether HCT is appropriate for the child, there is no inherent conflict between the treatment roles. In centers where roles overlap, the physician must be aware of potential bias and carefully explain the treatment alternatives, even offering the family the option of a second outside opinion.

Children with non-malignant disease

Although children with malignant disease comprise the majority of transplant candidates, patients with genetic and immunologic disorders may benefit from HCT, and these patients and their families require that the consent discussion be attuned to the specific disease. Non-malignant diseases eligible for HCT include conditions that are immediately life threatening, for example, aplastic anemia or severe combined immunodeficiency, and other conditions that, although not immediately life threatening, ultimately will lead to death or to devastation of the child's quality of life, for example, sickle cell anemia or adrenoleukodystrophy.

When patients are referred for HCT for these indications, it is critical that the transplant physician works closely with the primary specialist. Ideally, a coordinated effort will accurately portray the intent for HCT and what might be achieved. It also lays the groundwork for the patient's optimal care in the post-transplant setting. Even when HCT is successful, there may be delayed toxicities from therapy, or patients may remain susceptible to elements of the primary disease. Coordination of care is essential to maximize the transplant recipient's quality of life.

Patients with non-malignant disease rarely receive chemotherapy prior to HCT. Without this experience, patients and families lack a frame of reference to comprehend the side effects, both expected and rare, that may occur during HCT conditioning therapy. The physician must carefully explain the necessity for conditioning therapy and reassure families that all efforts will be taken to minimize discomfort and risk for their child.

Conditions affecting the child as a candidate for HCT

The evaluation of the patient as an HCT candidate reconciles the patient's current state of disease and overall physical condition. Both elements influence treatment outcome and prognosis. Occasionally, circumstances arise where there is no longer any benefit for HCT. In such cases, outside a clinical investigation, the recommendation should be against HCT. Initially, for most families this will be unacceptable, but with a careful and compassionate explanation, the physician can bring the family to understand that the discomforts and toxicities of HCT are not justified for their child when there is no benefit.

More difficult for the patient, family, and physician are those circumstances where the likely benefit from HCT is low but not altogether absent. Here too, candid discussion allows the family an opportunity to ask questions and discuss their concerns and expectations. Although it is rare that the family will decide not to proceed with HCT, knowing that refusing HCT is an option can be very important. It is possible that an older adolescent may refuse HCT or wish to delay hospital admission to fulfill an important life wish. These decisions, whichever way, should be respected and supported by the physician.

Table 29.7 The Lansky play-performance scale [130] (for use with persons aged 1–16 years)

Score (%)	Description
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both greater restrictions of and less time spent in play activities
60	Up and around, but minimal active play; keeps busy with quieter activities
50	Gets dressed but lies around much of the day, no active play; able to participate in all quiet play activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities
10	Unresponsive
0	Dead

Estimation of performance status is always subjective to some extent, and this applies even more so for children compared with adults. The Karnofsky scale, intended for adults, can be used for older children; school serves as a surrogate for work. For younger children, the Lansky scale (Table 29.7) [130] is more appropriate, but this scale relies heavily on parental reports. Even more problematic, the scale includes developmental skills that are not applicable to toddlers and infants. Although it has been demonstrated that pre-transplant performance measure may correlate with outcome, alone these scales lack sensitivity and specificity sufficient to counsel the family. Combined with organ-specific evaluations, performance scale measures can identify patients at greater risk for regimen toxicity [131,132].

For children with malignant disease, the most likely explanation for a low performance status is the effects of the disease for which HCT is being considered. Children have not lived long enough to suffer the morbidities that arise from aging and/or a lifetime of bad health habits. Organ-specific evaluation must anticipate the consequences of prior therapies, for example, myocardial injury secondary to anthracyclines or mediastinal irradiation. Complications from prior therapy may be exacerbated during the transplant course, as a patient with previous pulmonary fungal disease and reduced respiratory reserve is at grave risk from further lung injury. The physician's exposition of the performance status, organ evaluation, and prior morbidities enables the patient and family to comprehend better the risks undertaken during HCT and to understand the rationale of the treatment plan.

Organ system evaluation for the pediatric patient is similar to that for an adult but, depending upon the child's age, may require modification. For the youngest patients, pulmonary function tests may not be available [133]. Some children's hospitals can perform pulmonary function tests on infants, but their value in the transplant setting has not been established. If a child has experienced serious respiratory complications, imaging studies and consultation with a pediatric pulmonologist prior to transplant may be useful.

Two potentially important conditions, obesity and iron overload, may increase the risk for regimen-related toxicity, particularly liver toxicity. The increase in morbidity and mortality associated with excess liver iron has been established for patients undergoing HCT for thalassemia, but undoubtedly this association is pertinent to any patient with iron overload [120]. Thus, any child coming to transplant having received frequent red cell transfusions, for example, a patient with Diamond–Blackfan anemia or other marrow failure disorders, must be considered at heightened risk from regimen-related toxicities. Although iron accumulation by the heart can compromise cardiac function, adequate cardiac function determined by echocardiography diminishes the likelihood of clinically significant iron accumulation.

Poorly nourished children coming to HCT can usually be managed successfully with parenteral nutrition; less apparent is the risk for toxicity that HCT imposes on the obese child. For obese patients, elevated liver transaminase may be indicative of liver steatosis, or of steatohepatitis when insulin resistance and hypertriglyceridemia are also present [134]. The metabolic syndrome of obesity, hypertension, hyperlipidemia, and glucose intolerance is causally related to cardiovascular disease in adults, but the significance of these findings has not been established for children. It should be noted that children, particularly adolescents, are more prone to the hyperglycemic effects of prednisone and L-asparaginase [135]. Parents and caregivers compound the problem by ignoring or even promoting obesity by allowing the child indiscriminate amounts of high-calorie food.

The impact of HCT on the child's neurocognitive development concerns parents and physicians alike. If possible, neurocognitive testing should be obtained for all children before the start of conditioning [136]. Baseline assessment provides a reference to measure future development, which becomes important if remediation efforts are needed later. This is most relevant for children who receive TBI and/or CNS irradiation for conditioning, as such patients are most at risk for neurocognitive injury. Neurocognitive tests are absolutely essential to evaluate patients with metabolic storage disorders that cause neurocognitive deterioration. Poor neurocognitive performance index prior to HCT predicts an unsatisfactory outcome following HCT and should dictate against proceeding [137].

Counseling the patient and family

Emotional distress for patients undergoing HCT often is most severe as treatment commences and tends to diminish thereafter [138,139]. Likewise, parents' distress prior to HCT is greater than at any later time during the transplant course [140]. Therefore, the initial meeting with the family should attempt to address the causes for their distress. Not knowing what to expect from HCT is a major source of anxiety and fear. The physician, by providing details of the transplant course, allows the child and family to participate in and, to some extent, to control future events, which can help diminish stress. Additional consideration should be given to the parents' coping strategies and also the family environment, as a prospective study has shown that these factors are related to distress in children prior to HCT [141]. The dynamics of the family and specifically the parents sometimes affect the child/patient in a detrimental manner.

The discussion of medical matters should be tailored in a manner that is appropriate to the cultural and social background of the family. Although it is important to convey the rationale for HCT in addition to its risks, benefits, and long-term consequences, the discussion must ultimately meet the needs of the patient and the family. Some families prefer very detailed information and may even question the necessity for particular elements of the treatment, for example, the use of TBI. Yet other families become confused and anxious from too much detail and prefer to place their trust in the physician and be reassured that the physician will do the best for their child. If desired, other family members should be allowed to partake in the consultation process. Single parents, overwhelmed by the total responsibility for consenting, should be encouraged to bring a grandparent or other supportive person to this meeting [142]. If the treatment is a clinical trial, the physician must take care that the patient and family understand the experimental component, possible alternative treatments, and their right to refuse or withdraw [142]. The presence of a child life specialist at the consent discussion may be an effective means to assure understanding of the proposed treatment.

Every family brings hope for the future of their child to the HCT process. HCT is the means to the future, and at that moment the parents' focus is on the child safely passing through this treatment. Nonetheless, there are important long-term consequences arising from HCT of which parents must be made aware. Among these are the effects of HCT on intellectual development, growth, and fertility, and the risk for a second malignancy following HCT [143].

Problems arising from school are not uncommon for children post-HCT. Undoubtedly, patients come to transplant already challenged academically because of the school absence related to the primary disease and its treatment. Certain diseases for which HCT is performed may directly impact the CNS, such as brain tumors or demyelinating diseases. For hematologic malignancies, previous treatment, including intrathecal chemotherapy, may have affected neurocognition. HCT conditioning therapy with TBI adds to the risk for future learning difficulties, although it has been shown that cognitive function does not invariably decline post-HCT. Reassuringly, except among the youngest patients, many studies find preservation of pre-HCT neurocognitive function [136,144]. For children less than 3 years of age, most investigators document at least some fall-off in neurocognitive test performance. In relaying this information to parents, it is important to emphasize that a profound deterioration of intellect is rare and that coordinated efforts between neuropsychologists and educators may help the child compensate.

Linear growth depends upon intact bone and endocrine function; radiation therapy disturbs both systems. As is true for neurocognitive function, the patient's growth may have suffered prior to coming to HCT, and again it is the youngest patients who will be most seriously affected [145,146]. The physician must counsel the parents that while treatment with growth hormone may improve the child's linear growth, it is unlikely that the child will achieve what would have been the predicted adult height (see Chapter 102).

Although fertility has been discussed previously, there are several points unique to pediatric patients. Pubertal development may be delayed in up to half of children treated with fully ablative conditioning, and children receiving fully ablative HCT conditioning, particularly when the conditioning includes TBI, rarely are fertile [145]. For patients with malignancy and previously treated with intensive chemotherapy, fertility may have already been diminished. Often families indicate that they had not been aware of this possibility. Even as parents learn of and accept the risks for regimen-related toxicity and the potential for unfavorable or even fatal outcome following HCT, it may be heartbreaking for some to have to accept the responsibility for their child's infertility. For many cultures, the loss of the ability to conceive carries momentous impact, and the physician must be attuned to this. The development of reduced-intensity conditioning therapy may allow more children to retain their fertility, and the potential for advances in reproductive medicine offers parents hope for the future [147].

Successful HCT allows children the chance to live a normal and meaningful life but, unfortunately, not a life without persistent concerns. One undesirable consequence of surviving is the risk of secondary cancer. The risk is, in part, disease and HCT conditioning regimen specific [148]. Unfortunately, the risk of secondary cancer has not been adequately determined for every condition. HCT conditioning therapy that includes radiation has been associated with a higher incidence of new tumors, but heightened risk exists for patients receiving chemotherapy alone [149]. It may be important to note here that the estimate of developing solid tumors was 13% among patients transplanted for aplastic anemia and that, in the multivariate analysis, radiation, typically less than 10 Gy, was associated with that increased risk [150]. Although reduced-intensity conditioning regimens allow children with severe pre-transplant morbidity to undergo HCT, the risk for secondary cancers from reduced-intensity regimens has not been defined.

Advanced care planning (ACP)

HCT is an aggressive and intensive treatment and is intended to be life saving; however, it is associated with short-term morbidity and mortality. Physicians should establish during the initial sessions that other aggressive interventions, for example, dialysis or mechanical ventilation, may be needed to support the patient during the treatment course [3]. Discussion of ACP offers adolescents and some precocious younger children and their parents an opportunity to consider in what circumstances the decision would be to withhold or withdraw care. Rarely do adolescents provide an advanced directive; however, these matters may be discussed within the family, and parents will know of their child's wishes. These conversations will become very important should care ultimately become futile. Unfortunately, only 50% of adult transplant recipients are engaged in ACP. A retrospective survey indicated that patients who are not engaged in ACP have an inferior outcome compared with patients who are engaged in ACP [5], thus indicating that the patients who did not engage in ACP were the very patients who were most likely to need it.

Some final considerations

HCT requires a cooperative, educated, and insightful patient. Through the educational efforts of the transplant team and the informed consent process, the patient should have acquired the high level of understanding and knowledge that is a prerequisite for participation throughout the long HCT process, both at the transplant center and subsequently in the domestic environment. It is sometimes forgotten or ignored that the transplant process is not completed with the return of the white blood cell count to the normal range. Deficiencies of the immune system persist for many months and sometimes years following HCT.

Allogeneic marrow graft recipients especially are at risk for late infectious complications even if all in vitro tests are showing a return of normal immune function. The transplant patient should be instructed to seek medical advice for even relatively harmless-appearing symptoms. A well-functioning communication system between the transplant center, the patient, and the local physician has to be a permanent part of the post-transplant course. Most major transplant centers have a long-term follow-up department that assists local physicians caring for the post-transplant patient. It is important that the lines of communication between the local physician and transplant center remain intact. Patients serve as an important component of that link and they should always be encouraged to contact the transplant center if there are any complaints which they feel are not being adequately addressed by their local physician.

This chapter can never be complete. There are simply too many medical or social circumstances that cannot be described in detail or even be anticipated. There are no rules for common sense and no substitute for professional judgment or rapid communication.

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CHAPTER 30

Nursing Role in Hematopoietic Cell Transplantation

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Introduction

Caring for patients undergoing hematopoietic cell transplantation (HCT) is one of the most challenging and rewarding of all nursing subspecialties. These patients require nurses with expertise in assessing the acute, subtle, and unique clinical changes of HCT. In addition to the knowledge base of oncology nursing, the specialty of HCT nursing requires expertise in addressing unique diseases such as sinusoidal obstructive syndrome (SOS) and graft-versus-host disease (GVHD). Most of nursing's effort is focused on the post-transplant phase with managing side effects and toxicities, and preventing complications caused by the high-dose chemoradiotherapy given to prepare for HCT and the consequences of GVHD [1]. Nurses are the members of the HCT multidisciplinary team who spend the most time with the patients, and often are the first to identify changes in clinical status. Nurses must also have psychosocial skills as they support the patients and their significant others through decisions and acute clinical changes encountered during the HCT process [2].

Supporting medical research, the foundation of HCT care, is another factor that attracts nurses to this subspecialty. Integrating new research initiatives into clinical care and being on the front line as new therapies evolve to change standard practice are very exciting. Nurses experience satisfaction from working in a medical arena offering the best possible therapy to their patients. Those nurses who stay with this specialty over a number of years witness therapies used in phase I research studies become the standards of care or the basis for future research. These nurses can readily identify the improvements in patient outcomes that have occurred in the field of HCT over the past three decades in curing specific diseases, and also improvements in the prophylaxis and treatment of GVHD and SOS, infectious disease prevention and therapy, and symptom management. In addition, expanding HCT to treat autoimmune diseases and solid tumors has required nurses to become familiar with disease processes beyond hematologic malignancies. Treating patients using reduced-intensity conditioning regimens has presented new challenges to HCT nurses. Not only are these patients often older with more comorbidities than those eligible for myeloablative regimens, but also the patterns in their clinical courses in areas such as acute GVHD presentation can differ from those of myeloablative transplants. Nurses must be flexible and able to accommodate changes in medical therapy such as extracorporeal photopheresis for the treatment of chronic GVHD [3]. Another example is the recent increased use of umbilical cord transplants to expand the pool of patients able to be treated, which challenges nurses with a different level of immunosuppression experienced by these patients.

There has been a shift in the site of HCT care from inpatient units to outpatient infusion treatment facilities and clinics with the goal of containing costs while maintaining high-quality, safe patient care [4,5]. These changes were made possible by major improvements in oral antiemetic efficacy, antibiotics with less frequent dosing, and improved ambulatory infusion devices. Patients can receive all of their conditioning and hematopoietic stem cell (HSC) infusion in an outpatient setting, and are admitted only as their clinical picture dictates (usually fever or mucositis pain). Patients are also discharged from the hospital based on more liberal criteria than in the past. This change has resulted in increasingly complex ambulatory patients where the focus of nursing care shifts from first-hand assessment and care of inpatients to teaching the caregivers to provide some of these skills in the home setting [6]. This has resulted in the average acuity of the HCT inpatient populations to be markedly increased, with patients acutely ill with complicated multisystem issues. It has also led to the majority of patients having multiple unscheduled admissions and discharges in the months after transplant. Nurses are responsible for ensuring continuity of care and patient safety as the responsibility for care is handed off from one site to another [7].

The intensity of therapy requires subspecialization of HCT nurses to meet the care needs of these patients [8]. Transplant centers organize nursing support in a variety of ways depending on their larger operational structure. At most centers, specific nurses, or "coordinators," have a role in the initial intake of patients into the program. Most outpatient clinics have a case manager structure, which allows for continuity of care coordination throughout the transplant process. HCT patients often spend large amounts of time in outpatient infusion areas. These patients require nursing expertise different from that of other oncology outpatients. Inpatient care is provided either on a specific transplant unit or on a hematology–oncology unit. Each center needs to give serious consideration regarding whether to keep HCT patients requiring intensive care on the HCT unit or to transfer them to an intensive care unit (ICU). In either case, nurses with expertise in both HCT and intensive care are required. Discharging HCT patients from an inpatient unit to the outpatient setting is often complex, especially with more liberal discharge criteria, and many centers have specific nurses with expertise in managing these transitions[9].

Complex research protocols often require coordination by a nurse who works closely with the principal medical investigator.

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These nurses are responsible for ensuring documentation of protocol data and the reporting of adverse advents. They are key in ensuring that protocol coordination is seamless when patients transfer to and from the inpatient and outpatient settings. They are the primary resource for medical and nursing staff regarding protocol implementation. In addition, because of the relatively long length of stay in the medical centers with standardized treatments, HCT patient care provides ideal opportunities for nursing research. Nursing studies have been conducted in a number of areas including quality of life and symptom management [10–15].

The purpose of this chapter is to describe the nurse's role at the various phases of the HCT process. Tables detail the key specific nursing responsibilities of assessment, teaching, care coordination, and carrying out the medical care plan at each phase.

Assessment includes evaluating not only the basics of physical assessment of the patient, but also the ability of the patient and caregivers to cope with this intense experience.

Teaching must be individualized to each specific patient and caregiver situation. There is no substitute for one-on-one assessment and teaching by the patient's primary nurse. One-on-one encounters allow for assessment of the patient's assimilation of the required information and a time for the patient to ask whatever questions are of foremost concern. Teaching can be accomplished by a variety of methods, including written materials with photographs or illustrations and electronic learning formats. Classes are also an effective initial teaching method, followed by individual instruction for centers that perform a large number of transplants.

Coordination of care for these patients is extremely complex and critically important. Nurses assess the patient's and family's strengths and weaknesses, and plan interventions to assist them to integrate this intense therapy into their lives. Nurses must include clinical issues, operational resources, research requirements, and the patient's abilities, needs, and desires when planning care.

Carrying out the medical care plan is an important role of the transplant nurse. Specialized understanding of the patient's underlying illness, the transplant procedure, complications of transplant, and the typical treatments for each complication are important for successful implementation of the medical care plan.

Prework-up/prior to arrival at the transplant center (Table 30.1)

The first interface that many patients have with an HCT transplant program occurs at the time they come for an initial opinion about the role transplantation may play in the treatment of their disease. Patients may make a consultation appointment to compare a particular program with those of other centers they are considering. These patients meet with a transplant physician to review their options, a financial counselor, and a nurse. The nurse's role is to assist the patient with this decision by explaining the logistics of the program, including the usual length of stay on the inpatient unit and how much of the transplant process will be managed on an outpatient basis. Many patients are interested in the nursing services at the program they are considering and ask questions about the nurse-to-patient ratios. The responsibilities of the patient's caregiver are also reviewed. At that time, many families are concerned with the logistics of relocating and incorporating this intense therapy into their lives. These patients present with a myriad of questions, and the nurse must be versatile in meeting the patients' and families' informational needs in coordination with the transplant team. Nurses often are also responsible for giving information regarding the transplant program to physicians and nurses from the referring center in addition to third-party payers.

Table 30.1 Prework-up/prior to patient's arrival at transplant center

Assessment
Understanding of the overall transplant process and time commitment
Current symptoms from previous therapies or disease
Current coping ability
Current pain
Current blood product requirements
Sedation preference for procedures
Teaching
Length of time for work-up, mobilization, and transplant process
Role of caregiver
Care coordination
Confirm family/friend plan for caregiving
Contact referring physician's office to obtain report
Confirm financial clearance
Confirm housing plan
Carrying out the medical care plan
Maintain working knowledge of proposed transplant plan for patient

Table 30.2 Work-up

Assessment
Patient's current fears and concerns
Barriers to learning
Current pain
Knowledge of disease status
Knowledge of transplant process
Knowledge of patient's rights and responsibilities when participating in medical research
Usual coping strategies
Level of fatigue and usual sleep patterns
Patient's experience in other healthcare environments
Identified caregiver(s)' commitment
Identified caregiver(s)' barriers to learning
Allergies
Current medications and knowledge of purpose
Adherence in taking medications
Culture for presence of antibiotic-resistant organisms
Current central venous access
History of central venous access
Teaching
Logistics for working with home infusion companies and administration of home infusions
Clinic logistics, including how to access care after hours
Importance of having a caregiver during various phases of the transplant process
Purpose of procedures, laboratory tests, and scans required for work-up
Overall transplant process
Usual complications of transplant
Central venous catheter preoperative teaching
Care coordination
Assess ability to adhere to work-up schedule
Confirm financial clearance for transplant
Social work assessment
Nutrition assessment
Carrying out the medical care plan
Maintain working knowledge of proposed transplant plan for patient
Ensure all work-up studies are obtained in a timely manner

Work-up (Table 30.2)

Once a patient has met initial screening and has decided to undergo HCT at a specific center, the nurse's primary responsibility becomes education. The patient must understand the specifics of the rigorous evaluation. The coordination and timing of care during this phase are especially critical if the patient has an unrelated donor. The completion of work-up and the initiation of conditioning must start

Table 30.3 Preconditioning

Assessment
Knowledge of need for compliance during conditioning regimen
Knowledge of side effects to report during conditioning
Caregiver's ability to report symptoms
Caregiver's ability to utilize ambulatory infusion pump if required
History of antiemetics
Assure appropriate patient central venous access
Teaching
Treatment plan and schedule for chemotherapy and total body irradiation
Antiemetic schedule and importance of adherence
Purpose of viral and/or fungal prophylaxis
Purpose of prophylaxis for graft-versus-host disease if appropriate
Care of central venous access
Caregiver education for ambulatory infusion pump if hydration given in ambulatory setting
Importance of laboratory monitoring
Purpose of hydration
Symptoms to report
Differences between previous chemotherapy and conditioning chemotherapy
Importance of compliance with *Pneumocystis* prophylaxis
Care coordination
Research consents
Chemotherapy, total body irradiation and/or radioimmunotherapy order verification with protocol
Dosimetry and simulation appointment with radiation oncology if applicable
Pager/cell phone for patient if conditioning to start in the clinic
Carrying out the medical care plan
Ensure central venous catheter is inserted in timely manner
Ensure antivirals, antifungals, pneumocystis prophylaxis, immunosuppressants, and antiemetic medications are initiated as ordered

on an exact date to allow HSC infusion on the planned date of HSC procurement. The generosity of the volunteer donors must be respected and accommodated.

Preconditioning (Table 30.3)

The nurse plays an important role in the informed consent process, supporting the medical staff's explanations and plans to ensure, as much as possible, that the patient is making an informed decision regarding HCT.

Donor preparation (Table 30.4)

Donors have been called the "forgotten patients" of transplant. The HCT recipient, appropriately, is the center of focus for the transplant team. However, donors also have concerns about their own health and the procedures they will undergo. It is ideal for donors to have a primary nurse with whom they can establish a relationship and who can prepare them for the hematopoietic cell collection and monitor them throughout the procedure. The Foundation for the Accreditation of Cellular Therapy (FACT) has also determined that the donor should be cared for by a different physician than the transplant recipient.

Table 30.4 Donor work-up

Assessment
Current fears and concerns
Barriers to learning
Donor screening per American Association of Blood Banks (AABB) requirements
Knowledge of donation process
Assessment of adequacy of veins for apheresis
Knowledge of patient's rights and responsibilities when participating in medical research
Allergies
Teaching
Donation plan and schedule
Importance of laboratory monitoring
Preoperative teaching if applicable
Symptom management
Care coordination
Assess ability to adhere to work-up schedule
Research consents
Storage of autologous red cells if marrow donation planned
Carrying out the medical care plan
Maintain working knowledge of proposed transplant plan for patient
Ensure all work-up studies are obtained in a timely manner

Table 30.5 Mobilization and collection (autologous patients and allogeneic donors)

Assessment
Knowledge of care of central venous access (if applicable)
Teaching
Purpose of monitoring CBC, CD34$^+$ cells, and electrolytes
Purpose and care of central venous catheter (if applicable)
Specific medications used to mobilize, and usual side effects if applicable
Subcutaneous injections if applicable
Temperature monitoring
Importance of increasing calcium intake
Medications to avoid (e.g. aspirin)
Care coordination
Coordinate home administration of subcutaneous medications
Manage side effect of growth factors
Coordinate stem cell collection and storage with Cellular Therapy Laboratory
Confirm adequate venous access
Carrying out the medical care plan
Administer subcutaneous injections in clinic
Central catheter management
Collect stem cells

Mobilization and collection (autologous patients and allogeneic donors) (Table 30.5)

Nursing care during mobilization must be individualized to the specific medical protocols being utilized in addition to the response of the donor or autologous patient. The nurse must stay in daily contact with the donor or autologous patient during this time as collection schedules can change based on cell yield. The nurse needs to assist in determining whether it is necessary to have a long-term central venous access catheter inserted before mobilization, if a short-term central venous access catheter is sufficient, or if vein-to-vein access may be possible. The donor should be educated regarding monitoring and reporting side effects of administered cytokines and chemokine antagonists (i.e. bone pain, headache and gastrointestinal symptoms) in addition to providing the appropriate interventions for symptom relief [16].

Conditioning (Table 30.6)

Prior to the infusion of stem cells, the patient will receive conditioning therapy to eliminate residual disease (autologous and allogeneic transplants) and prepare the patient's system to accept donor stem cells by creating "space" in the bone marrow and suppressing the patient's immune system (allogeneic transplants) [17]. Conditioning can be chemotherapy (high-dose regimens or reduced-intensity regimens), biotherapy, total body irradiation, radioimmunotherapy, or a combination of these modalities. Chemotherapy used for traditional myeloablative transplants is administered at higher doses than conventional chemotherapy to treat cancer. These high-dose regimens will lead to acute toxicities that may manifest more rapidly and intensely than with standard-dose therapy [18]. The nurse's role is key in safe administration of high-dose therapy and in supporting the patient and caregiver in the management of acute toxicities from the high-dose regimen. Toxicities associated with

Table 30.6 Conditioning

Assessment

Knowledge of potential side effects of conditioning regimen
Response to high-dose chemotherapy, immunotherapy, radioimmunotherapy, and/or total body irradiation
Ability to perform self-care and infection control preventative measures (hand washing, personal hygiene, oral care, etc.)
Renal and fluid status: intake and output; postural vital signs; daily or twice-daily weights; breath sounds, heart sounds; skin turgor; laboratory tests: serum electrolytes, blood urea nitrogen, creatinine
Nutritional status: weight; amount, content, and patterns of nutritional intake
Outpatients:

- Patient/caregiver ability to assess, report, and manage side effects of conditioning regimen
- Knowledge of telephone numbers to access support and emergency care 24 hours per day

Teaching

Treatment plan and schedule, including administration of intravenous hydration, antiemetics, uroprotectants, and other medications
Potential side effects of conditioning regimen (nausea, vomiting, mucositis, diarrhea, fever, etc.)
Prevention and management of side effects
Level of caregiver support required
Guidelines for preventing infection:

- Hand hygiene
- Personal hygiene, oral care, CVC care, etc.
- Nutrition/diet guidelines for immunosuppressed patients
- Avoiding activities with high risk for infection

Outpatients:

- Signs/symptoms requiring inpatient hospitalization
- Access to 24-hour triage and emergency care telephone numbers

Care coordination

Clinic appointment schedules or admission to inpatient unit
Total body irradiation schedule and coordination of care with radiation oncology department
Patient and caregiver education: one-on-one instruction or attendance at caregiver classes

Carrying out the medical care plan

Verification of chemotherapy orders
Administer conditioning therapy, intravenous hydration, and medications (antiemetics, uroprotectants, etc.)
Chemotherapy administration
Drawing and monitoring laboratories
Provide supportive care

CVC, central venous catheter.

high-dose chemotherapy and/or radiation therapy require vigilant monitoring with special emphasis on assessment of fluid, gastrointestinal tract, and renal status. Reduced-intensity regimens will be better tolerated, but will still require expert nursing monitoring to detect toxicities that need to be managed to reduce negative sequelae. Conditioning regimens may be administered in the outpatient or inpatient setting, based upon the type of preparatory regimen chosen, the availability of 24-hour triage and supportive services, and the available level of caregiver support.

Care must be available to outpatients 24 hours per day, 7 days per week in order to provide safely for patients whose conditions may rapidly deteriorate. In general, successful management in the outpatient setting during the conditioning phase of transplant requires a high level of continuous caregiver support. Outpatient teaching places special emphasis on use of ambulatory infusion pumps, patient and caregiver ability to assess, report, and manage the side effects of high-dose therapy, and access to support telephone numbers and 24-hour emergency care. Printed patient and caregiver teaching reference materials containing outpatient guidelines and how to access emergency care are useful tools for the reinforcement of patient and caregiver teaching. Patients and caregivers must understand which symptoms require immediate medical attention (Table 30.7) . Caregivers should also be supported during this physically and emotionally taxing time [19].

Table 30.7 Quick reference for symptoms

Area of concern	Critical emergency DIAL 911	Call clinic now
Alertness, consciousness, activity	• Unconscious • Unable to arouse • Seizure	• New or increased confusion • Change in level of alertness • Mood changes: irritable, tearful, agitated • Sleeplessness • Falling down • Numbness, tingling or loss of movement in limbs • Dizziness • Lethargic • Change in energy level • Tremors/shakiness • Not able to get around • Difficulty swallowing
Bleeding	• Uncontrollable, consistent bleeding • Patient is unconscious	• New or increased bleeding • Bloody urine • New bruising • Unable to stop nosebleed • Bloody diarrhea • Vomiting of blood • 1 or more feminine pad per hour is used • Little red or purple spots on the skin
Blood sugar	• Blood sugar lower than 50 and unable to awaken	• High or low blood sugar • Hard to wake up – use glucagon
Breathing	• Not breathing • Choking – not moving air into chest	• Trouble breathing • Gets "winded" more easily with minimal activity • Feeling as if can't get enough air • Trouble breathing when laying flat • Wheezing with breaths • New or recurrent cough • Persistent continuous cough • Coughing blood or green/yellow material
Central venous catheter (Hickman line)	• Line open to air and patient short of breath • Clamp line immediately	• Line broken or leaking • Face, neck, exit site swelling • Inability to flush • Line fell out • Headache related to infusions • Redness, swelling, or tenderness at exit site • Drainage from exit site
Diarrhea		• Constant or uncontrolled diarrhea • New-onset diarrhea • Diarrhea with fever and abdominal cramping • Whole pills passed in stool • Greater than five times each day • Stool that is bloody, burgundy, or black • Mild abdominal cramping
Fall	• Hit head during fall and • Change in consciousness after fall	• Did not hit head and no changes in consciousness after fall
Fatigue	• Unable to wake up	• Dizziness • Fatigue is getting worse • Too tired to get out of bed or walk to the bathroom • Staying in bed all day
Fever/chills		• Fever greater than 1 degree above usual • Cold symptoms (runny nose, watery eyes, sneezing, coughing) • Shaking chills, temperature may be normal • Temperature greater than 38.3 °C or 100.9 °F by mouth
Mouth pain/ mucositis	• Not breathing	• Having difficulty breathing • Bright red blood in mouth • Pain not controlled by medication • White patches or sores appear on gums or mouth • Difficulty swallowing food or fluid

(*Continued*)

Table 30.7 *(Continued)*

Area of concern	Critical emergency DIAL 911	Call clinic now
Nausea/ vomiting		• Nausea persists without control from antiemetic medications • Projectile vomiting • Uncontrolled, constant nausea and vomiting • Blood or "coffee ground"-appearing material in the vomit • Medicine not kept down because of vomiting • Weakness or dizziness along with nausea/vomiting • Severe stomach pain while vomiting
Pain	• Severe chest/arm pain • Severe squeezing or pressure in chest	• New or uncontrolled pain in body • New headache • Chest discomfort • Pounding heart • Heart "flip-flop" feeling • Painful central line site or area of "tunnel" • Burning in chest or stomach • Pain or burning while urinating • Pain with infusion of medications or fluids into central line
Rash		• Rash with severe pain and or itching • New rash
Swelling		• Sudden swelling with or without pain • Swollen legs, arms and hands
Urination		• Unable to urinate for more than 8 hours • Bloody urine • Pain or burning while urinating

Source: Fred Hutchinson Cancer Research Center/Seattle Cancer Care Alliance Patient Education Program.

Table 30.8 Bone marrow harvest

Assessment
Results of preharvest donor work-up
Preharvest physical reassessment, as indicated
Verification of informed consent
Understanding of bone marrow harvest process and potential complications
Understanding of signs/symptoms to report to healthcare team postharvest
Ability to access 24-hour triage and emergency care telephone numbers
Teaching
Bone marrow harvest process and procedures:
- Hospital admission location, schedule
- Marrow aspiration sites
- Autologous blood transfusions
- Hospital discharge criteria
- Site care post-bone marrow harvest
- Postharvest iron supplements

Potential complications:
- Pain
- Infection
- Anemia related to blood loss
- Nausea and vomiting (if general anesthesia used)
- Dehydration

Signs/symptoms to report to healthcare team
Access to 24-hour triage and emergency care telephone numbers
For patients receiving unrelated donor marrow:
- Marrow harvested at donor residence (state/country)
- Identity of donor remains unknown
- Marrow arrives via courier
- Anticipated time of unrelated donor marrow arrival

Care coordination
Operating room scheduling
Type and cross-match for autologous stored unit, if stored
Time and location of hospital admission for day surgery
Discharge teaching
Discharge medications
Postharvest outpatient appointment and laboratory draws (generally, complete blood count with differential and platelets)

Bone marrow harvest (Table 30.8)

The technique of harvesting marrow is essentially the same for allogeneic related donors, unrelated donors, and autologous patients. Education will prepare donors or autologous patients for anticipated side effects, outlining those to report to healthcare providers. Nurses educating donors or autologous patients should emphasize the expectations for the marrow harvest, including information about anesthesia and the aspiration procedure, admission to hospital or day-surgery settings, anticipated recovery period and discharge from the hospital or day-surgery to home, pain management plan for marrow aspiration site pain, site care post-harvest, and follow-up visits.

Patients receiving unrelated donor marrow will need instruction regarding when and how the donor marrow will arrive for infusion. Donor marrow is harvested at the medical center closest to the donor's residence, and the marrow is flown to the patient's location accompanied by an Unrelated Donor Program courier. Patients will also be told that the identity of their unrelated donor must remain unknown for a period of time to protect the donor.

Autologous patients will have their marrow harvested and cryopreserved (frozen) for later use. The bone marrow will be thawed and infused after they receive conditioning therapy.

Transplant phase (Table 30.9)

The stem-cell product infusion is very similar to a blood transfusion. The rate of infusion, method of administration, and potential side effects depend on whether the product is cryopreserved or not. Dimethyl sulfoxide (DMSO) is generally used as the preservative for cryopreserved marrow, peripheral blood stem cells, and umbilical cord blood. Infusion of products cryopreserved with DMSO tends to cause a significant number of transient, self-limited side effects amenable to premedication [14,15,20]. These side effects are rarely severe (less than 1% of all complications), although life-threatening complications have been reported [21]. Side effects to be expected with cryopreserved cells are nausea/vomiting, tickle in the throat, facial flushing, hypertension or hypotension, bradycardia or tachycardia (can be delayed), cardiac dysrhythmia, chest tightness, cough, flushing, chills, fever, abdominal cramping, diarrhea, headache, transient taste and smell of DMSO (oyster, garlic, or creamed corn odor), and/or the appearance of red urine post-infusion. Complications from infusion of non-cryopreserved products may include volume overload and pulmonary abnormalities from fat or cellular debris emboli. In addition, patients may experience symptoms similar to blood transfusion reactions (chills, urticaria, fever, etc.) or hemolysis related to ABO incompatibility.

Patients and caregivers benefit from nursing education about the infusion procedure, potential side effects, and premedications. Important care coordination considerations for nurses include ensuring initiation of GVHD prophylaxis at the appropriate time for allogeneic patients and timing stem-cell product prehydration and premedications with the arrival of the products on the unit or the patient care area.

Pre-engraftment (Table 30.10)

The majority of complications that occur prior to engraftment are the result of toxicities associated with the high-dose conditioning regimen and/or the administration of immunosuppressive medications. Nursing care is directed at strategies to prevent potential

Table 30.9 Transplant

Assessment

Preinfusion:
- Knowledge of stem-cell infusion process
- Renal and fluid status: intake and output; postural vital signs; weight; breath sounds; skin turgor; laboratory tests: serum electrolytes, blood urea nitrogen, creatinine
- Response to prior red blood cell transfusions
- Adequacy of venous access for infusion of stem cells (central venous access required for infusion of cryopreserved stem-cell products due to DMSO; central venous access preferred for non-cryopreserved products)
- Availability of emergency medications

During infusion:
- Frequent vital sign monitoring and assessment to detect the early onset of potential infusion-related complications

Postinfusion:
- Frequent vital sign monitoring and assessment to detect postinfusion-related complications
- Tolerance of cell infusion
- Renal function and fluid status: intake and output (general goal is to maintain urine output at 2–3 mL/kg/h); presence of hematuria due to red-cell lysis

Teaching

Location for stem-cell infusion (inpatient versus outpatient setting)
Anticipated timing of stem-cell product arrival on unit or in outpatient department
Schedule for stem-cell infusion, including multiple days if necessary
Stem-cell infusion procedure:

Cryopreserved
- Prehydration
- Premedications (e.g. antiemetics, diphenhydramine, hydrocortisone, acetaminophen)
- Transport of cryopreserved cells
- Thawing of cells at bedside
- Infusion of cells via central venous catheter

Non-cryopreserved
- Premedication (if patient previously experienced transfusion reactions to red blood cell transfusions)
- Transport of cells to patient care area
- Infusion (generally via CVC)

Potential complications:

Cryopreserved
- Reactions to cryoprotectant DMSO including nausea/vomiting, tickle in throat, facial flushing, hypertension, hypotension, bradycardia, tachycardia, cardiac arrhythmia, chest tightness, cough, flushing, chills, fever, abdominal cramping, diarrhea, headache, transient taste and smell of DMSO, appearance of red urine postinfusion
- Prevention/treatment of throat tickle and nausea secondary to DMSO by antiemetics, sucking on hard candy, eating or smelling an orange
- Transfusion reactions (if allogeneic)
- Volume overload
- Allergic reaction
- Pulmonary microembolism (clumps of cell debris may occur after thawing): shortness of breath, dyspnea, cough

Non-cryopreserved
- Fluid overload
- Pulmonary microembolism secondary to fat emboli (may occur with infusions of bone marrow): shortness of breath, dyspnea, cough
- Transfusion reactions (if allogeneic)
- Allergic reactions
- Excessive anticoagulation: may occur with rapid or large-volume infusions (marrow and blood cells anticoagulated with heparin and/or citrate solutions)

Care coordination

Initiation of graft-versus-host disease prophylaxis pre-transplant
Coordinate arrival of stem-cell product with department or service handling and/or processing cells:
- Apheresis unit (related allogeneic peripheral blood cells)
- Cryopreservation laboratory [cryopreserved cell products (bone marrow, umbilical cord blood, peripheral blood cells)]
- Operating room (related allogeneic bone marrow)
- Unrelated donor program (unrelated donor peripheral blood cells or bone marrow)

Carrying out the medical care plan

Administer prehydration and premedications appropriately timed with arrival of stem-cell product
Administer stem-cell product and monitor for complications

DMSO, dimethyl sulfoxide.

complications, and also early detection and prompt implementation of appropriate interventions if complications develop. Systematic and astute assessment skills are required to identify subtle clues indicative of multiple potential complications [1]. For the pancytopenic patient, transplant nurses play a critical role in assisting the patient in preventing infections and bleeding complications and in managing fatigue. Nurses are also critical to help the patient manage pain from mucositis and to monitor fluid, electrolyte, and nutritional status.

Coordination of care can be very complex during this phase. Neutropenia and thrombocytopenia are perhaps the most dangerous complications during the pre-engraftment period. Nurses should follow evidence-based guidelines for the care of the neutropenic patient [22–24]. Patients who are outpatients during all or part of the pre-engraftment phase require frequent, often daily, clinic visits, with intense patient education to ensure that they can manage the complications and report promptly symptoms requiring care. Patients often require administration of multiple red blood cell and platelet transfusions, intravenous fluids and parenteral nutrition, and multiple intravenous medications. Whether in the inpatient or ambulatory clinic setting, infusing all required intravenous medications and fluids presents a major challenge. Many transplant centers place double-lumen central venous catheters for use throughout the transplant. Patients may require additional intravenous access during the pre-engraftment phase in order to administer all of the required intravenous medications and fluids. Additionally, patients usually require frequent collections of blood specimens, diagnostic procedures, examinations, or appointments with various members of the healthcare team, coupled with ongoing needs for education, completing activities of daily living, and psychosocial support.

Table 30.10 Pre-engraftment

Assessment

Assessments for potential conditioning-related toxicities:

- **Mucositis:** color/integrity of oral mucosa; volume and consistency of oral mucus; airway patency; general level of consciousness; subjective complaints of pain and/or difficulty swallowing; ability to perform oral care and maintain fluid/nutritional needs
- **Nausea/vomiting:** subjective reports of nausea; frequency and appearance of emesis, including gastroccult for presence of blood; abdominal assessment; fluid status, including intake and output, postural vital signs; effectiveness of antiemetic therapy
- **Diarrhea:** amount, frequency, appearance of stool, presence of blood; abdominal assessment; fluid status, including intake and output; subjective complaints of fullness, cramping, pain, microbiology culture reports, effectiveness of antidiarrheal therapy
- **Infection:** fever and/or chills unrelated to blood products or other medications; skin integrity, including oral and perineal areas; presence of adventitious breath sounds or cough; subjective reports of pain, weakness, fatigue; microbiology culture reports; hematologic laboratory values; chest X-ray report
- **Bleeding:** appearance and integrity of skin, sclera, mucous membranes; appearance of oral secretions; presence of frank or occult blood in urine, stool and emesis; mentation and level of consciousness, vital signs noting hypotension or tachycardia; laboratory values (hematocrit, platelet count, coagulation studies, hepatic and renal function tests)
- **Sinusoidal obstructive syndrome:** weight gain; increased abdominal girth; abdominal distention; right upper quadrant pain; hepatomegaly; mentation and level of consciousness; jaundice (skin and/or sclera); laboratory values (liver function tests including serum bilirubin, SGOT, alkaline phosphatase)
- **Pain:** onset, location, description, intensity (using rating scale), aggravating and relieving factors; effectiveness of interventions; psychosocial assessment; effect of pain on activities of daily living
- **Renal insufficiency:** strict intake and output; daily or twice-daily weight; postural blood pressure and heart rate; pulmonary assessment including respiratory rate, quality and presence of adventitious breath sounds; cardiac assessment, neck vein distention; presence of edema or ascites; administration of nephrotoxic drugs; laboratory values (serum blood urea nitrogen, creatinine, electrolytes; urine specific gravity)
- **Pulmonary:** respiratory rate, rhythm, depth, quality; breath sounds; existence and quality of cough; sputum production; mentation and level of consciousness; presence of cyanosis or skin mottling; subjective complaints of shortness of breath, dyspnea, or pain; oxygen saturation

Knowledge of patient/caregiver of potential complications

Ability to participate in self-care measures to treat and/or prevent complications

Ability of caregiver to participate in patient support

Teaching

Recognition, reporting and management of post-transplant complications:

- Mucositis
- Nausea/vomiting
- Diarrhea
- Infection
- Bleeding
- Sinusoidal obstructive syndrome
- Pain
- Renal insufficiency
- Pulmonary complications

Reinforcement of guidelines for preventing infections:

- Hand hygiene
- CVC care
- Personal hygiene, oral care, etc
- Nutrition/diet guidelines for immunosuppressed patients
- Avoiding activities with high risk for infection

Reinforcement of guidelines for preventing bleeding episodes:

- Careful oral hygiene with a soft-bristled toothbrush
- No rectal maneuvers
- Avoid medications that increase risk of bleeding
- Avoid falls
- Use electric razors for shaving
- Avoid nose blowing or picking
- Women with menses should use pads, not tampons

Rationale and procedures for administration of blood products, parenteral fluids and nutrition, medications

Anticipated time to engraftment

Signs and symptoms of engraftment

Care coordination

Coordinate multiple healthcare provider appointments (outpatient) or healthcare provider examinations and tests (inpatient)

Provide patient and caregiver education: one-on-one instruction or attendance at caregiver classes, for new caregivers

Carrying out the medical care plan

Blood product administration:

- Platelet transfusions (often daily or multiple times per day)
- Packed red blood cell transfusions

Intravenous fluids:

- Total parenteral nutrition/lipids
- Hydration

Medication administration:

- Graft-versus-host disease prophylaxis (allogeneic patients)
- Antibiotics, antivirals, antifungals
- Antiemetics
- Opioid/non-opioid analgesia (patient-controlled analgesia in inpatient setting)
- Investigational drugs

Multiple laboratory draws

Frequent physical assessments and vital signs (inpatient, every 4 hours and as needed; outpatient, every clinic visit and as needed)

Physical care:

- Bath and skin care
- Oral care
- CVC dressing changes
- Exercise/ambulation

CVC, central venous catheter; SGOT, serum glutamic-oxaloacetic transaminase.

Early post-engraftment (Table 30.11)

Engraftment typically occurs between days 14 and 28 post-transplant, depending on the stem-cell source. The most common issue in the early post-engraftment period for allogeneic transplant patients is acute GVHD. Nurses play an important role in assessing patients for this condition and in teaching patients and caregivers how to monitor for its signs and symptoms. Transplant nurses must have a thorough understanding of the pathophysiology, clinical manifestations, and management of GVHD [25].

The nurse plays a key role in preparing the patient and family for discharge from the hospital. Criteria for transition from inpatient to outpatient settings will differ based on institutional policies, the patient's condition, the availability of skilled outpatient teams, and family caregiver support. Caregivers must be knowledgeable in recognizing, managing, and appropriately reporting complications, and be competent in performing aspects of the patient's care. Detailed and structured discharge teaching is necessary to ensure adequate understanding of critical information by patients and caregivers [26].

Hospital readmissions after initial hospital discharge to the ambulatory care setting frequently occur during the early post-engraftment phase [7]. Coordination and communication of patient care issues between the nursing staff of the inpatient unit and the ambulatory care setting allow for smooth transition and continuity of care. Particular attention needs to be given to caregivers' ability to cope continuously with their multiple demands and roles.

Table 30.11 Early post-engraftment

Assessment

Ongoing assessment for conditioning-related toxicities (see Table 30.10)

Signs and symptoms of GVHD:

- **Skin**: itching; redness or maculopapular rash on palms of hands, soles of feet, ears, trunk, extremities; erythroderma
- **Liver**: right upper quadrant pain; hepatomegaly; jaundice; laboratory values (elevated serum bilirubin, SGOT, alkaline phosphatase)
- **Gut**: nausea; vomiting; anorexia; diarrhea; abdominal cramping and pain

Readiness for discharge from hospital:

- Availability of caregiver on discharge
- Competency of patient and caregiver to: (1) recognize, report, and manage post-transplant complications; (2) manage outpatient schedules and treatments; and (3) administer outpatient IV fluids and medications
- Knowledge of telephone numbers to access emergency care and support 24 hours per day on discharge

Caregiver ability to provide support

Teaching

Signs and symptoms of acute GVHD

Rationale and procedures for diagnostic testing for GVHD if signs and symptoms develop (e.g. skin biopsy, liver biopsy, and/or endoscopy)

Management of GVHD (adding immunosuppressive medications to current GVHD prophylaxis), dietary restrictions

Hospital discharge planning:

- Criteria for discharge from inpatient to outpatient setting (e.g. nausea/vomiting/pain controlled; blood counts supportable; number and type of IV infusions or medications)
- Level of caregiver support required on discharge (generally constant support required post-hospital discharge)
- Outpatient administration of IV fluids and medications
- Discharge medication schedule: immunosuppressant medications for GVHD prophylaxis and/or treatment (allogeneic patients), prophylaxis for *Pneumocystis carinii* pneumonia, antivirals, antibacterials, antifungals, electrolyte replacements, antiemetics, etc.
- Importance of reporting inability to take medications prescribed
- Outpatient appointment schedules on discharge

Recognition and management of post-transplant complications

Likelihood of readmission to hospital post-initial discharge

Care coordination

Coordinating multiple healthcare provider appointments (outpatient) or healthcare provider examinations and tests (inpatient)

Scheduling procedures (marrow aspirations, chest-X-rays, etc.)

Transitioning care from inpatient to outpatient setting

Carrying out the medical care plan

Blood product administration:

- Platelet transfusions (often daily or multiple times per day)
- Red blood cell or whole blood transfusions

Intravenous fluids:

- Total parenteral nutrition/lipids
- Hydration fluids
- Patient-controlled analgesia (inpatients)

Medication administration:

- GVHD prophylaxis
- Antibiotics, antivirals, antifungals
- Opioid/non-opioid analgesics
- Electrolyte replacements
- Investigational drugs
- Antiemetics

Multiple laboratory draws

Frequent physical assessments and vital signs (inpatient, every 4 hours and as needed; outpatient, at each clinic visit and as needed)

Physical care:

- Bath and skin care
- Oral care
- CVC dressing changes
- Exercise/ambulation

CVC, central venous catheter; GVHD, graft-versus-host disease; IV, intravenous; SGOT, serum glutamic-oxaloacetic transaminase.

Intensive care management of the transplant patient

Most transplant patients require complex, high-acuity levels of care throughout their inpatient stay [27]. Additionally, it is estimated that up to 40% of patients undergoing HCT will require nursing skills typical of most ICUs, including hemodynamic monitoring, non-invasive cardiac monitoring, administration of inotropic and vasoactive medications to support blood pressure, and mechanical ventilatory support [28]. The need for ICU support will vary based on the type of HCT performed and how ill the patient is prior to transplant. All transplant nurses, whether ICU trained or not, need to be aware of early signs and symptoms of common complications of transplant so as to intervene early and prevent morbidity and mortality [27,28].

Management of critically ill transplant patients combines the complexity of oncology and critical care nursing skills, presenting significant challenges to the provision of expert nursing care. Oncology nurses generally are not trained or skilled in the management of critically ill patients. Conversely, critical care nurses generally do not have an oncology background. Patients with critical care complications may be transferred to a critical care unit. However, many institutions have worked towards merging the two specialties of oncology nursing and critical care nursing to meet the needs of these patients by training transplant nurses to provide intensive care on the transplant unit or by cross-training ICU staff to specialized transplant procedures [29]. Challenges to each approach include the maintenance of nurses' competency. Transplant nurses may find it difficult to keep critical care skills up-to-date if the volume of transplant patients requiring critical care is low. Critical care nurses may face similar issues in maintaining transplant competencies and skills. Each institution must evaluate the available resources, outcomes of care, and cost-effectiveness of cross-training staff and maintaining clinical competencies when determining models for providing critical care to transplant patients [30,31].

Substantial progress has been made in treating the complications of HCT. Past reports of close to 100% mortality rates in HCT patients requiring ICU care are no longer the reality; the current mortality rate in ICUs is less than 50% [32–36]. Mortality remains highest for those HCT patients admitted to ICU for sepsis or respiratory failure [32,33].

Relapse post-transplant

Some patients may fail to respond to the HCT or, after initial response, relapse of underlying disease may occur [37]. For patients with disease relapse, options for care may include standard or experimental chemotherapy, donor lymphocyte infusion or other cellular therapies, discontinuation of immunosuppressive therapy, a second HCT, or palliative care [37] (see Chapter 71). Nurses play a significant role in clarifying the treatment options presented by the medical staff. Nurses also serve as advocates for the patient and family, helping to communicate the patient's individual goals clearly to the health-care team.

Discharge (Table 30.12)

Preparation for long-term recovery begins during the treatment phase and is part of the ongoing nursing care process. Discharge education is provided by nurses to review symptoms of possible late effects of transplantation, such as chronic GVHD and infectious risks, and make recommendations related to activities of daily living for the first year after HCT (Table 30.13). It is also a good opportunity to discuss possible cognitive, sexual, and emotional concerns. Some large centers accomplish this educational goal through individual classes. This effort facilitates the hand-over of care from the transplant center to the care of the referring physician.

Table 30.12 Discharge from transplant program

Assessment
Knowledge of infectious risks
Knowledge of expected follow-up office visits and laboratory testing
Nutritional needs
Need for continuance or removal of CVC
Allogeneic patients: • Current symptoms of GVHD • Visual examination of skin • Skin biopsy • Schirmer's test • Knowledge of chronic GVHD
Teaching
Symptoms of chronic GVHD
Recovery of immunity
Recommendations for minimizing daily infectious risks (see Table 30.13)
Recommendations for returning to work or school
Management of fatigue
Management of possible problems related to sexual function
Management of cognitive and emotional issues
When to report fever
Necessity for consistent medical follow-up for life
Care coordination
Medication list
Nursing care plan for local nursing care provider
Home healthcare agency if needed
Special transfusion requirements and preparation of products (irradiation and CMV screening if CMV negative)
Interface with health insurance carriers as needed
Assist with research coordination
Time after transplant

CMV, cytomegalovirus; CVC, central venous catheter; GVHD, graft-versus-host disease.

Patients often express feelings of anxiety when leaving the security of the transplant program that they have relied on so heavily. If the patient is being discharged from the transplant center to their referring physician, identifying a designated contact person or group at the transplant center for both the patient and the referring physician for consultation on transplant-related problems is essential. This contact person can decrease the anxiety of the patient and caregiver and provide the referring physician access to transplant expertise and continuity of the medical plan. General guidelines should be provided by the transplant center for the referring physician that details the important aspects of long-term care of the transplant recipient [38].

Long-term recovery (Table 30.14)

Nurses, under the guidance of the transplant center medical team, can successfully act as liaison between the referring physician and the transplant physicians. They can collect important and pertinent information necessary to triage transplant-related problems and, after discussion with the transplant physicians, relay possible solutions to the team providing care at home. One organized approach is to conduct long-term follow-up rounds on a regular basis to discuss non-urgent problems. Issues most frequently requiring the expertise of the transplant team are the diagnosis and treatment of chronic GVHD, pulmonary complications, gastrointestinal symptoms, engraftment issues, treatment of serious and life-threatening infections, booster immunization, recurrent disease, and the development of secondary malignancies [39].

Table 30.13 Common questions asked by hematopoietic stem-cell transplantation (HSCT) and long-term follow-up (LTFU) patients utilized at the Fred Hutchinson Cancer Research Center

Questions often asked	**Time after transplant**			
The general guidelines below may not apply to your case. You must discuss with your physician to assess if these rules apply to you.	**All patients Less than 6 months**	*Not* **receiving immunosuppression 6 months to 1 year**	**Receiving immunosuppression 6 months to 1 year**	**Receiving immunosuppression More than 1 year**
School	No	No	No	OK
Hot tubs (1)	No	OK (1)	No	No
Swimming (1) (avoid head submersion and diving, use sun-screen)	No	OK (1)	No	OK
Gardening (digging in soil); mowing the lawn; raking leaves	No	No	No	No
Having plants in the home (not handling)	OK	OK	OK	OK
Making/kneading yeast breads	No	OK	OK	OK
Carpenter work	No	No	No	No
Occasional woodworking (sawdust)	No	OK	OK	OK
Animals, birds, reptiles, fish, other (not handling feces, litter boxes, cleaning utensils, cages/tanks, etc.):				
• New pets in patient's household	No	No	No	No
• Cats/dogs (not sleeping with pets)	OK	OK	OK	OK
• Domestic birds (parakeets, parrots, etc.) (not with respiratory problems)	No	OK	OK	OK
• Poultry and wild birds (pigeons, chickens, ducks, geese, other wild birds)	No	No	No	No
• Small cage rodents (gerbils, rabbits, hamsters, guinea pigs, hedgehogs, prairie dogs, etc.) (do not handle)	No	OK	OK	OK
• Reptiles (snakes, turtles, lizards, iguanas, etc.); ferrets	No	No	No	No
• Farm animals (pigs, horses, cows, llamas, etc.) (do not handle; stay out of barns full of hay)	No	OK	OK	OK
• Wild and game animals (deer, elk, squirrels, bears, etc.); exotic animals (monkeys, etc.) (do not handle)	No	No	No	No
• Zoos and petting zoos	No	No	No	No
• Public aquariums (do not touch marine life in handling tanks) (3)	OK (3)	OK (3)	OK (3)	OK (3)
• Animal trophy mounts in the house	OK	OK	OK	OK
• Fishing (fresh and salt water) (OK to handle fish if wearing gloves; do not bait hooks)	OK	OK	OK	OK
• Horseback riding (stay out of barns full of hay)	No	OK	OK	OK
• Hunting – patients without CVC (wild game and birds) and sport shooting (2) (wear disposable medical gloves when handling game; do not clean game)	No	OK (2)	OK (2)	OK (2)
• Golfing (2) (sun protection required; without venous catheter device)	No	OK	OK	OK
• Spectator events and crowds (3) (church, movies, games, etc.) (no hand shaking)	No (2)	OK (3)	OK (3)	OK (3)
• Sexual activity	OK	OK	OK	OK
• Working with mechanical equipment (oil changes, working on cars and engines, etc.)	OK	OK	OK	OK
• Camping and hiking	OK	OK	OK	OK
• Down comforters (with cover)	OK	OK	OK	OK

CVC, central venous catheter.

(1) *NO* swimming if venous access device still in place (i.e. catheter). No patient history of sinusitis. Chlorinated and well-maintained pools preferable over uncertain water conditions of lakes, rivers, and sea for swimming, Do not recommend swimming in pools used by non-toilet-trained babies and children. Water aerobics OK.

(2) Shooting of rifles and shotguns and golfing are not recommended if venous access device still in place (i.e. catheter).

(3) Recommend caution and an understanding of the risks involved when participating in public events or going to locations with large crowds.

(4) Real Christmas trees: in the water reservoir of the tree stand, use a solution of 1 part chlorine solution (Clorox) in 10 parts water.

Other reminders:

Minimize: **exposure to dirt and aerosolized material**.

Minimize: **sun exposure and use of sun-screen (SPF >15%)**.

Use: **hat, long-sleeve shirts** and **pants** if outside for long periods of time.

Avoid contact with people with **respiratory illness** or other **transmissible diseases**.

Source: Fred Hutchinson Cancer Research Center/Seattle Cancer Care Alliance Patient Education Program.

The need for the transplant team to act as a resource may persist for a few months to several years. While patients who live at a distance from the transplant center benefit from periodic follow-up visits to the transplant center, the general oncologist or hematologist at home can provide the routine follow-up care. Timely and consistent communication between the transplant centers and community practices is essential for safe continuity of care [40]. Transplant nurses can also provide ongoing support in the outlying communities by offering educational programs and by sending out educational material when the need is recognized. Facilitation of support groups is another way for transplant nurses to provide ongoing education and support for local patients in the long-term recovery phase.

If the transplant center is involved in ongoing research, the research nurse plays an integral role in continuing data collection and adherence to research protocols. In some research centers, patient health questionnaires are regularly mailed to post-transplant patients to update demographics, quality-of-life information and survival documentation. Research nurses can triage problem issues that are identified from the questionnaires.

The role of the nurses working with the HCT patients during long-term recovery requires patience and perseverance when considering the recurring nature of chronic GVHD and other long-term issues. As in other phases of HCT nursing, the work is rewarding and challenging, and it allows for the establishment of long-term and trusting relationships. While nurses working in the office setting may find the problems of this population to be daunting at times, the eventual recovery and return of many patients to a satisfying and full life is a reward experienced by those who are fortunate enough to work with these patients.

Table 30.14 Long-term recovery

Assessment
Fatigue
Pain
Functional status
Complete blood count
Kidney function
Liver function
Magnesium level
Drug levels
Chronic GVHD symptoms (allogeneic patients):

- **Skin**: erythema, dryness, macular or urticarial rash and pruritis, hyperpigmentation, vitiligo, mottling, lichenoid plaques, hyperkeratosis, exfoliation scleroderma or morphea
- **Mouth**: dryness, mucositis, erythema, ulcerations, lichenoid changes, striae, tightness around the mouth, sensitivity to hot, cold, or spicy foods, gingivitis
- **Eyes**: dryness, grittiness, blurring, excessive tearing, photophobia, and pain
- **Liver**: elevated liver function tests not attributable to other causes and sometimes jaundice
- **Gastrointestinal system**: anorexia, nausea, vomiting, diarrhea, dysphagia, malabsorption, weight loss, early satiety
- **Lungs**: cough, wheezing, dyspnea on exertion, history of recurrent bronchiolitis or sinusitis, bronchiolitis obliterans
- **Nails**: ridging, onychodystrophy, onycholysis
- **Hair**: premature graying of scalp hair, eyelashes, eyebrows, thinning of scalp hair, alopecia, decreased body hair
- **Vagina**: dryness, dyspareunia, stricture or stenosis, erythema, atrophy or lichenoid changes not induced by ovarian failure or other causes
- **Myofascia**: stiffness and tightness with restriction of movement, sometimes with swelling, pain, cramping, erythema, and induration, most commonly affecting the forearms, wrists and hands, ankles, legs, and feet, contractures
- **Muscles**: proximal muscle weakness, cramping
- **Skeletal**: arthralgia of large proximal girdle joints and sometimes smaller joints
- **Serosal**: unexplained effusions involving the pleural, pericardial or peritoneal cavities
- **Laboratory:** Thrombocytopenia, eosinophilia, hypogammaglobulinemia
- **Energy level**: unusual fatigue

Medication adherence
Nutritional needs
Symptoms of infection
Hormonal issues
Neurological/cognitive symptoms
Sexual dysfunction

Teaching
Symptoms to be reported: skin changes, nausea, anorexia, weight loss, diarrhea, dysphagia, dry or sensitive mouth, dry, gritty eyes or excessive tearing, coughing, wheezing, or shortness of breath, thinning of scalp hair, ridged nails, loss of range of motion or stiffness, vaginal symptoms
When to report fever
How to access medical help after hours
Review of infection prevention
Importance of medication adherence
Importance of reporting abnormal lesions or lumps, bowel changes, or abnormal vaginal bleeding
Importance of regular exercise

Care coordination
Healthcare provider appointments
Medication administration
Laboratory draws
Return visit to transplant center at 1-year anniversary of transplant and thereafter as needed
Vaccination schedule post-transplant
Allogeneic patients:

- Plans for work-up of chronic GVHD symptoms based on assessment
- Plans for treatment of chronic GVHD

GVHD, graft-versus-host disease.

Nursing practice issues in hematopoietic stem-cell transplant

Various nursing roles are needed to successfully care for transplant patients. The organization of nursing roles usually parallels that of the center in which the program resides and is based on that institution's nursing philosophy and care delivery model. In the ambulatory setting, nursing models must complement the medical model for successful care delivery. For example, primary care in the outpatient setting is often structured by having a specific registered nurse work with a specific provider caring for one group or team of patients. Table 30.15 gives descriptions of the various nursing roles.

Achieving and maintaining an expert nursing staff is a constant challenge for institutions with HCT programs. Initial orientation of a nurse with oncology experience generally takes 6–8 weeks. After this time, the nurse can be counted in staffing patterns but is not considered fully competent until they have completed their first year. The nurse-to-patient ratio is also an issue that must be considered when planning an HCT unit. Most inpatient units staff on a ratio of one nurse to two or three patients. This is usually a much lower ratio than on other oncology units and may need to be justified to hospital administrators. Patients develop strong feelings of trust and confidence in specific nurses, and facilitating work schedules to maximize these relationships can be a challenge for nurse managers.

Continuing education must be built into HCT nurses' work schedules. This education includes updates and refreshers on nurse practice topics and also education on new medical protocols. It is also important for the nurses to remain up-to-date with the results of medical research. Each unit should identify topics of high risk and high complexity that qualify for annual competency review and testing. Examples on an inpatient unit include "Administration of high-dose chemotherapy" and "Administration of stem cells." Examples in an outpatient clinic include "Steroid-induced diabetes teaching" and "Donor screening." These nurses are at high risk for experiencing job-related stress, moral distress, and compassion fatigue as they work in fast-paced units with acutely ill patients with uncertain outcomes [41]. Flexible scheduling

Table 30.15 Nursing role descriptions in hematopoietic stem cell transplantation

Nursing title	Role description
Inpatient staff RN	Cares for patients requiring acute care. Includes assessment, patient teaching, and carrying out the medical care plan. Inpatient nurses may care for patients on a designated transplant unit or on an oncology unit
ICU RN	Cares for patients requiring critical care. Includes assessment, patient teaching, and carrying out the medical care plan. ICU nurses may care for patients in an ICU incorporated into the transplant unit or in a main hospital ICU
Nurse coordinator	Coordinates pre-transplant preparations including donor identification. Initiates patient education regarding transplant process. May continue as case manager during patients' transplant course. This position is required of National Marrow Donor Program designated centers
Discharge planning RN	Assists team in planning and coordinating patients' discharge from hospital to ambulatory setting. Provides patient and caregiver education required to manage care at home
Outpatient clinic RN	Cares for patients requiring ambulatory care. Includes assessment, triage, care coordination, patient teaching, and carrying out the medical care plan
Outpatient infusion RN	Cares for patients receiving infusions and transfusions. In many centers the stem cell infusion occurs in the outpatient infusion unit
Home infusion RN	Cares for the patients receiving infusions in the home setting. Educates patients and caregivers to administer intravenous solutions in the home
Apheresis RN	Cares for patients and donors undergoing apheresis procedures
Long-term follow-up RN	Cares for patients after the acute phase of transplant. Provides education and triage for ongoing complications or late effects of transplant
Research RN	Partner with the principal investigator to implement research protocols. Coordinates research activities with primary care team
Nurse manager	Responsible for nursing care delivered in a specific unit or clinic. Ensures appropriate staffing levels for the safe delivery of care
Clinical nurse specialist	Responsible for advancing transplant nursing practice by promoting evidence-based practice, conducting nursing research, creating nursing care guidelines, and evaluating outcomes of nursing care at the population level
Nurse educator	Responsible for orientation and continuing nursing education for transplant nurses
ACP (Advanced Care Provider)	Provides medical care under the direction of the attending physician. Performs procedures such as lumbar punctures and bone marrow biopsies

ICU, intensive care unit; RN, registered nurse.

and supporting time-off requests assist many nurses to keep their resilience to continue to work with these patients. It is also important that the nurses have time to share their experiences with each other and members of the healthcare team as they may not be understood by other support people in their lives, such as family members or even other healthcare professionals. It is important for HCT nursing leaders to have a proactive strategy to prevent "burnout" in their staff. The emotional labor of caring and compassion fatigue can be alleviated by creating a community of caring at work, professional boundaries, and enriched personal life [42].

The need for HCT nurses to network on a national level has been identified since the 1980s, when many transplant units were established. Nurses in the initial transplant centers organized national conferences, including conferences in Seattle, Minneapolis, and Omaha. The conferences were very well attended but a stress on the resources of the individual centers. In 1989, the Oncology Nursing Society acknowledged the unique needs of several subspecialties in oncology nursing, and created "Special Interest Groups" (SIGs). The Blood and Marrow Stem Cell Transplant (BMSCT) SIG of the Oncology Nursing Society (ONS) was one of the initial 10 SIGs and remains one of their largest.

HCT nursing requires unique expertise, and efforts have been made to delineate standards of nursing care. The ONS has created the "Fundamentals of Blood and Marrow Transplant Webcourse," which covers basic information for nurses entering the field of HCT. The ONS has announced a new certification in HCT nursing for experienced nurses, The first Blood and Marrow Transplantation Certified Nurse (BMTCN™) Examination was offered in February 2014. Nurses who are successful in passing the test will earn the credential "BMTCN" [43,44].

In 1998, the Center for International Bone Marrow Transplant Research/American Society for Blood and Marrow Transplantation (CIBMTR/ASBMT) agreed to have a concurrent nursing conference with their "Tandem" annual medical conference coordinated by the ONS BMSCT SIG. This nursing conference has been presented for over a decade, and has grown from a half day to three full days. In 2008, the ASBMT Board of Directors identified a need for nursing input in their initiatives, and requested that Registered Nurse members establish a SIG within the Society. This group was initiated in 2009. Nursing SIG members sit on several subcommittees of the ASBMT and also co-Chair the annual nursing conference with leaders from the ONS BMSCT SIG.

Conclusion

Expert nursing care is essential to the success of an HCT program. In most HCT programs there is teamwork, respect, and mutual appreciation among the multidisciplinary staff. Dr. E. Donnall Thomas, founding Medical Director of the Fred Hutchinson Cancer Research Center's Transplant Program and joint recipient of the 1990 Nobel Prize in Physiology or Medicine, called transplant nurses "his secret weapons" [45]. Nurses who are drawn to this subspecialty within oncology are rewarded by intense, intimate, and long-term relationships with the patients and their families. There is great satisfaction in knowing that they have made a positive difference in the lives of these patients.

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CHAPTER 31

Ethical Issues in Hematopoietic Cell Transplantation

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Introduction

Many of the ethical issues raised in hematopoietic cell transplantation (HCT) are common to those involved in other forms of medical therapy utilizing advanced technologies. These issues are informed by the traditional principles of patient autonomy and the importance of informed consent and confidentiality; justice, that is, the fair allocation of limited resources; beneficence and non-maleficence; and fidelity or non-abandonment when the goals of treatment shift from cure to supportive care.

However, certain unique features of HCT compound the complexity of these ethical issues, including: the young age of most patients; the fact that most of the diseases are life threatening and often fatal within a short time in the absence of an HCT, yet are potentially curable with this treatment; the fact that a living donor is required who is often a minor; the ability to select potential donors for an affected sibling by utilizing preimplantation genetic diagnosis; the great financial expense of the procedure and controversies about reimbursement; and the fact that survivors of HCT may be cured of their primary malignant disease but may face other serious complications, such as sterility, chronic graft-versus-host disease (GVHD), second malignancies, psychologic distress, sexual dysfunction, cognitive impairments, and occupational disability. In this chapter, some of the ethical issues in HCT are reviewed under the following general headings: who is eligible for HCT, informed consent for the patient, informed consent for the donor, alternative sources of stem cells, HCT for non-malignant diseases, and end-of-life issues.

Who is eligible for HCT?

Case scenario 1

A 49-year-old man with Stage II multiple myeloma has a human leukocyte antigen (HLA)-identical sibling. He is eligible for a protocol utilizing tandem autologous HCT followed by a reduced-intensity regimen allogeneic HCT. His physicians feel that this approach represents his best chance for long-term, disease-free survival and possible cure of his myeloma. His insurance plan considers this treatment plan experimental, and will only authorize a single autologous HCT.

Case scenario 2

A 32-year-old man with advanced chronic myelogenous leukemia (CML) has no sibling donors available for allogeneic HCT. Although his company's insurance plan covers the costs of procurement and the actual transplant for a matched unrelated donor HCT, it will not pay for the search costs, which the patient cannot afford. Although the company sponsors a donor drive in the name of this patient, he cannot access any of the donors who might be identified.

These two scenarios, which are based on actual cases, help to highlight some of the difficult issues involved in determining who undergoes an HCT procedure. This process involves both medical decision-making based on clinical and protocol-determined eligibility criteria and third-party payer decision-making. Each of these processes is ethically complex. First, the medical decision-making: in the interest of justice, it is imperative that transplant teams develop standardized sets of criteria by which patients are selected for HCT. Some of these criteria are straightforward, such as the patient's diagnosis and stage of disease, age, and adequacy of major organ function. Other criteria are more subjective and often relate to psychosocial issues that may influence the patient's ability either to tolerate the HCT procedure or to comply with the long-term follow-up regimen. Multidisciplinary teams are needed that include not only physicians, but also nurses, psychologists, and social workers.

It is important to avoid arbitrary and inconsistent decision-making in deciding when not to offer HCT to a patient. For example, an HCT center may decide that a young patient with acute leukemia who has Down syndrome is not an acceptable candidate because of expected psychosocial management problems that might compromise the outcome. In one survey of 58 pediatric HCT centers, 16 leukemic children with Down syndrome had been transplanted at 10 centers. This was only about 20–25% of the predicted number based on incidence data. In fact, the outcomes were no different than those expected from a similar cohort of patients with acute leukemia who did not have Down syndrome. The author cautioned against physician bias towards these patients [1]. In an accompanying editorial, physicians were advised to use a utilitarian approach, that is, to recommend against HCT for children with Down syndrome only if data show poorer outcomes for them.

Many HCT teams are concerned that patients with a history of active substance abuse may be poor candidates because of expectations of higher complication rates and poor compliance in their long-term care. A retrospective study from one transplant center identified 17 of 468 HCT patients as lifetime substance abusers (alcohol in 71%, marijuana in 30%, and opiates in 30%). When these 17 patients were paired with matched control subjects, a significant difference in survival probability was seen, 60% versus 10% ($p = 0.0022$) [2]. More data may be needed to substantiate these findings, but they do support the current practice at many HCT centers of excluding such patients.

A survey of 597 HCT professionals (physicians, social workers, and nurses) was conducted in which 17 case vignettes were posed

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that highlighted a range of psychosocial issues that could potentially impact the long-term outcome of the transplant procedure [3]. The issues included suicidal ideation, use of addictive illicit drugs, and a history of non-compliance. At least 10% of responders indicated that they would not proceed in all 17 vignettes, but there was a lack of unanimity for most of the cases in terms of recommendations to proceed or not. The decision-making seemed contingent on perceptions of the severity and currency of the psychosocial issue and the ability to manage the patient's potential non-compliance.

A challenging case raised the issue of whether an incarcerated minor with relapsed acute leukemia should be offered an HCT, and who should pay for it [4]. This type of case highlights the sharp conflicts between a physician's obligation to provide optimal care versus social justice. Is it cruel and unusual punishment to deny a prisoner a potentially life-saving treatment when the crime was not judged to be severe enough to justify the death penalty? Is it right for the state to pay for such a procedure when other law-abiding citizens without insurance or other resources are denied this type of treatment? There is a need for Federal and state-specific guidelines to establish when it is appropriate medically and socially for prisoners to receive technologically advanced and expensive healthcare.

Decision-making regarding third-party payer reimbursement is seen as a conflict over fairness and justice. Physicians as patient advocates are often in conflict with insurers who argue that HCT for certain diagnoses and disease stages is experimental and unproven. Physicians often believe that it is unfair to have patients accepted or denied access based on geography, their position in society, or their ability to pay when a therapy is investigational, expensive, or both. In analyzing patterns of utilization in four states, Mitchell et al. found that black patients, those enrolled in health maintenance organizations, those covered by Medicaid, and self-pay patients were less likely to receive HCT when admitted for either leukemia or lymphoma [5]. Insurers believe that it is unfair, and uneconomical, to be pressured by physicians and patients to pay for care that is not standard therapy [6].

Uniform and fair methodologies are needed to bridge these two views, rather than relying on costly litigation [7]. One approach is taxonomic, with a treatment modality labeled as experimental or standard. Third-party payers would cover experimental therapy if it met certain criteria, such as a phase III trial approved by the appropriate government agency, for example, the US Food and Drug Administration (FDA), and if the treatment were likely to benefit the patient. The treatment should be medically necessary, safe, and effective. The therapy must be as beneficial as any established alternative, and the improvement must be attainable outside the investigational setting [8].

It is necessary to balance the premature dissemination of poorly studied, toxic, and expensive therapies to desperate patients by accepting limitations to HCT on the one hand, and by accepting that a patient's best interests may be served by an experimental therapy on the other. Legal authorities caution that, although insurers may deny autologous transplant for a patient with cancer because the treatment is experimental, the more relevant question may be, "Is it the best option available for that particular patient with that particular disease?"

The controversy related to autologous HCT for breast cancer over a decade ago exemplifies these dilemmas. Welch and Mogielnicki highlighted some of the lessons that could be gleaned from this experience, including: (1) it is premature to discuss the cost-effectiveness of an intervention when its clinical effectiveness is unknown?; (2) the National Institutes of Health should have an important role in determining what is experimental therapy; (3) public officials should not mandate coverage in the absence of clear data; and (4) the news media watchdog role should be extended to healthcare [9].

An alternative approach is to reach a consensus based on available data of efficacy, duration of expected benefit, and the quality of wellbeing after the treatment [10]. Panels of expert physicians and insurers could review available data on a regular basis and develop grids that designated which diagnoses and disease stages would be covered, based on outcome analyses. Different categories could be considered, such as (1) diseases for which HCT is curative and may be the only curative therapy available, for example, CML; (2) diseases for which HCT may not be curative but definitely prolongs survival, for example, autologous transplant for CML, and possibly breast cancer and multiple myeloma; and (3) diseases for which the benefits of HCT are not yet known, for example, autologous transplant for autoimmune diseases such as multiple sclerosis. One model is the Oregon Medicaid system, which adopted such an approach and covered HCT for all major hematologic malignancies, including chronic lymphocytic leukemia, but did not cover chemotherapy for metastatic renal cell carcinoma. Such an approach is followed in California for Medi-Cal patients and by Blue Cross/Blue Shield. Another component of this approach is to restrict payments to "centers of excellence" that have demonstrated track records in their field.

One of the most difficult challenges that the medical profession and the public at large face is how to assure that progress in developing new therapies will continue in a managed care system designed to minimize healthcare expenses and to reimburse only for medically necessary and proven treatments. Who is going to pay for ongoing research that will lead to the breakthroughs of the future? One could argue that third-party payers have an ethical obligation to participate in this process by helping to underwrite the expenses involved in clinical research, and thereby to help establish which new treatment modalities are in fact effective and superior to established methods. Such new therapies may in fact be cheaper in the long run if they lead to definitive cures for patients and obviate the need for years of expensive, supportive chronic care.

Informed consent for patients undergoing HCT

Case scenario 3

An 18-year-old woman with relapsed acute myelogenous leukemia (AML) has an HLA-matched brother available for allogeneic HCT. She has expressed an interest and willingness to undergo a transplant. However, during the informed consent process, she refuses to listen to any discussion about possible risks and complications from HCT. Can her doctors assume that she has given her consent and proceed to HCT?

Case scenario 4

A 13-year-old girl presents with pancytopenia and is diagnosed with M6 AML. Her physicians recommend treatment with induction chemotherapy followed by allogeneic HCT from a matched sibling or unrelated donor. The patient and her parents are Jehovah's Witnesses and they refuse to accept transfusion of blood products.

Respect for patient autonomy is reflected most critically in the informed consent process, in which patients are informed of their diagnosis, prognosis, potential benefits and risks of the proposed therapy, and alternative treatments available. Although this statement is true for any type of medical intervention, in the setting of HCT there are extra complexities, including the facts that many patients are minors, that almost all patients being considered for HCT have diseases that will likely be fatal without a transplant and are potentially curable with the procedure, and that patients who survive their original disease and the early transplant mortality are at risk for development of a wide variety of chronic complications that may impair their quality of life in many ways.

After a patient has been told that he or she has a fatal disease and that a potentially curative procedure is available, how much does the patient really hear and/or retain about the potential risks and complications? How accurate are the available data about details of mortality and morbidity – including risk of sinusoidal obstructive syndrome (SOS), acute and chronic GVHD, cytomegalovirus and other infections, chronic radiation effects, avascular necrosis, sterility, the risk of second malignancies, and quality of life after transplant – and how many of these data are revealed to prospective HCT patients? If patients choose not to hear all of these specifics and make choices solely on the options of life and death, is this really informed consent? Must we insist on inflicting them with these truths? Can patients exercise their autonomy by choosing not to be fully informed?

Forsyth et al. coined the phrase “decision-making in a crowded room” to describe the process of a patient’s deciding to proceed to allogeneic HCT. This is not a simple, individual cognitive process, but rather a complex, relational interactive process that needs to reflect the multiple roles that an individual plays not only as a patient, but also as a parent, spouse, child, etc. These decisions are “embedded in social relations of obligations and reciprocity” [11].

One study compared the process of informed consent as viewed by physicians or nurses in comparison with the view of patients [12]. For HCT nurses, it was most important that patients know about the side effects and complications of the procedure. The physicians wanted the patients to know about the diagnosis, therapeutic options, and outcomes of treatment. The physicians’ views matched the patients’ most closely, as they made decisions based mainly on outcome and life or death. In another study of adult and pediatric patients and their physicians, the three main reasons why patients and their parents chose HCT were: (1) trust in their physician; (2) fear that their illness would get worse without the transplant; and (3) belief that the HCT would be a cure.

Most of the adult patients remembered that complications could occur, but they could recall fewer than half of those mentioned. Most of the patients believed they had been given adequate information and that it was not too technical, although the physicians thought the information conveyed was in fact too technical and excessive. Most of the patients believed that their physician wanted them to undergo a bone marrow transplantation (BMT), and the patients made their decision based on that advice. One HCT nursing director suggested that the role of the HCT nurse in this process may be to ensure that patients have been completely informed, although the nurse must realize that patients may not be able to comprehend the complex details of treatment, and that the intensity of the choice between life and death may overshadow the risks of potentially life-threatening complications. The nurse can function as a patient advocate, which may create conflicts with the physician at times, and the nurse must not take away hope from the patient [12].

Lee et al. analyzed the degree of concordance or discordance between 313 patients and their physicians in estimating the chances of success after HCT [13]. There was significant concordance between patients and physicians when the outcomes were likely to be favorable, but when patients had more advanced disease, they tended to fail to recognize the higher risks of relapse and death. The authors speculated that physicians may tend to minimize the risks in their discussions with patients who have advanced disease. Divulging more information about these risks may not change the patients’ decisions to move forward with the HCT, but it may promote better psychologic adjustment after HCT.

Andrykowski et al. focused on the question of whether patients had “returned to normal” following HCT, and examined differences between their expectations and actual outcomes, and the impact that these differences might have on their sense of wellbeing [14]. They studied 172 disease-free survivors from five HCT centers and found that only a minority felt they were back to normal. Before HCT, only 19% of patients had not expected to return to normal, and 47% anticipated they would. Following HCT, 32% (possibly up to 52%) stated that they had not returned to normal. The discordance between pre-HCT expectations and their current functional status was associated with greater current psychologic stress. Despite this discrepancy, the survivors’ evaluation of their decision to pursue HCT was generally fairly positive.

The issue of involving children in medical decision-making is important since many candidates for HCT are minors. Most investigators recognize that children’s capacity for decision-making and autonomy develops over time. The parents or guardians must give consent for their minor child, but the wishes and concerns of the child should be taken into account [15]. The American Academy of Pediatricians states that one “should not exclude children and adolescents from decision-making without persuasive reasons” [16]. It is generally accepted that, starting at 7 or 8 years of age, children are capable of giving assent to any proposed intervention, and that their assent should be required in order to proceed. The assumption is made that the parents will act as surrogate decision-makers for their children and make choices based on the best interests of the child. The courts may need to become involved if parents do not seem to be following this approach, as in case scenario 4 if a parent is a Jehovah’s Witness and refuses blood transfusion for the child.

Informed consent for donors of hematopoietic cells

Case scenario 5

A mother leaves the country with her 16-year-old son who is HLA matched with his 22-year-old brother who has acute leukemia, because she fears that if her younger son donates stem cells then he will die or also get leukemia. She feels that she is going to lose one son and does not want to lose the other. The older son dies from refractory leukemia without HCT.

Case scenario 6

In performing HLA typing on members of a large family because a relative has leukemia, it becomes evident that one of the siblings has a different father from the rest of the children.

The source of cells for an allogeneic transplant is usually a living donor who may be closely related to the patient, who may be a minor, or who may be an unrelated volunteer. In each of these situations, the autonomy of the donor must be respected, as reflected in an appropriate informed consent process. Donors must be informed of the specifics of the procedure that they may undergo, especially the potential risks involved. The process must be free of coercion and must respect the confidentiality of the donor and the family when it is a family donor in question. Cultural diversity issues may complicate the search for a potential donor if unusual or irrational beliefs are brought to bear. Strict confidentiality related to HLA typing results must be maintained to protect the privacy of all potential donors, including the possibility that previously hidden facts about paternity may be brought to light to an unsuspecting family. Revelation of such information could have devastating effects on the entire family unit.

Special and important considerations are raised when the donor is a minor. It is generally accepted that the risks involved in being a marrow donor are very small. Estimates of the incidence of life-threatening or incapacitating complications are about 0.29% for adult donors at several centers and 0.4% in a pediatric population from a single institution [17]. The European Group for Blood and Marrow Transplantation reviewed 27,770 bone marrow collections from 1993 to 2005 and found only one fatal event (pulmonary embolism) and 12 serious adverse events, mostly cardiac [18]. A potential benefit of the donation process is that pre-existing medical problems that require intervention may be detected in the donor. In this study in Seattle, 206 medical problems were observed in the 1549 donor evaluations, with hypertension, obesity, and cardiac problems the most common (see Chapter 42) [19].

Many collection centers are using granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood cells (PBCs) from matched siblings or unrelated donors as their preferred source of cells [20–22]. Many of the risks associated with marrow harvests are minimized or prevented, such as complications from general anesthesia, the pain from multiple iliac crest punctures, local hematomas, and/or infection at the aspiration sites. The use of G-CSF for this purpose is considered to be generally safe except for donors who have certain underlying medical conditions such as hemoglobinopathies, autoimmune disease, or coronary artery disease.

In a randomized study comparing PBC with bone marrow donations, the donors found the PBC collections to be the less burdensome and preferred method [23]. In a study of sibling donors for patients enrolled in a randomized trial comparing PBCs with bone marrow cells, Rowley et al. found that the intensity and nature of pain were very similar for the two groups, although all the PBC donors returned to normal function by 14 days compared with 80% of the bone marrow cell donors [24]. It is still not settled whether there is any overall advantage to the recipient from one source of cells over the other. Donor preference may be the deciding factor if there is no clear advantage between the two cell sources.

In reviewing the experiences and attitudes of the first 20 volunteer unrelated donors at the University of Minnesota, Stroncek et al. found no serious physical or emotional after effects [25]. Nine of the 20 donors reported that a friend or family member had discouraged them. Nineteen said they would donate again, and 17 would advise others to donate.

The benefits of being a marrow donor for a family member or an unrelated recipient are a matter of debate. At one extreme, it is argued that there are no benefits to the donor, and therefore even the small risk of complications becomes more significant. Others state strongly that there are remarkable benefits to the donor of a psychologic nature, especially when donating to a family member, such as the satisfaction of helping to save a loved one's life [26]. On balance, the very low risks associated with marrow donation and the fact that marrow is a renewable resource like blood, and that significant benefits are derived by the donor, make it ethically appropriate to encourage PBC and marrow donation.

Of course, it is imperative that any coercion of potential donors be avoided. To that end, the HCT center should keep results of HLA typing confidential (or not even initiate testing) until all family members have had the opportunity to discuss privately their desire to proceed [12]. To protect further the rights of a potential related donor, the current edition of the Foundation for the Accreditation of Cellular Therapy–Joint Accreditation Committee of the International Society for Cellular Therapy and European Group for Blood and Marrow Transplantation (FACT–JACIE) *International Standards for Cellular Therapy*, Standard B6.3.1.2 now requires that "allogeneic donor suitability should be evaluated by a licensed healthcare professional who is not the primary transplant physician or healthcare professional overseeing care of the recipient" [27]. This has been the standard practice for consenting of minor related donors and adult unrelated donors for many years.

The psychologic state of the donor must be respected. If the outcome is poor, especially if the patient fails because of GVHD, there will be a natural tendency for donors to blame themselves for the patient's death. Appropriate counseling before and after the HCT can help lessen this burden for the donor. In light of these concerns, it has become the legal standard that no court can force anyone to undergo medical testing or a procedure that is intended only to benefit another individual.

Confidentiality and non-coercion are especially important issues in relation to unrelated donors. The National Marrow Donor Program has very explicit policies and procedures designed to respect potential donors' autonomy by requiring informed consent at every step of the process, including the time at which a donor is first listed in the registry; before collection of blood for confirmatory typing, infectious disease markers, and research samples; prior to notification of the transplant center that a donor is willing to proceed to donation; and before general anesthesia [28].

The donor's privacy is of the utmost importance to avoid any extraneous influences or pressures as the donor decides whether or not to donate. The identity of the donor is known only to the individual donor center, and many precautions are put in place to ensure this. Generally, the recipient and donor are allowed to contact each other directly only by mutual consent 1 year after the HCT. The National Marrow Donor Program considers these measures to be critical in promoting increased participation in the program by altruistic individuals. The World Marrow Donor Association reviewed the ethical issues for unrelated donors, focusing on respect for the donors' autonomy, wellbeing, and needs. They stressed the importance of non-coercion and adequate informed consent, especially when requests for donation of a second cellular product are being considered [29].

The process by which decisions are made to use minors as marrow donors is somewhat controversial [30–32]. Some authors have questioned the manner in which informed consent is obtained to collect marrow from minors and have asked whether parents can truly give informed consent. The need to involve the minor in the consent process by seeking his or her assent is a widely accepted concept, especially for children over the age of 7–8 years. The American Academy pf Pediatrics Committee on Bioethics has developed five criteria that must be met for the ethical utilization of a minor sibling donor: (1) a medically equivalent adult donor must not be available; (2) an actual or anticipated strong personal and positive emotional relationship between the donor and recipient must exist; (3) there is a significant likelihood that the recipient will benefit; (4) the clinical, emotional, and psychologic risks to the donor must be minimized; and (5) parental consent and the child's assent must be given [33,34].

Delany, in an article with several separate contributions, questioned whether it is really legal to harvest marrow from a minor and labeled the process "altruism by proxy" [32]. One legal concept permits a medical procedure only if it serves the best interests of the child who undergoes it. If there is disagreement between caregivers, it may be necessary to go to court. A second concept argues that parents can give valid consent to treatments that are "not against the interests of the child." This approach was developed to allow blood drawing from children whose legitimacy or parentage was at issue. Some have argued that this principle is relevant to any medical intervention carried out on children purely for other people's benefit.

Delany argued that marrow donation is not in the best interests of the donor, and that it may be against the child's interests, especially if the potential donor is too young to have established an emotional bond with the intended recipient. She also contended that the parents are not well suited to give informed consent for the donor owing to conflict of interests related to the sick child, and that an informal tribunal or other forum independent of the parents and medical advisors should be responsible for approving each proposed donation. This procedure would be analogous to the strategy developed by the Law Commission of England and Wales to safeguard mentally incapacitated adults from being inappropriately volunteered for marrow extraction.

Month, in the same joint article, disagreed strongly with this interpretation of the issues [32]. She believed that the positive aspects for the child donor are considerable and include the saving of a sibling's life and the benefit of many years of a whole family not burdened by the psychologic trauma of the death of a child. Further, since HCT is an accepted therapeutic option that can be lifesaving, and often offers the best cure rate, is there really any reason a morally competent sibling would not want to donate? The risks of donation are minimal, especially when compared with the risks of not donating marrow, that is, the death of a sibling.

Lyons described the donation of stem cells by a minor to a sibling as "the appropriation of bodily tissue from one unconsenting human and its conveyance to a third, albeit related, party." He challenged the legality of this exercise of power by the parents. He argued that for parental consent to be "legally permissible, it must be on the basis of an implicitly acknowledged property right in the (donor) child" [35].

Savulescu, in the cited joint article [32], argued that although the bone marrow donation is not in the donor's medical interests, it may be in his or her overall interest. To save the life of a loved one is one of the most important things one can do. Even for donors who are too young to understand at the time, the potential for a future sense of achievement and the love and gratitude of the saved sibling is important. There are also benefits to the family unit. Parents should be the ones to give informed consent after they are educated about the risks and benefits of the donation, just as parents often make decisions with conflicting interests of their children, but with a commitment to the overall good of the family.

An unusually difficult case was published involving a 15-year-old boy with relapsed AML who was incarcerated for sexually assaulting his 9-year-old sister [4]. The conflicts involved in the decision-making about whether or not to offer the boy HCT were discussed in a preceding section. The 9-year-old sister was the only HLA-matched relative available. Should she be asked to donate stem cells to her brother? Is there a reasonable alternative source of stem cells available for the patient, for example, a matched unrelated donor? Is the mother the right person to give informed consent on her behalf? Should there be a legal guardian appointed to protect the interests of the sister? Would the potential harm to her, psychologic and physical, outweigh the benefits? Although these are questions relevant to almost every case involving a minor donor, the conflicts were clearly more acute in this case.

The formal process for approving the use of a minor for marrow donation varies between countries and between centers within the same country. A survey of 52 HCT centers in Europe and the United States found that some centers had an ethics committee decide the validity of the therapeutic proposal [31]. In France, there is a law that requires a committee of three experts to assess the psychologic and medical consequences of donation of the organ by a minor, and notes that "the refusal of the minor donor to accept the removal shall always be respected." In the United States, a Washington State court ruling stated that the advantages to the donor are undoubtedly greater than the risks, and defers to the parents to give informed consent. Some institutions require an authority outside the family and hospital, for example, a "tutelary judge" or commission of experts, to make the decision.

A questionnaire was sent to 70 HCT centers in North America by pediatricians at the M.D. Anderson Cancer Center to poll the HCT teams on their approaches to marrow donations by minors [36]. The impetus for sending the questionnaire was a case brought to the M.D. Anderson pediatricians in which they were asked to collect marrow from a young child to be donated to the child's parent. There was a significant difference in size between the child and parent, and three HLA mismatches. The parent had relapsed AML. Ethical concerns were raised by hospital staff members. As a result, the M.D. Anderson Center developed a process in which the minor donor and surrogate are interviewed by a social worker, pediatrician, and anesthesiologist, none of whom is involved in the care of the recipient. If any concerns are raised, the case is referred to the ethics committee for consultation.

Fifty-six (80%) of the centers responded to the questionnaire. There was consensus in endorsing the validity of parental consent. Most centers would use donors as young as 6 months old and would use them more than once if needed. The centers were willing to use minor donors for patients on experimental protocols, and the projected outcome of the HCT did not affect the decision to use the minor as a donor. Issues raised included the non-therapeutic nature of the donation, the vulnerability of the minor, and the potential conflict of interest when a parent acts as a surrogate for both the donor and a sick family member. Even greater conflict was seen if a parent was the intended recipient of the marrow, and most agreed that the other parent or both should be involved in the informed consent procedure.

The majority of the centers and physicians had policies or practices of using the parents as the surrogate; a minority preferred to use a parent plus a child advocate or child protection agency. If there were disagreements between the parents, eight centers would cancel the HCT, and the rest would either seek consultation with the ethics committee, use an unrelated donor, refer the parents for another opinion, or refer the decision to a child protection agency. When asked if the use of a child intentionally conceived as a marrow donor was ethical, most of the physicians answered that they would be willing to use such a donor. They would consider using umbilical cord blood (UCB) in that case as being safer for the donor.

The participation of pediatric donors in clinical research raises additional issues beyond standard clinical practice [37]. When can Institutional Review Boards (IRBs) approve such activities? The IRB must focus on whether the proposed research procedure is different for the donor compared with a standard stem-cell collection. If the proposed research adds or replaces procedures to the standard clinical donation, and thereby increases the risks to the donor, such a protocol must be approved by the Secretary of Health and Human Services with the advice of a panel of experts (category 46.407 of the Federal Regulations). Some have argued that minors who are asked to donate G-CSF-primed PBC should be considered to be research subjects, since the data about long-term risks in this setting are not well known. The parents would be the best persons to decide for their child donors, but IRB approval would be required [17,38].

The participation of unrelated donors in research activities is also an issue of concern in terms of advancing the science of such transplants, and protecting the rights of these altruistic individuals. There must be efficient mechanisms in place to ensure that research review boards approve such protocols in the countries of both the donor and recipients. The World Marrow Donor Association has called on all donor registries to participate in research acitivities that can lead to improved outcomes for patients and to clearly define what constitutes research versus standard clinical practice. King et al. cautioned that the health and wellbeing of the donor must take precedence over the interests of science and society [39]. Donors must be carefully informed about what will be done with their cells, especially if there is any intended use for research purposes [39,40].

Alternative sources of hematopoietic cells

Case scenario 7

A couple decides to reverse a vasectomy to create a new baby to be a possible donor for their teenage daughter with CML who has no family or unrelated donors.

HLA-matched siblings are usually considered to be the primary source of cells for HCT. For the 70% of patients who lack such a donor, alternative sources of stem cells include matched related donors other than siblings, unrelated volunteer donors, UCB cells, and possibly autologous stem cells in the appropriate clinical setting. In this section, the ethical issues raised by two potential sources of stem cells are discussed, namely UCB [15,41–43] (see Chapter 39) and "children conceived to give life" [44] or so-called "savior siblings"[45,46].

The advantages of cord blood over adult marrow include the lower risk of carrying viruses, a reduction in procurement time from the 4–6 months needed for an unrelated marrow donor to 1–2 weeks, and the fact that cord blood cells are potentially usable across greater HLA disparities. The major disadvantages are the limited number of cells available, the fact that one cannot go back to the donor for more cells if needed, and the risk of transmitting a genetic disease not previously diagnosed in the donor's family.

Marshall raised several ethical issues related to cord blood cell collections and cord blood banks, starting with consent and privacy [47]. Is informed consent required for a product that is otherwise discarded as waste? Is the mother (or parents) the appropriate one(s) to give consent? Is consent required to conduct a battery of tests to detect possible infectious or genetic diseases, or both? Should donors, that is, the parents, be informed of the results of such tests? Who owns the cord blood? Obviously, the donor themself cannot give informed consent, yet an extensive medical profile about the donor may be generated, raising concerns about invasion of privacy. Should consent be obtained for follow-up tests that are not consented to directly? What should be done with abnormal results from such tests? Should banks maintain medical files and genetic data, but in such a way that names and identifiers are delinked from the donated sample [48]? What are the obligations to inform the family if evidence of human immune deficiency virus or an abnormal gene is found?

Some authors argue that the infant donors should be the first to benefit from use of their own cord blood cells [49]. Records should be kept of diseases detected and parents informed. Therapy should be offered for treatable diseases. Commercialization of cord blood cells for therapy should include a financial benefit for infants; for example, royalties from each donation should be placed in a national trust fund for research and treatment of children with serious diseases. Kurtzberg et al. argued that cord blood that was previously discarded material should be a public resource with no financial gain to any party [50]. Regulation of donor banks by an agency such as the FDA is needed. For example, there are companies that try to persuade parents that they should store the cells as insurance against future needs, when in fact the chances are remote that the donor would ever need or want to use the cord blood cells. There is a pressing need to protect the confidentiality of the information collected, to notify parents and children of the test results, and to assure equitable access to cord blood samples. For the health of the mother and baby, the collection process should be restricted to full-term, uncomplicated pregnancies, and should not interfere with good obstetrical practice at delivery.

The American Academy of Pediatrics has addressed the issue of cord blood banking and issued a set of guidelines to help physicians regarding types and quality of blood banks (for-profit versus not-for-profit), ethical and operational standards including informed consent policies, financial disclosures, and conflict-of-interest policies [51]. Their three main recommendations are: (1) the storage of cord blood in private banks for later personal or family use should be discouraged unless there is a full sibling in the family with a known malignant or genetic condition that might benefit from cord blood transplantation; (2) cord blood donations should be encouraged when stored in a bank available for public use; and (3) private storage of cord blood as "biologic insurance" should be discouraged.

Considerable attention and debate were generated in response to the actual case outlined above as case scenario 6. The practice of "conceiving a child to give life" is not a rare event [52,53]. Some question whether a good outcome for the recipient lessens the ethical dilemmas posed. It was argued that such a practice does not impose harm on persons or relationships within the family, and does not show lack of respect for the child conceived [12]. Others raised their concerns about performing prenatal testing to diagnose the genetic disease affecting the intended recipient and for HLA typing, and the potential for aborting in the case of a mismatch.

One hematologist's view of the concept is that such a child is conceived not to replace the sick sibling, but to give life to the sibling who would not survive without a transplant [53]. The benefits of this approach include the fact that the cord blood is disposable material, and that no anesthesia or blood transfusions are needed for the donor. From the recipient's perspective, the transplant can be performed earlier since a marrow transplant could require a delay of 6 months or more.

Alby described three characteristics of pregnancies conceived for HCT: (1) families in that situation face the trauma of the impending death of the sick child; (2) the pregnancy outcome with respect to HLA matching and the HCT results are both uncertain; and (3) biology has intruded into the family dynamics [44]. A family may attempt a cure at any cost, especially in an emergent situation such as acute leukemia. A child may be viewed subconsciously as a replacement child, but should never be reduced to the role of a therapeutic tool. In terms of family dynamics, a child may be viewed as bad or good based on HLA matching. Many are concerned that such a child is being conceived only for transplantation. Yet a child is always born for something: to maintain the integrity of a couple, to gratify the parents' need for a family, to repair their vulnerability. All parents must go beyond the idealized view of their children and accept that they have an identity of their own, and all children have some symbolic role in the family fantasy. In that light, is there really anything wrong with conceiving a child to donate cells for the purpose of saving the life of a sibling?

Advances in in vitro fertilization techniques have opened the door to preimplantation genetic diagnosis (PGD). Candidate embryos can be screened in vitro for HLA matching [54] and for various genetic traits [55–57] as the basis for selecting which embryos to implant. HLA typing of embryos to select a potential stem cell donor for an afflicted relative has been proposed as a legitimate means of securing a donor for a patient who is in need of a lifesaving transplant.

The ethics of this approach have been reviewed, and the conclusions were that using PGD to choose a stem-cell donor is unlikely to cause harm to anyone, is likely to be beneficial to some, is a reasonable use of limited health resources, and should be permitted in countries where PGD is already allowed [58]. Morgan et al. also concluded that the use of assisted reproductive techniques to create a stem-cell donor can be considered to be ethically acceptable [59]. As a case in point, in the United Kingdom, the Human Fertilisation and Embryology Authority gave permission for PGD to be utilized to select an unaffected embryo to serve as a future hematopoietic cell donor for a sibling with thalassemia [60]. The technique has been used in the United States since 2000. Pennings et al. argued that PGD-guided selection of embryos for this purpose is morally defensible on the condition that the procedure to be performed on the future child is acceptable for an existing child, and that the "instrumentalization" of the donor child does not demonstrate disrespect for the child's autonomy and intrinsic worth [61].

Devolder considered the risks and benefits of utilizing preimplantation HLA typing for selecting embryos to be used as donors of stem cells for sick siblings and concluded that it would be unethical not to allow this procedure, since there is no indication of harm to the donor child, and some lives would be saved [62]. She argued further that it should be up to the parents to decide whether their current or *future* children can act as a donor for a loved one, and that the pool of potential recipients should not be restricted to siblings or other family members, but should be expanded to include anyone whom the couple loves.

The ability to create multipotential stem cells in vitro from pluripotential embryonic stem cells is a subject of wide public debate. Legal and ethical distinctions have been drawn between human reproductive cloning and nuclear transplantation for the production of stem cells for therapeutic applications. Only the latter will be discussed here as a potential source of hematopoietic cells for use in HCT in the future. Many scientists, ethicists, government bodies, and medical organizations in the United States, Canada, and Europe have advocated for the legalization of research for the purpose of therapeutic cloning [63–68]. The potential to utilize such cells to treat a variety of medical conditions appears to be great, although significant technical hurdles are yet to be overcome before there can be clinical applications. Many serious ethical issues must be considered, including the protection of human dignity, the source of unfertilized eggs and/or embryos to be used for research, informed consent from potential donors, the privacy of donors, and financial incentives to donate eggs and/or embryos.

Transplantation for non-malignant diseases

Most HCT procedures are performed to treat malignant diseases for which HCT often represents the only curative option. Many non-malignant diseases are also treated by HCT, especially in the pediatric patient population, and include primary marrow failure such as severe aplastic anemia and a variety of congenital immune deficiency syndromes (see Chapters 71–76). The balance of risk and benefit for patients with these diseases is clearly in favor of HCT since the diseases are often rapidly fatal, and curative alternative options are generally not available.

The balance may not be so clearly defined for a variety of diseases that affect marrow-derived cells, which may be a source of significant morbidity for affected patients and possibly shortened life span, but are not considered to be rapidly fatal. Further, the data that demonstrate the long-term benefit from HCT for these conditions may be inconclusive or even non-existent. Examples of this category of diseases include hemoglobinopathies such as sickle cell disease, certain metabolic storage diseases, autoimmune diseases such as multiple sclerosis and rheumatoid arthritis, and acquired immune deficiency diseases, specifically AIDS. How does one select the right patient to be treated by a procedure that carries significant risk of morbidity and possibly early mortality? How can one demonstrate the efficacy of HCT in diseases that usually progress over years rather than weeks or months? How does a parent decide to consent to an HCT for a child with such a disease?

This discussion focuses on hemoglobinopathies as a paradigm for these issues since there has already been a broad experience in performing transplants for thalassemia [69], and a body of literature about the ethical dilemmas involved is available. These issues were discussed at an international meeting on HCT in children held in March 1994 [41]. With regard to hemoglobinopathies, the difficult questions are whom to treat and when, and whether to offer HCT as a curative procedure to all affected children as early as possible. Some participants favored HCT for all patients with sickle cell disease who have an HLA-matched sibling in early childhood, given the high risk of morbidity in adult life. Others argued that it should be reserved for those who show clinical indicators of poor prognosis, as adopted by the ongoing national trial to recruit 30–60 children over a 5-year period. For thalassemia, the clinical course is more variable. Some proposed that HCT should be offered before any morbidity has occurred, whereas others would reserve it for children whose iron overload was well controlled and who were without liver disease.

Giardini discussed the ethical issues of HCT for thalassemia in an editorial in 1995 [70]. He argued in favor of HCT since the risk of mortality from disease-related complications remains high even with desferrioxamine chelation. Giardini argued that it is ethically and economically appropriate to recommend HCT for thalassemia, especially given the progress in dealing with GVHD and cytomegalovirus, the availability of newer antibiotics, and so forth; and the fact is that currently HCT offers the only chance of cure of the disease.

Kamani et al. reviewed several issues related to HCT in the pediatric population [71]. They posed three questions that need to be addressed when considering allogeneic HCT for non-malignant diseases in children such as hemoglobinopathies. These were: (1) how bad is the health quality of life for affected patients?; (2) are there prognostic factors to predict who is likely to suffer serious complications from the underlying disease?; and (3) what is the likelihood of success from the HCT in light of the quality of the matched donor available? More data are needed to answer the second question in particular to help select candidate patients in a way that would improve the risk/benefit balance of this approach.

Kodish et al. examined the issue of parents' decision-making concerning HCT for children who were affected with sickle cell disease [72]. A questionnaire was presented to parents with hypothetical chances of cure versus mortality to see how they balanced these two outcomes. In the study of 67 parents, 54% were willing to accept some risk of short-term mortality, 37% would accept at least a 15% mortality risk in the short term, and 12% were willing to accept a 50% or more risk. Sixteen (24%) of the 67 parents would not accept HCT even if there was a 0% mortality risk.

Differences were found between the group of parents who would accept some risk and those who would not. Parents who were high-school graduates, who were employed outside the home, or who had more than one child with sickle cell disease were more willing to take the risks. Of note, parents were more willing to take risks for girls than for boys. The parents' decisions were not related to the clinical severity of their children's illness, unlike the way the doctors might make such a decision. For parents with more than one child, their willingness may reflect their knowledge of the disease, or the burden of caring for children with sickle cell disease. Clearly, the concerns and values of the parents must be considered along with weighing the medical issues of risks and benefits.

In a recent report by Hansbury et al., a cohort of 113 pediatric patients with sickle cell disease receiving chronic transfusions was analyzed with respect to options and preferences for allogeneic HCT [73]. Forty of these patients had unaffected full siblings who could possibly serve as a stem-cell donor. The families of 23 of these 40 patients agreed to HLA type the sibling(s) and eight of these 23 were matched. Only three of these eight underwent HCT. The reasons for declining HLA typing or HCT included fear of toxicities, inadequate support for the procedure, including time and resources, and belief that the current transfusion therapy was adequate.

Recent advances in HCT, such as the use of peripheral blood or umbilical cord blood hematopoietic cells from allogeneic donors and reduced-intensity conditioning regimens, have been utilized in the treatment of hemoglobinopathies [74–76]. These advances help reduce the risks of short- and long-term toxicities for either the donor or the recipient, and thus create a more favorable risk/benefit ratio. Parents of minors with hemoglobinopathies will need to be informed about these innovations to help them make an appropriate decision for their children who may be candidates for HCT.

End-of-life issues

Case scenario 8

A 50-year-old physician with CML is dying from SOS and GVHD with liver and respiratory failure early after HCT. He has no written advanced directive. He made his second wife promise that she would not let him stay on life support for more than 1 week if he had no hope of recovery. She is devoutly Catholic, but feels she made a commitment to her husband to withdraw life support. His adult children from his first marriage, who are atheists, say their father would never agree to withdrawal of life support, that he is ardently opposed to euthanasia, and that to him withdrawal of life support is no different from euthanasia.

Respect for patient autonomy requires caregivers to determine the wishes of their patient with respect to resuscitation and life support. Advanced directives, such as a Power of Attorney for Health Care, are extremely effective vehicles to facilitate discussions between patients and their doctors, for patients to convey their wishes, and to name a surrogate to speak for them when they become incapacitated.

A new type of document called Physician Orders for Life Sustaining Treatment (POLST) has been developed to augment other traditional methods of advance care planning [77,78]. Currently 34 states either have or are developing POLSTs. Unlike Do Not Resuscitate (DNR) orders, these documents allow patients to specify their treatment preferences before the event of a cardiopulmonary arrest. POLSTs are particularly helpful for elderly or incapacitated patients who may be shuttled between various nursing homes and hospitals with many different healthcare providers involved in their care.

Yet very few patients execute such documents or discuss these matters with their doctor. Such discussions about end-of-life issues are difficult for most physicians, nurses, and patients. They are even more difficult in the context of HCT, where the patients are generally young and, although they have potentially fatal diseases, often have a significant chance for cure with this treatment. During the informed consent process that leads up to the HCT, physicians are obligated to discuss the risks and complications of the planned therapy, including the chance of early mortality. As discussed above in the section on informed consent for patients, when faced with life and death choices, patients often block out the details of possible complications in the decision-making process. Physicians have trouble raising end-of-life issues in the context of proposing a potentially lifesaving therapy, in part for fear that patients will take away a negative message about their prognosis [79].

The fact remains that up to 15–20% of patients may succumb to early allogeneic transplant-related mortality. HCT patients often decompensate rapidly and unpredictably, for example, from severe SOS or GVHD. There may not be an opportunity to discuss end-of-life issues when the patient is in respiratory failure on the way to the intensive care unit. The quality of patient care would be much enhanced if such discussions and appropriate documentation of patients' wishes were carried out well before this point, and were then acted upon as dictated by the patient or the surrogate. Many of these patients will be maintained by intensive life support for extended periods of time, despite generally poor chances of recovery. Some centers have developed ICU admission policies that exclude HCT patients who meet certain criteria, such as critically ill patients who have relapsed disease with no treatment option for their malignancy, multiorgan system failure with respiratory failure unresponsive to standard treatments short of intubation, and refractory grade III–IV acute GVHD with respiratory failure requiring intubation. For those patients who are admitted to the ICU, it is imperative to update their surrogates/families on a regular basis with multidisciplinary meetings. The caregivers need to discuss current status in addition to short-term goals of ongoing therapy, and then reassess the patient's progress towards those goals every few days. At some point in the course of the ICU stay, the goals of therapy must shift from cure and aggressive interventions to palliation and supportive care for a dying patient. Caregivers are obligated to recommend when it is time to do so based on the principles of patient autonomy and non-maleficense. Rigorous attention to pain control is essential throughout the HCT process and certainly during the period of dying.

The principles of fidelity and non-abandonment require caregivers to make it clear to patients and their families that they will not be abandoned as the goals of treatment shift from cure to caring for the dying patient. Although it may be appropriate to withhold interventions that are medically futile or ineffective, how is this to be determined? How much of our limited healthcare resources should be expended providing what is ultimately futile medical care? How great an emotional cost do families have to pay? These are difficult questions that need to be addressed at both the macrolevel of society and the microlevel of individual patients and families. It is the patient (or surrogate) who is best able to understand and decide on the issues of his or her ability to pay for a treatment and the possibility of financial destitution of a family by continuing futile care. The surrogate must utilize substituted judgment to relay the decisions that the patient would have made if he or she had the capacity to do so. In cases in which no surrogate is legally designated, an ethics committee may need to be involved to consider quality of life issues and death with dignity.

To explore the variables involved in instituting DNR orders for HCT patients, a retrospective review of 40 patients who died in the Seattle Veterans Administration Medical Center HCT unit was carried out [80]. This center has a DNR policy that requires physicians to document the discussion held with the patient/surrogate, and for the patient/surrogate to sign a DNR consent. Patients with fatal diseases often suspend their feelings about terminal illness in the hope of a cure through HCT. The dying trajectory of these patients is often sudden and unpredictable. The healthcare providers often focus on aggressive medical management when these situations arise and, as a result, the topic of death is avoided. The process of executing DNR orders was believed to facilitate the transition from aggressive care to supportive care in critically ill HCT patients.

In 11 studies of HCT patients who required mechanical ventilation, the rates of survival to discharge from the hospital ranged from 0% to 11.1%, with a mean of 4.7% [81]. It would be clinically useful to define a subgroup of patients whose chance of survival is so low that both patients/surrogates and physicians can agree that intensive care can no longer fulfill the original goals of the HCT. Criteria to identify patients who are destined to die after HCT are needed to help reduce emotional and financial expenditures of families and institutions. At the Fred Hutchinson Cancer Research Center from 1980 to 1992, 25% of all HCT patients were ventilated. Patients who were more than 20 years old, had disease in relapse, and had grafts that were not HLA identical had a 50% chance of needing ventilation. Only 6.1% survived. To identify predictors of death in mechanically ventilated HCT patients, Rubenfeld and Crawford conducted a nested case–control study comparing all 53 survivors of ventilatory support with 106 matched control subjects who did not survive [81]. These patients were selected from the 865 individuals who were mechanically ventilated for at least 24 hours during that 12-year period. Survivors were defined as those who were alive for 30 days after extubation and who were discharged from the hospital.

Survival was statistically associated with younger age, lower score on the APACHE (Acute Physiology, Age, Chronic Health Evaluation) III scale, and shorter time from HCT to intubation. Of the 106 control subjects who died, 82% died while on the ventilator, and the remaining 18% died in the hospital a median of 18 days after extubation. Of the 53 survivors, median survival after extubation was 634 days (range 54 days to 12 years). Eighteen (34%) survived for less than 6 months and five (9%) for less than 1 year; 30 (57%) lived for more than 1 year. No patient who was intubated for more than 149 days survived. The survival rates changed from 5% to 16% over the most recent 5 years, which was not explained by changes in the age of patients, the rate or timing of intubation, or the percentage of allogeneic transplants that were not HLA identical. The improved survival may be due to better antimicrobials and the use of cytokines.

There were no survivors among the 398 patients who had lung injury and either required more than 4 hours of vasopressors or had sustained hepatic and renal failure. Using these three criteria, an accurate prediction of death could be made in the first 4 days for more than half of the patients who did not survive. Of the 60% of patients who developed any two of these three risk factors, half had done so by day 2 and 90% by day 4. On average, these risk factors developed six ventilator days and nine hospital days before death. If life support had been withdrawn on the first day that two out of three risk factors were met, and if death had followed swiftly, more than 7300 hospital days and 4800 ventilator days could have been avoided for the 812 patients who died.

A multi-institutional study confirmed these observations in adult HCT patients, for whom the combination of mechanical ventilation and hepatic and renal dysfunction was associated with a probability of death of 98–100% [82]. This experience was confirmed in a recent report from a single institution which reviewed the outcomes for 68 patients who required mechanical ventilation after allogeneic HCT [83]. The overall mortality rate was 86.8%. The Sequential (or sepsis-related) Organ Failure Score (SOFA) [84] was used to predict mortality in this group of patients. The SOFA score assesses the function of six major organs and generates a score of 0 to 24. The mean SOFA score for surviving patients was 7.7 and for non-survivors it was 11.6 ($p = 0.009$). The presence of renal failure or liver dysfunction at the time of intubation was associated with a 100% mortality rate, as was reported previously by Rubenfeld and Crawford [81]. The SOFA score on the day of admission to the ICU was also predictive of survival in other studies in Germany [85] and Sweden [86]. A study in Belgium confirmed the poor prognosis for allogeneic HCT patients who require intensive care [87]. The overall ICU mortality, in-hospital mortality and 6-month mortality rates were 61, 75, and 80%, respectively. Bacterial infection as a the cause of multiorgan failure was associated with a lower mortality rate compared with other causes.

A scoring system for pediatric patients undergoing allogeneic HCT called the Oncologic Pediatric Risk of Mortality (O-PRISM) may be useful in predicting fatal events, and thus help parents decide to establish a supportive care strategy for their children [88]. Outcomes for pediatric patients who require mechanical ventilation after allogeneic HCT may be somewhat better than for adult patients. In a study in The Netherlands [89], 35 out of 150 patients required mechanical ventilation between 1999 and 2007. The case fatality rate was 42%, and mortality was associated with multiorgan failure on day 2 of ICU admission, and with the use of high-frequency oscillatory ventilation. The 6-month survival for patients discharged from the ICU was 82%.

Although most physicians and ethicists may agree that futile, inappropriate, or ineffective medical care should not be provided even if requested, how are these terms to be defined, and by whom? In cases in which the patient/surrogate continues to request treatments that the caregivers consider futile, the physician is not ethically required to provide that care, but is obligated based on the principle of non-abandonment to facilitate the transfer of the patient to another physician who will. Consultation with the institutional ethics committee may be helpful in these most difficult situations. Several medical and legal societies such as the AMA, CMA in California [90] and LACMA/LACBA in Los Angeles County have published guidelines or policies stating that physicians are not obligated to provide care that is considered medically ineffective by community standards. Some medical centers have policies and procedures to define the process by which conflicts arising over medically ineffective care will be managed. In cases where patients/families demand treatments that the healthcare team deems to be medically ineffective, a sequence of steps is initiated including consultation with other physicians and the ethics committee. If there is still disagreement, then the burden falls on the family to seek a court injunction in a timely manner to stop the withdrawal of life-sustaining treatments.

Conclusion

The indications for HCT have expanded over the years to encompass a wide range of diagnoses and disease stages, including patients with early stages of hematologic malignancies considered to be at high risk for relapse, solid tumors, congenital disorders, and autoimmune diseases. Potential sources of stem cells have also expanded to include autologous and allogeneic cells from bone marrow, peripheral blood, and cord blood from related or unrelated HLA-matched donors. It is imperative that the rights of both patients and donors be respected in step with these technologic advances. The ethical principle of justice requires caregivers and insurers to provide potentially life-saving yet high-risk procedures to HCT candidates in an open and equitable manner. Patient autonomy, non-maleficence, and non-abandonment are guiding principles in making decisions about end-of-life care.

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CHAPTER 32

Psychosocial Issues in Hematopoietic Cell Transplantation

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Case Study: *MB is a 55-year-old married female 18 months after autologous HCT for multiple myeloma currently on disability and living at home with her spouse and two high-school age sons. The patient had two kyphoplasty procedures and two cycles of Revlimid, dexamethasone and Velcade in addition to mobilizing therapy prior to HCT. She tolerated transplant well, experiencing mucositis, controlled with pain medication.*

At her initial counseling session, she described physical symptoms including fatigue, neuropathy, back pain, occasional muscle cramping, and profuse sweating up to six times per day similar to a hot flash. Additionally, she reported poor short-term memory, occasional crying spells, and inability to function in her role as a college professor owing to memory deficits, physical limitations, and fatigue. She also worried about the high cost of her maintenance drug therapy that was covered by her husband's insurance policy.

Introduction

Hematopoietic cell transplantation (HCT) is an effective treatment for many life-threatening illnesses such as multiple myeloma, non-Hodgkin lymphoma (NHL), and hematologic malignancies. In this chapter, we define HCT as a group of procedures that involve the collection of stem cells from an individual and their administration to a recipient with a defective bone marrow or immune system caused by a myeloablative preparative regimen. HCT remains a high-risk, intensive, life-prolonging treatment that may be the best option for patients with few alternatives, even when the outcome includes the possibility of significant morbidity. HCT may be associated with a variety of serious biological, psychological, social, and spiritual complications. The above case study illustrates many of the complex and interrelated biopsychosocial issues confronting HCT recipients and their caregivers for months and years post-transplant. We organize potential psychosocial issues facing patients around four thematic stages from the decision to undergo HCT through the trajectory of long-term recovery (Table 32.1) Several studies have identified the temporal trajectory of psychosocial concerns and recovery for HCT recipients [1–3] and many have assessed health-related quality of life (HRQOL) changes over time in this population.

The term "psychosocial" refers to issues that are psychological and social in nature. Our case example depicts a recipient who is unable to return to work following HCT; she encounters both psychologic (e.g. depressive symptoms related to a change in identity from primary bread winner to disabled professor) and social issues (e.g. changes within co-worker network). Table 32.1 lists issues that can become problems over the course of the HCT process. The distinction of when an issue becomes a problem is somewhat arbitrary and largely dependent on the interaction of patient coping skills with the specific issue. For example, if, in our case vignette, the patient had a less demanding work role, perhaps her cognitive deficits would not have led her to apply for disability from her valued position. We focus on the literature identifying psychosocial issues for HCT recipients across the treatment and recovery time period and review the literature on informal caregivers.

Factors influencing psychosocial issues in HCT

Over the past decade, there have been shifts in how the HCT process unfolds for recipients and family members. Below, the forces that continue to shape the HCT process and hence associated psychosocial issues are discussed briefly.

The transplantation of older recipients. The reduced-intensity allogeneic transplantation includes a preparative regimen that is comparatively less toxic than the standard allogeneic transplant. Transplanting older recipients may mean that older loved ones, with less physical strength and stamina, become primary caregivers for a spouse, sibling, parent, or friend.

The collection of peripheral stem cells has nearly replaced bone marrow harvesting. Rather than an operating room procedure with general anesthesia requirements and associated complications of bone marrow harvest, autologous transplant candidates are seen in an apheresis unit after receiving granulocyte colony-stimulating factor. Stem cells are collected over a period of hours on one or more occasions. This change likely reduces the potential anxiety generated by an operating room procedure necessary for bone marrow harvest.

Ready access to information through Internet websites. Information about HCT is readily available to potential recipients with computer access. An ever-increasing database of HCT recipients is tracked through the Center for International Blood and Marrow Transplant Research (CIBMTR) (http://www.cibmtr.org/). Here, any family member or potential recipient can look up detailed information on

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Table 32.1 Themes, tasks, and psychosocial issues associated with four stages of HCT

Stage	Issues[a]
I. The decision to undergo HCT	*Theme: active decision-making*
	Confronting mortality and the possibility of death
	Managing the uncertainty of treatment outcome
	Considering alternative treatments
	Financial considerations/insurance limitations
	Psychosocial evaluation
	Informed consent process
	Symptoms of anxiety, depression, and distress
II. Pre-HCT preparation and post-HCT hospitalization	*Theme: aggressive treatment and watchful waiting*
	Adopting the patient role and managing hospital routines
	Managing isolation and acute treatment side effects
	Confronting unfamiliar procedures and treatment
	Separation from family and friends
	Adjusting to altered body image
	Waiting for engraftment
	Heightened physical and emotional vulnerability
	Maintaining morale and hope; managing discouragement
	Dealing with life-threatening complications
	Encountering acute psychological distress
III. Hospital discharge and early recovery	*Theme: transition from intense medical surveillance*
	Managing the loss of daily psychosocial support of medical team and allied healthcare professionals
	Contending with the stress of frequent medical appointments, readmissions, and set-backs
	Reintegration into valued social roles
	Managing unexpected sequelae
	Fear of recurrence
	Adapting to potential frustration, depressive symptoms, and anger
	Complying with self-care guidelines and daily medicine regimen
IV. Long-term recovery	*Theme: establishing the new normal life*
	Re-establishing primary identity/relinquishing patient role
	Recovery of valued roles in community, at work, and at home
	Adjust to losses associated with transplantation
	Return to employment
	Accepting the possibility and reality of long-term effects

[a] Many of these issues continue over the course of the four stages. For example, confronting mortality and the fear of recurrence may be revisited more or less intensely each time the patient returns to the transplant center for ongoing evaluation over many years. Anxiety, depressive symptoms, and distress and also delays in developmental milestones may emerge over the four stages of HCT.

survival statistics and other information. The National Bone Marrow Transplant link (nbmtLINK) (www.nbmtlink.org/) and Blood and Marrow Transplant Information Network (BMTinfonet) (www.bmtinfonet.org) are other rich sources of medical and psychosocial information for recipients and caregivers.

The emphasis on survivorship care planning and post-transplant survivorship monitoring. National and international organizations, e.g. the National Marrow Donor Program, have recommended specific screening and preventive practices for long-term HCT survivors [4]. For cancer survivors in general and HCT recipients in particular, survivorship care plans and the monitoring of clinical outcomes have become the recommended standard of care, even though it has yet to be proven that such monitoring improves outcomes [5,6]. The goal of such efforts is to help patients manage their long-term survivorship. Several important studies of recovery following HCT conducted with relatively large cohorts of recipients and caregivers have identified key problem areas and pointed to potential areas for psychosocial intervention [3,7–9].

The increasing number of unrelated volunteer donors and cord blood products given to recipients without family donors. With the availability of a larger pool of unrelated donors, more prospective recipients can proceed to transplant. Research on the experience of related versus unrelated donors is needed.

Further scrutiny of the costs of transplantation [10]. Financial difficulties are the source of great distress from the early decision-making stage of HCT to long-term survivorship where ongoing medications and lost wages can be extremely costly.

Prominent psychosocial stressors in HCT

HCT recipients and caregivers are confronted with two major sources of psychosocial distress that are more or less prominent throughout the lifespan of the HCT recipient: (1) their disease is life threatening and (2) the treatment itself can be both quality and quantity of life threatening at all stages of treatment, potentially a great source of distress.

Many patients have read or heard from their healthcare provider the prognosis for their particular disease, the fact that it is life threatening. Such an encounter with "mortal time" can debilitate some, invigorate others, and do both at various times along the survival trajectory [11].

A second source of distress for recipients is pre-transplant anxiety about expected emotional and physical side effects of treatment. Acute side effects, such as nausea, mucositis, hair loss, and diarrhea, and long-term effects, including graft-versus-host disease (GVHD) and secondary malignancies, are sources of significant distress. Treatment of many of these side effects requires the use of multiple drugs and therapies with their own side effects ranging from mild discomfort to debilitation. Even when the transplant is medically successful, the ever-present possibility of medical complications can be a source of chronic anxiety.

Stages of HCT and associated psychosocial issues

From early decision-making through long-term survivorship, HCT recipients face a predictable sequence of treatment-related events. We use the term "stage" to refer to a variable period that is characterized by specific tasks and also physical and psychosocial stressors that recipients and their caregivers must manage. In the cancer survivorship literature, the period from diagnosis through long-term survival has been described as the "seasons of survival" [12,13]. In Table 32.1 we identify four relatively distinct stages: (i) the decision to undergo HCT; (ii) pre-HCT preparation and post-HCT hospitalization; (iii) hospital discharge and early recovery; and (iv) long-term survivorship. These stages represent a general map of the HCT process and not an invariant sequence of events and stressors facing all recipients. Some psychosocial issues that appear during one stage of HCT may surface again at a later time. For example, fear of recurrence and what it portends is a significant source of distress for a proportion of HCT patients [14]. However, there is great variability in how patients navigate the HCT process, and few will proceed directly though the stages we describe.

Stage I: the decision to undergo HCT

Deciding to undergo HCT can be very stressful. Most research on decision-making relative to HCT focuses on the provision of written informed consent, a formal stage of decision-making that occurs after the decision to proceed with HCT. Some researchers suggest that the decision to undergo HCT is made prior to formal consent [15]. Furthermore, it is important to realize that in addition to the patient and their loved ones contemplating the life-changing HCT treatment, the medical and psychosocial team is also evaluating candidacy of prospective recipients.

Referral for treatment and selection of candidates

HCT may become a potential treatment option through a formal medical referral, via personal research, or from knowledge gleaned from the treatment experience of acquaintances. Current and comprehensive information is essential at this stage, especially if HCT is seen as the only curative possibility. Patients may be quick to sign on for a curative treatment option and thereby make a rapid psychological commitment to the procedure that is resistant to change [16,17].

The American Society for Blood and Marrow Transplantation (ASBMT) HCT guidelines for clinical centers include a psychiatric/psychosocial assessment as part the general medical workup for all HCT candidates [18]. The assessment is designed to enhance the healthcare team's knowledge of a recipient's psychosocial status, including psychologic distress, psychiatric history, social support, coping style, compliance, and drug abuse tendencies. Information obtained can be used to develop an individualized plan of care over the course of HCT. Standardized approaches to the pre-HCT psychosocial evaluation have been developed that may assist in identifying psychosocial concerns before transplantation and guiding intervention afterwards. Examples include the Transplant Evaluation Rating Scale (TERS) [19] and the Psychosocial Assessment of Candidates for Transplantation Scale (PACT) [20].

Universal psychosocial assessment and selection criteria have not been adopted across transplant centers. However, some researchers have highlighted the prognostic value of psychosocial screening and also the emergence of a high-risk patient profile. Foster et al. administered the PACT to 120 allogeneic transplant patients and identified significant associations between PACT subscale scores and future medical outcomes [21]. Select findings include significant relationships between compliance and reduced inpatient mortality, length of stay and engraftment, drug or alcohol use and engraftment, and receptiveness to education and decreased risk of mortality. Although the overall PACT rating score was not associated significantly with medical outcomes, subscale scores may be good predictors of future outcomes. Several other studies suggest an association between psychosocial status at the time of HCT and important post-HCT outcomes, such as survival [22–25] and risk for medical complications [24,26]. In spite of these findings, considerable uncertainty remains about specific screening variables and the level of risk necessary to rule out HCT candidacy, especially when HCT is the only treatment option [27].

Informed consent

The decision to undergo HCT is formalized in a face-to-face meeting with the transplant physician and the recipient before and/or at the time of pre-HCT hospitalization. At this meeting, the consent form is reviewed in detail (depending on recipient information preferences) and signed, thus becoming a legal document. In theory, the consent process allows patients to make an informed, voluntary decision regarding whether to proceed [28]. In practice, however, this ideal is unlikely to be realized in the HCT setting, for numerous reasons [16,29]. The nature and amount of information required to make an informed decision, the stressful context within which a decision is made, and the reluctance of potential recipients to engage actively in risk consideration all impact the consent process.

The process of obtaining informed consent occurs within a context of considerable distress. Most consent forms contain exhaustive descriptions of medical risks associated with HCT. Researchers have documented that many recipients are considerably distressed in the days prior to HCT [1,30–34]. Distress can compromise the consent process by inhibiting communication with healthcare providers, limiting sensible consideration of risks and benefits, and inhibiting comprehension or memory for information communicated during consent discussions.

Patients may be reticent to engage in discussion of the risks and benefits of HCT [35]. In fact, in the absence of particulars associated with treatment, some patients may enter the consent process with their decision already made. Shannon-Dorcy and Drevdahl reported that a strong desire to avoid death may lead patients to decide to participate in treatment well before they know actual treatment specifics [36]. Discussion of information regarding mortality and morbidity risks has the potential to be threatening, triggering attempts to avoid or minimize important information [37]. Although it may seem maladaptive for patients to distort or avoid information provided in the consent discussion, such avoidance may be critical to the mobilization of the psychological resources necessary to start the arduous, uncertain course of HCT [16]. Prospective candidates may be confronted with other consent and treatment options in addition to information about advanced care planning (ACP) at this juncture.

Advanced Care Planning (ACP)

ACP is particularly relevant for HCT candidates since HCT is an elective procedure that has life-threatening complications and significant mortality rates, and candidates spend time in a consideration phase pre-HCT [38]. Joffe et al. surveyed 155 adult patients pre-HCT and found that 69% reported designating a healthcare proxy, 44% reported completing a living will, and 63% reported discussing life support wishes with personal contacts [38]. Only 39% had formal advance directives (ADs) in their charts and only 16% reported discussing life support wishes with their doctors. ACP was more common in older, college-educated, and allogeneic transplant patients. Ganti et al. [39] found that those most likely to face negative outcomes (death within 1 year) also were those who were less likely to have prepared formally by completing ACP documents. Additional studies should evaluate the nature of this association and should seek modifiable explanatory factors that could be the target of interventions.

ACP has psychosocial implications for patients, loved ones, and formal healthcare providers, as it requires those involved in the process to consider their own mortality and the possibility of becoming incapacitated. The existing psychosocial complications related to chronic disease and pronounced lifestyle changes within transplant recipients are well documented [40]; however, candidates may experience great difficulty anticipating how their lives will change as a consequence of these variables.

Prospective transplant candidates are confronted with the task of making numerous treatment-related decisions at once. Furthermore, decision-making typically occurs in the context of the multiple stressors associated with the HCT setting. Patients and healthcare providers have much to gain from continued research on patient decision-making and optimal methods of informed consent provision.

The case of MB *– The decision to proceed was relatively straightforward and uncomplicated for MB. HCT appeared the best treatment option, and she had excellent social support, financial resources, and job flexibility.*

Stage II: preparative regimen and recovery of marrow function

Preparative regimen

The second stage of HCT begins with the myeloablative preparative regimen consisting of either high-dose or reduced-intensity chemotherapy, often in combination with total body irradiation. The preparative therapy is administered within a 3–10-day period immediately prior to the transplantation of stem cells in either the inpatient or outpatient setting. HCT-related side effects can be severe and vary as a function of the type and intensity of the preparative regimen. Even with noted improvements in supportive care (i.e. antibiotics and infection control procedures), significant and debilitating side effects can continue after preparative therapy has been completed.

When the preparation and recovery phase of HCT is conducted in the inpatient setting, the primary psychosocial issues experienced include adapting to hospitalization and its routines, developing trusting relationships with professional caregivers, loss of privacy, awaiting engraftment, dealing with complications, and physical malaise even in the best of circumstances. Simple hospital routines, such as vital sign monitoring, are essential for management of the painful and potentially demoralizing side effects of preparative therapy. However, the constant intrusions can be a source of significant irritation to patients accustomed to privacy and control of their immediate environment. Pain and nausea typically are managed aggressively through the use of antiemetic and analgesic medications. Cognitive–behavioral therapy (CBT) also can be used as adjunctive therapy for symptom control, especially with patients who are averse to antiemetic and analgesic medications [41,42]. Some evidence suggests that a single-session psychoeducational intervention designed to enhance coping with treatment side effects can be effective in reducing nausea, fatigue, and anxiety in autologous HCT recipients [43].

Some studies have measured distress experienced during this stage II of the HCT process. When psychological distress was assessed in 50 allogeneic and autologous HCT recipients prior to transplantation [34], a significant percentage of recipients reported clinically significant levels of distress (50%) and anxiety (51%). Coordinators underestimated the number of patients with high levels of emotional distress; this suggests a need for periodic, formal assessment of distress in recipients.

Recovery of marrow function

After completion of cytotoxic preparative therapy, new stem cells are infused. While most recipients experience the infusion of stem cells as simple, anxiety is greatly relieved at completion of this central medical HCT procedure. This phase is characterized by physical vulnerability given the recipient's lack of a functioning immune system. During the weeks after HCT, recipients are monitored daily for evidence of stem-cell engraftment and immune system reconstitution. Since many recipients pay close attention to daily laboratory results, both recipients and family members' moods may fluctuate daily depending heavily on the upward or downward movement of their "counts."

Once the side effects of preparative therapy have resolved, and if no serious infectious complications develop, the recipient enters a period of hopeful waiting. Weakness and fatigue are common. Should serious medical complications develop, however, this quiescent state quickly ends. Recipients often react to medical complications with considerable distress. Furthermore, the number and extent of post-HCT medical complications is a good barometer of the length of post-HCT hospitalization required. A prolonged hospitalization can be demoralizing, especially if a tentative discharge date had been set, only to be postponed. At these times, the psychosocial team can play a critical role by helping the recipient to cope with the stresses, disappointments, and lack of control characteristic of this stage.

Additional psychosocial issues during this early period center on the promotion of continued effective coping with the physical and psychological stresses associated with HCT and management of any emergent psychological symptoms. In addition to anxiety and depressive reactions [44], recipients may experience neurocognitive symptoms or neurological complications [45–47]. Episodes of delirium may occur, lasting several hours to several days. Symptoms are diverse and can include alterations in level of consciousness, altered sensory or perceptual function (e.g. hallucinations), impairments in memory, concentration, and higher cognitive processes, mood swings, impaired judgment, and sleep disturbance [48]. Management of these symptoms is challenging owing to their multifactorial etiology.

HCT recipients are more likely to have psychosocial difficulties associated with prolonged hospitalization, including boredom, loss of control and identity, social and physical isolation, and interrupted sleep. These all may be reduced when HCT is an outpatient procedure. However, different problems may develop for outpatient HCT recipients, including perceived lack of immediate availability from the medical team. Research examining the different psychosocial challenges posed by HCT in the outpatient and inpatient settings may be critical to the provision of appropriate support to recipients and families [49].

Despite the presence of multiple and significant stressors during stage II of HCT, there is some relevant research on this time period. Using the M.D. Anderson Symptom Inventory, Anderson et al. studied 100 patients longitudinally in order to determine the severity of symptoms and develop overall symptom profiles in NHL and lymphoma patients [50]. Reported symptom means were mild at baseline, intensified during conditioning, peaked at nadir, and decreased by day 30. The most severe symptoms reported were lack of appetite, fatigue, weakness, feeling sick, disturbed sleep, nausea, and diarrhea. Different diagnoses, conditioning regimens, and performance statuses at HCT presentation make it difficult to predict severity of symptom patterns for individual patients. Nevertheless, this study is a step towards better management when predictable difference can be assumed with different diagnoses.

Garcia et al. compared early quality of life (QOL) outcomes in 105 autologous and allogeneic transplant patients over the course of hospitalization [51]. Despite clinical differences, autologous and allogeneic recipients demonstrated decrements from all subscales at reinfusion relative to baseline and return to near baseline at discharge, with the exception of the functional wellbeing and BMT (symptom) subscale, which indicated worse QOL for patients at discharge. This observation confirms the significant mean effect of time on nearly all QOL subscales seen in other research. The preparatory and hospitalization stage of HCT is taxing physically and emotionally.

Since psychological distress during the HCT hospitalization period is common, periodic monitoring of distress is recommended with short-term counseling and/or psychopharmacologic intervention utilized if needed. There are significant challenges to the accurate assessment of mood and psychosocial status during this stage since recipients' eating and sleep–wake cycles often are disrupted to some extent, and fatigue and lack of energy are common side effects of intensive preparative regimens. A lack of energy also may be the result of anemia, sleep disturbance, and/or nutritional deficits. Persistent fear and worry also can be a significant energy drain. Often, patients mislabel themselves as depressed, especially if they have no prior experience with how an intensive treatment regimen affects mood and outlook. Healthcare providers must consider the variety of underlying explanations for the significantly depressed or fatigued HCT recipient and prescribe the appropriate treatment. When recipients are medically stable, engraftment has occurred, and blood counts are near normal, they are ready for discharge.

***The case of MB** – MB's major problems during stage II were (1) painful mucositis, controlled for the most part with medication, and (2) confinement to one small area of the inpatient setting, described as "… just about the hardest thing I have ever done."*

Stage III: hospital discharge and early recovery

Hospital discharge to home is beginning of the long process of recovery and life reintegration. There are a number of important issues associated with this period, including coping with anxiety generated by leaving the care of the multidisciplinary treatment team, coping with profound physical debilitation, and adjusting to the need to follow strict self-care behaviors, such as avoiding crowds, designed to minimize the risk of infection. Paradoxically, returning home can also be distressing. The possibility of life-threatening difficulties that might emerge when medical assistance is not immediately available can temper excitement about leaving the hospital. Furthermore, frequent clinic follow-up visits can be inconvenient and yet are crucial during early recovery.

Recipients usually leave the hospital physically and psychologically depleted. Rischer et al. studied sleep disturbance and emotional distress during the acute course of HCT [52]. The prevalence of sleep disturbance was 32% before admission, 77% during the hospital stay and 28% after discharge, with difficulty maintaining sleep being the most intense sleep problem during hospitalization. Not unexpectedly, sleep problems were significantly worse during the hospital time relative to other assessment time points. Increased fatigue, decreased physical functioning, and increased treatment-specific distress also were correlated positively with sleep disturbance. The authors concluded that sleep disturbances for HCT patients may be associated more with subjective physical functioning, fatigue, and treatment-specific distress. Furthermore, the significant number of interruptions, and also the frequent need to go to the bathroom, likely contributed heavily to disruption in the overall sleep cycle.

Physical debilitation and concomitant fatigue are a significant challenge for nearly all patients during early recovery, with patients often reporting generalized weakness and fatigue up to 12 months after HCT [1,53,54]. These symptoms can interfere with resumption of routine activities and valued life roles. The anticipated time line for post-HCT recovery varies considerably [55]. Many recipients and family members often are overly optimistic in their expectations, anticipating a quick return to fully functioning, "normal" life. Frustration, depression, and anger can result when these expectations are not met [16]. Family and friends often are puzzled by the tenacity of post-hospital symptoms and may lose patience, perhaps thinking that the recipient is not motivated.

In order to reduce the risk of infection and regain physical strength, recipients are asked to adhere to specific healthcare behaviors. Patients may be required to take several dozen pills each day with varying schedules. Despite the importance of appropriate self-care after HCT, little research has examined this issue. Our experience suggests that adherence to self-care guidelines can be a significant problem after HCT. We have known of one male HCT recipient who acquired a mortal wound infection after shaving with a forbidden straight razor and one patient who contracted a fatal fungal infection after smoking. Although these are notable examples of non-adherence, less flagrant violations of self-care guidelines are common and can result in equally disastrous consequences. In a unique study examining predictors of non-compliance in HCT patients treated in an outpatient setting, Mumby et al. found that 121 patients (80%) were non-compliant with some aspect of their treatment regimen on one or more days of the study period [56]. Depression, psychological distress, and severe nausea and vomiting all were related to increased non-compliance on items such as oral hygiene. There is some indication that screening might identify those at risk for non-adherence with medication regimens [57].

***The case of MB** – The patient's knowledge of her illness, proximity to the transplant center, and supportive social community helped the transition to the new normal begin smoothly. However, fatigue and short-term memory deficits made returning to the classroom quite difficult.*

Stage IV: long-term recovery

Successful recovery after HCT is characterized by adaptation to the residual biopsychosocial and spiritual effects of HCT, diminishment of physical side effects and symptoms, a return to better psychosocial and physical functioning, and resumption of valued life roles. This process usually begins 3–6 months after HCT and can continue for years [55]. There is wide variability in the "trajectory of recovery" following HCT, with some recipients merging back into "normal living" and others never fully recovering, either physically and/or psychosocially.

Support of HCT patients has grown as specific long-term late effects have been identified as affecting an increasingly large number of patients. Jim, in an article with three separate contributions, identified fatigue and lack of energy as significant problems for patients at all stages of the HCT process [58]. However, fatigue can be most distressing during the immediate post-hospitalization stage and long-term survivorship as patients' expectations for returning to normal are not fulfilled. There are multiple mechanisms that contribute to fatigue and reduce physical functioning in HCT patients. These complex biological mechanisms likely account for much of the source of fatigue. Psychosocial distress is likely a reaction to fatigue rather than a causative factor in its etiology. However, both anxiety and depression may contribute to a sense of psychological fatigue. Pharmacologic and behavioral interventions have been developed to address fatigue and the general physical debilitation that occurs.

Syrjala, in the same joint article [58], summarized three aspects of cognitive and psychological health of recipients following HCT: (1) patients are highly resilient, and most will report competent cognitive functioning and emotional stability; (2) during intensive treatment, the majority of recipients will have some decrements in emotional health and also cognitive functioning; (3) approximately 10–40% of all recipients will report long-term neurocognitive and psychological problems. These general statements are highly dependent upon outcome measures used and the subgroups evaluated. Syrjala noted that the pre-HCT assessment can have significant positive and lasting effects in that it establishes a normative baseline for patients talking about their emotions and can normalize future psychosocial distress.

Thygesen et al. conducted a thorough and systematic review of the literature on the impact of HCT on sexuality [59]. A significant number of patients experience long-term sexual problems, including decreased libido and sexual activity, genital alterations, erectile and ejaculatory dysfunctions, and altered sexual /physical appearance. Women reported a greater number of problems than men across all time points and women were less likely to recover from sexual dysfunction. In general, treatment of sexual dysfunction includes education, hormone evaluation, hormonal therapy where indicated, topical or behavioral strategies, and couples interventions. These conditions typically are under-treated in most settings for a variety of reasons, including patients' reluctance to call attention to such a private area of their lives. This is unfortunate since affectionate relating and physical intimacy serve many purposes, including reducing emotional distress, promoting wellbeing, and providing both partners with a sense of vitality.

Physical and psychosocial late effects of HCT

One of the benchmarks used by recipients to gauge the progress of their recovery is the extent to which they perceive they have "returned to normal" [16,60]. Clinical experience and empirical research both suggest that many, if not most, disease-free HCT survivors will experience the sense that they have returned to some version of normal within the first several years following transplant [1,16]. However, the recovery process may take as long as 3–5 years [55]. What is "normal," of course, is dependent upon each recipient's unique perspective.

After the intense experience of HCT, it is common for even disease-free HCT recipients to recognize that their lives "will never be the same." At the same time, paradoxically, many acknowledge that they have "returned to normal," sometimes referred to as a "new normal." Even with the return of what they might consider a normal lifestyle, few recipients are left unchanged by their HCT experiences. Recipients may evidence a variety of late effects, such as neurocognitive deficits, pulmonary problems, sterility, endocrine dysfunction, and weakness/fatigue [61,62]. Even in the absence of such late effects, most recipients experience some interruption in normal developmental tasks (e.g. maintaining a career and establishing satisfactory family relationships).

Despite the presence of physical and psychosocial late effects, the majority of long-term HCT survivors report relatively satisfactory global QOL [63,64]. Broers et al. found that 90% of their HCT recipient sample reported good to excellent QOL 3 years post-HCT despite the fact that about 25% continued to experience significant functional limitations [65]. Recipients often acknowledge having a life of quality while at the same time experiencing significant physical or psychosocial difficulties. This apparent paradox can be explained partially by some evidence that suggests that patients with a chronic disease may alter their perspective on QOL, establishing a lower ceiling of acceptability and redefining what constitutes "good" QOL [66]. This apparent "response shift," where internal ratings of what constitutes a life of quality can change over the long course of treatment [67]. also might be explained by the potential of the transplant experience to exert a positive impact upon recipients' lives. As in any life-changing event, the long-term impact of HCT may not be uniformly negative. Recipients often experience positive psychosocial sequelae of their transplant experience [68,69].

Bishop summarized findings regarding psychosocial sequelae of both HCT recipients and caregivers at least 1 year post-HCT [70]. She surmised that psychosocial recovery can take longer than physical recovery and, like Mosher et al. [64], named specific long-term psychosocial issues that have been identified by patients as problematic, including distress, depression, anxiety, post-traumatic stress disorder, sexual problems, and cognitive difficulties. She concluded that (1) addressing psychosocial sequelae is important since these issues, especially elevated distress and depression levels, can predict adherence and poorer return of function; and (2) early identification and referral for treatment of psychosocial problems could result in positive outcomes, including better satisfaction with care, improved doctor–patient communication, and fewer complications.

Neurocognitive deficits

Neurocognitive deficits following HCT pose a significant psychosocial stressor for many patients, depending upon the extent of the deficit and how it impairs daily functioning. We saw in the case of MB that memory difficulties resulted in an inability to function as a college professor. Syrjala et al. studied the trajectory and extent of long-term cognitive dysfunction from 1 to 5 years after treatment in 66 allogeneic HCT recipients and matched controls who completed standardized neuropsychological tests [62]. HCT recipients recovered significant cognitive function from the 80-day assessment to 5 years on all tests except verbal recall. Between 1 and 5 years, both verbal fluency and executive function improved but motor dexterity did not, remaining below controls and more than half a standard deviation below population norms. On the global deficit score, 41.5% of survivors and 19.7% of controls had mild or greater deficits.

Survivorship following HCT

Survivorship programs and care plans in general [71], and those specifically designed for HCT recipients [72], have been described as the number of survivors has increased and the recognition of late effects grows. Survivorship programs have gained increasing legitimacy with the proposal to have a specific written or digitized medical history of the patient and recommended health maintenance and surveillance appointments. The survivorship care plan document has been applauded universally and yet evidence to support its influence on outcomes is sparse in the general cancer population [5,6] and non-existent in the HCT literature. Nevertheless, a logical, rational argument can be made for the effectiveness of such information to empower patients to seek the best care possible following such an intensive treatment regimen as HCT.

After reviewing 22 prospective studies, Mosher et al. reported on physical, psychological, and social sequelae following HCT [64]. They found that a significant proportion of HCT recipients experienced persistent anxiety and depressive symptoms in addition to fatigue, sexual dysfunction, and fertility concerns over the long term. Nevertheless, the majority of HCT survivors resumed work, school, or valued household roles. The HCT recipient's life-threatening disease and transplantation can be conceptualized as a psychosocial transition, defined as a major life experience that requires individuals to restructure ways of looking at the world and plans for living in it. As noted earlier, these transitions can present recipients with a paradoxical combination of personal growth *and* deficits in physical, social, and emotional wellbeing. Such a paradox is consistent with the psychosocial transition paradigm offered by Mosher et al..

Long-term QOL and symptoms

Grulke et al. reviewed literature that included a total of 2804 HCT recipients [73]. This comparatively large sample size yields important information. QOL was lowest during inpatient hospitalization. Patients returned to pre-transplant QOL levels at approximately 1 year, yet fatigue, dyspnea, and insomnia remained elevated. The authors concluded that group data may provide general guidelines for patients; however, they cautioned that it is impossible to know for how many patients these predictions will hold true. A host of moderating factors impacts the pace at which QOL returns.

Recognition of psychosocial late effects is achieved best through routine and periodic monitoring. The CIBMTR, the European Group for Blood and Marrow Transplantation and the ASBMT developed screening guidelines for long-term HCT survivors [74]. These guidelines recommend that a mental health provider conduct a psychosocial assessment at 6 and 12 months and annually thereafter, assessing the following: depressive symptoms, psychological distress, sexual functioning, dietary and exercise habits, substance abuse, family functioning, and the adjustment of the primary caregiver. In addition to their impact upon general physical health, certain health behaviors may be linked to the experience of distress and hence may be useful indicators of psychosocial difficulties.

Physical activity and exercise

There is a growing body of research examining the impact of physical activity and exercise interventions on functional and psychosocial outcomes. In some of the earlier studies on structured programs of physical activity, participation by HCT survivors has been associated with better QOL, less distress, and less fatigue [75–78]. Almost all HCT recipients undergo significant physical debilitation due to intensity of the treatment, length of hospital stay, and reduced physical activity, and impairment of daily sleep and eating patterns. Wiskemann and Huber reviewed 15 studies and concluded that physical exercise could be a potentially promising intervention to ameliorate many of these side effects [79]. The most common interventions were isolated aerobic activity during and after HCT. They proposed that physical exercise interventions could affect endurance and strength performance, psychosocial parameters, body composition, and immune system/functions and concluded that there is a need for randomized controlled trials (RCTs) with larger sample sizes. Additional studies indicate that exercise could have a beneficial effect on side effects and HRQOL both during and after HCT [80–83]. One study improved on previous research by studying the effects of a partially self-administrated exercise program [83]. Investigators conducted an RCT where allogeneic patients were placed in either an exercise or social contact control group. Training was conducted in a home-based setting before hospital admission, during inpatient treatment, and during a 6–8-week period after discharge. All exercise participants were asked to participate in three endurance and two resistance training sessions per week, including warm-up and cool-down periods of aerobic activity. Every effort was made to standardize the exercise intervention, particularly during the inpatient stay, and to provide some guidance both prior to and after HCT. The exercise group showed improvements in fatigue, physical fitness functioning, and ratings of global distress.

The Wiskemann study, like others noted, indicates that early and structured physical rehabilitation programs can make a difference for a significant percentage of HCT recipients. The extent to which prior exercise behavior and other factors influence patients' motivation for these programs is unknown. Somerfield and Rizzo [84] called for a large multicenter trial powered sufficiently to answer the question of whether exercise programs in the early period after transplantation can improve both physical functioning and QOL. Questions remain as to whether exercise program benefits endure beyond the initial short-term effects. In the absence of a more elaborate multicenter RCT, the authors concluded that there is enough evidence to support structured exercise programs for HCT participants and that transplant centers could introduce simple, structured, and low-impact exercise programs into clinical practice.

Long-term recovery from HCT is characterized by gradual passage through a series of psychosocial transitions [64,85] dictated by the changing physical and psychosocial concerns of primary importance to the HCT recipient [1,2]. Early in the course of post-HCT recovery, recipients likely are concerned most with surviving and achieving a measure of medical stability. As months and even years pass, recipients focus more on psychosocial issues revolving around sexuality, occupational adjustment, social relationships, fear of recurrence, and coping with physical limitations. Ultimately, most surviving recipients achieve a level of psychosocial equilibrium and move on with their lives [85].

The case of MB *– 18 months after HCT, MB learned to accept her new normal, which included chronic pain, reduced stamina, and low energy relative to her pre-HCT state, permanent disability due to cognitive changes, and the high cost of maintenance medication. Yet, her depressive symptoms responded to counseling and her general outlook on life improved.*

Psychosocial Issues in HCT: informal caregivers

Informal caregivers are the family members, friends, and acquaintances who often serve as an "essential extension" of the family and healthcare team [86]. Throughout the transplant and recovery processes, they can offer emotional and logistical support and motivation, and help with daily tasks of living and even the provision of medical assistance. At the same time, most caregivers continue to balance other life roles, such as parenting or elderly care, work, and home management. The informal caregiving role may extend for a significant length of time post-transplant and even change the very nature of the caregiver–HCT recipient relationship [87]. Some preliminary data suggest that the presence of a caregiver during HCT hospitalization is linked to better survival during the first year post-HCT for recipients [88]. Even if supportive caregiving does not influence quantity of survival, it certainly affects its quality. This section highlights current research on these unsung heroes.

There are several important developments in the literature on HCT caregivers. Researchers have tried to identify the specific needs of HCT caregivers, document the nature and consequences of the burden they experience over time, perform interventions to ease burden, and examine caregivers in inpatient versus outpatient settings.

Beattie and Lebel investigated the emotional impact and high distress reported by caregivers [89]. It is not surprising that distress among caregivers was highest prior to transplant and decreased over time, since the informal caregiver is exposed to many of the same psychosocial stressors as recipients themselves. The vicarious experience of watching a loved one suffer can be profoundly difficult. Wulff-Burchfield et al. highlighted numerous caregiver issues that may persist long after the transplant process, including psychological symptoms (e.g. anxiety and depression), physical symptoms (e.g. fatigue and weight loss), and persistent familial, marital and social structure changes [86]. Typically, the bulk of the care and support are centered on the HCT recipient. Unlike the HCT recipient, however, caregivers usually are not permitted to assume the "sick role" that permits dependence upon others for daily assistance. On the contrary, caregivers often experience the imposition of increased expectations by the HCT recipient and professional health team.

Caregivers report distress levels that are equivalent to or even higher than those reported by patients [89]. Variables associated with distress include female sex, higher patient distress levels, and higher perceived levels of burden. Caregivers reported fears related to the future, uncertainty, the need to balance personal needs with those of their loved ones, and difficulty with role transitions. Alongside these concerns were positive aspects of being a caregiver, including personal growth and positive relational changes. In a study of 56 caregiver and allogeneic transplant recipient dyads, caregivers reported the most difficulty relative to tending to the emotional health of their loved ones as well as talking about the future and the possibility of death [90]. As strain increased in any life domain, QOL correspondingly decreased. QOL also was related positively to predictability in their caregiving, highlighting the importance of caregiver preparedness, clear information provision, and predictability, when possible. Similarly, in a study of 58 caregivers, the most common needs were those related to information, particularly regarding home care after discharge and diagnosis, fear, and leisure activity deficits [91]. Together, these results highlight the high distress levels experienced by caregivers alongside positive aspects of this active process.

Comparison of the HCT caregiver with healthy controls or normative data also places the status of the HCT caregiver in context. Langer et al. studied 131 spouse caregiver–recipient dyads from pre-transplant through 24 months post-HCT and incorporated norm data from a sample of healthy controls [92]. While negative affect declined over time among both caregivers and recipients, caregivers reported more depression and anxiety relative to recipients and controls. Although similar levels were reported pre-transplant, caregivers reported lower levels of marital satisfaction relative to the recipient at 6 and 12 months post-transplant. Similarly, Bishop et al. compared current HRQOL and post-traumatic growth in 177 spouse/partner caregivers and HCT recipient [roughly 2–19 years post-transplant (mean = 6.7 years)] dyads and 133 recipient-matched, healthy controls [7]. Caregivers and healthy controls reported better physical health than HCT recipients. However, both partners and recipients reported more depressive symptoms and sleep and sexual problems than controls. Caregivers reported less social support, dyadic satisfaction, and spiritual wellbeing and more loneliness than *both* recipients and healthy controls. Furthermore, caregivers reported less post-traumatic growth than recipients. While reporting better physical health status, caregivers reported equal or significantly worse psychological and social status relative to HCT recipients.

Demonstrating the possibilities in interventional work, Langer et al. developed an RCT based on an expressive talking paradigm for the partners of HCT survivors, measuring physiologic, subjective, and expressive variables related to disclosure of their deepest feelings about their partners' transplant experiences [93]. Relative to controls, participants endorsed more negative emotion and more negative emotion words, and believed that the exercises were more meaningful and useful, suggestive of more emotional processing. Even though participants rated the emotional expression exercise more "emotionally effortful," they also described it as meaning-laden and helpful.

The trend towards the provision of HCT care in an outpatient, ambulatory setting has led to a need to identify how this movement affects caregivers [94]. For outpatient models of HCT care delivery to be successful, the presence of a suitable caregiver is essential. Caregiver outcomes therefore must be important considerations in the evaluation of care delivery settings. From pre-transplant to 12 months post-transplant, Grimm et al. compared the emotional responses and needs of 26 HCT caregivers from an inpatient setting with 17 caregivers from an inpatient/outpatient setting [95]. Few differences were found in caregiver distress between the settings, yet some evidence suggested that the inpatient/outpatient setting might be associated with less caregiver mood disturbance. Given the small sample size and the lack of random assignment to setting, the results warrant replication.

In summary, informal caregivers are essential team players for recipients and the healthcare team. The available research suggests that the caregiving experience in the HCT setting can be physically, emotionally, socially, and financially stressful. Examining caregiver outcomes based on inpatient or outpatient setting will continue to be important as the most efficient models of care delivery are developed. Additional studies are needed on the unique needs of homogeneous caregiver groups (e.g. spouse vs. friends), both the intrapersonal and interpersonal (i.e. dyadic) experience of caregiving, changing needs over time, and the incorporation of theoretical frameworks into caregiver research [89].

Conclusion

HCT is an intense treatment procedure that can extend over many weeks, with side effects lasting months to years after the initial conditioning and recovery period. It is utilized for patients who differ by disease site, age, gender, performance status, amount of previous chemotherapy, and coping and supportive resources. Hence broad generalizations about the experience of recipients and caregivers have limited utility. Nevertheless, the basic experience is similar for nearly all HCT recipients over the trajectory of care beginning with decision-making and culminating in long-term survivorship. Even with continual improvement in medical treatment regimens and supportive care, HCT remains a life-threatening treatment for life-threatening diseases that can produce a range of serious physical and psychosocial morbidities including ongoing fear of cancer recurrence [96,97]. We have summarized the important psychosocial issues that arise over the course of transplantation. There is a pressing need to develop simple interventions to help recipients and their caregivers adapt to the ongoing challenge of survivorship across the trajectory of treatment and recovery over the course of a lifetime.

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CHAPTER 33

Assessment of Quality of Life in Hematopoietic Cell Transplantation Recipients

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Introduction

Health-related quality of life (QOL) has been well-described in myeloablative hematopoietic cell transplantation (HCT) patients from pre-transplant through at least 10 years after transplantation [1–7]. As a result, much is known about QOL outcomes over time for myeloablative autologous and allogeneic sibling and unrelated transplant recipients within and across many diagnoses. The central consistent findings of these studies are that (1) physical function returns to pre-transplant level for a majority of survivors by 1 year, (2) by 3 years, 75–90% of survivors are back to full-time work, and (3) the majority of patients navigate the challenges of HCT with good psychological health. Although the QOL outcomes of non-myeloablative HCT recipients have been less well described, research indicates that symptoms and overall QOL during acute treatment is poorer for those receiving myeloablative versus non-myeloablative or reduced-intensity treatment [8,9], whereas QOL is comparable in the long term for those who are disease free after either transplant type [7,10]. Notwithstanding these generally positive outcomes, a majority of patients will have residual problems in specific domains of QOL such as fatigue, sexuality, fertility, and musculoskeletal symptoms. Other transplantation QOL outcome comparisons are not as clear, such as comparison of umbilical cord blood with other types of HCT outcomes, long-term QOL after HCT for multiple myeloma, or non-malignant diseases other than anemias.

Excellent measures and methods for evaluating QOL have been validated with HCT patients. Population-normed instruments are available for comparison with HCT-related outcomes. Computer-adapted online assessments are validated that facilitate routine, rapid assessment and provide immediately accessible results, enhancing the potential utility of QOL assessments for clinical care.

Progress has been made in previously under-considered groups affected by HCT. Research has described pediatric outcomes, caregiver impacts, and QOL in patients living with chronic graft-versus-host disease (cGVHD). Despite progress, a dearth of research remains in two areas: clinical trials to improve QOL outcomes, and QOL outcomes research following relapse after HCT. In addition, research is lacking in documenting outcomes for bereaved families of patients who die.

This chapter summarizes what is known about QOL during and after HCT, and considers assessment issues necessary to understand when interpreting results or doing research that includes QOL outcomes. Other chapters discuss the application of QOL within outcomes research, in the psychosocial domain, and with sexual problems (see Chapters 28, 32, and 34).

Definition of health-related QOL

Most QOL researchers would concur that QOL refers to “the extent to which one’s usual or expected physical, emotional, and social wellbeing are affected by a medical condition or its treatment” [11]. Elements essential in any definition of QOL are *multidimensionality* and the *patient’s perspective*. With time, QOL has grown to encompass not only subjective evaluation, but also those behaviors that may be reported by patients but go beyond subjective experience to include functional impact in addition to activity reports. Whereas medical outcome studies often define outcomes in terms of peak toxicity or time to an event such as relapse or death, QOL is usually described in relation to non-transplant controls or as a change from pre-transplant status. A central tenet is that QOL is *relative* to the current situation and the *expectations* of patients within the circumstances in which they find themselves. As an example, patients with major toxicities during transplant can report good psychosocial QOL and satisfaction with QOL because they believe they are doing as well as possible in a difficult situation, and they have the support they need from people important to them [5]. Despite this subjectivity, QOL correlates with medical, functional, and observational outcomes including both severity of cGVHD and comorbidities [12–15]. However, clinician ratings and toxicity scoring do not substitute for patient reports in evaluating changes in QOL during and after HCT [16].

An important element in QOL outcomes research is the concept of patient-reported outcome (PRO). This concept has been instituted by the US Food and Drug Administration (FDA) for medical product development and labeling [17]. PRO measures include reports of disease symptoms, treatment of adverse effects, functional status, or overall wellbeing [18]. A useful guideline for determining appropriateness of a PRO is offered by Lipscomb et al.: “the concept measured by the PRO instrument should be relevant and specific to the population, condition, and treatment to yield clinically valid and useful information” [19]. This definition is somewhat narrower than that of QOL and linked more explicitly to medical outcomes. However, the term “PRO” is being broadly accepted as covering aspects of health status evaluation that come directly from those individuals being evaluated, without modification or interpretation by another observer. It seems likely that, over time, PRO will replace QOL as a broad term and end-point of clinical trials, given its

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importance to medical treatment and potentially to reimbursement decisions. Indeed, PRO data may even be more useful as a clinical trial end-point than conventional toxicity information in some cases [20]. However, components of QOL not directly linked to medical decision-making, such as spirituality or social function outcomes, impact on caregivers, and return to work, will always be of interest to patients and families preparing for treatment, recovery, and long-term survival needs.

Dimensions of QOL

Concepts of QOL continue to adhere broadly to a three-level "pyramid" first described by Spilker [21]. He tops the pyramid with global assessment of wellbeing. The middle level includes multidimensional assessment of the physical, psychological, economic, spiritual, and social domains. The base details subcomponents of each domain; for example, work and symptoms are aspects of the physical domain. Other subcomponents include social and recreational activities, sexual function, relationships with the medical team, religion, perspective on life, personal control, and components of health and treatment that contribute to stress or worry for the patient. Ferrell et al. [22] interviewed 119 adult bone marrow transplant survivors at least 100 days post-transplant to determine what QOL meant to these patients. They categorized the many positives and negatives noted by survivors into themes and then into four domains matching those of Spilker: physical, psychological, social and spiritual/existential wellbeing. Consistent with these definitions, the multidimensional QOL measures used in most HCT studies include physical, mental, and social function domains, in some cases with the addition of items specifically relevant to HCT symptoms and other areas of interest (Figure 33.1).

Smith et al. found that patients perceived their health status and QOL as distinct from each other [23]. Physical function weighed more heavily in assessments of health status, whereas emotional health weighed more heavily in judgments of QOL. Social function was not prominently considered in either global rating. Consistent with this emphasis on health outcomes, medical studies incorporating a QOL assessment have successfully used the shortened version of the Functional Assessment of Cancer Therapy – BMT Module (FACT-BMT) with only the physical wellbeing, functional wellbeing, and transplant-specific items. This abbreviated measure, called the Trial Outcome Index, is useful in studies when targeted outcomes are focused on physical function and symptoms [24,25].

Researchers have documented resilience as they recognize that most patients with cancer and those receiving HCT do very well in the psychological domain. Four concepts examined are spirituality, optimism, benefit finding and "post-traumatic growth." In essence, the ability to find positive meaning beyond the diagnosis and to perceive gains in addition to losses in the disease and transplant process predict better long-term adaptation [4,26–28]. Most often gains are reported as greater appreciation for life, closer interpersonal relationships, reprioritizing "what really matters," feeling closer to God, inner strength, and a sense of peace and thankfulness [4,22,29,30]. Although these concepts do not appear in most health-related QOL measurements, they are usually noted as important outcomes by patients, clinicians, and researchers examining qualitative outcomes of HCT [31,32].

In summary, QOL is always evaluated from the patient's perspective. It is rated in part by internal comparisons with how much worse things could be. Thus, patients with poor physical health may report good psychological or overall QOL. Increasingly, studies are measuring optimism, benefit finding, spirituality, and resilience as

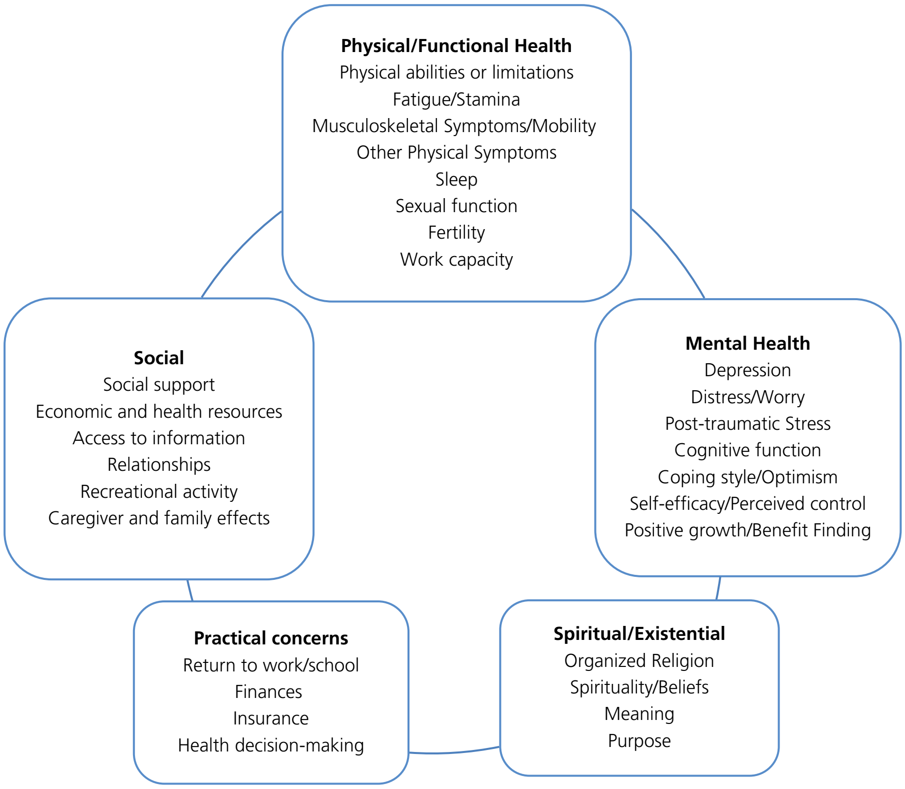
Physical/Functional Health
Physical abilities or limitations
Fatigue/Stamina
Musculoskeletal Symptoms/Mobility
Other Physical Symptoms
Sleep
Sexual function
Fertility
Work capacity
Social
Social support
Economic and health resources
Access to information
Relationships
Recreational activity
Caregiver and family effects
Mental Health
Depression
Distress/Worry
Post-traumatic Stress
Cognitive function
Coping style/Optimism
Self-efficacy/Perceived control
Positive growth/Benefit Finding
Practical concerns
Return to work/school
Finances
Insurance
Health decision-making
Spiritual/Existential
Organized Religion
Spirituality/Beliefs
Meaning
Purpose

Figure 33.1 The principal dimensions of quality of life, and elements of each dimension that have been evaluated in HCT.

outcomes that may predict both mental health and medical outcomes. Although many dimensions of QOL can be considered for evaluation, physical and mental components are nearly always included.

Phases of HCT and related risk factors for QOL outcomes

Transplantation is a dynamic and individual experience. While consistent patterns can be described, a patient will have his or her own trajectory of illness and recovery depending on both medical and psychosocial factors. Research has more recently been examining QOL changes over time based on different treatment types, complications such as cGVHD, and baseline QOL domains [1,16,33–36]. Although 60–80% of transplant survivors return to pre-transplant levels of function and QOL by 6 months to 5 years post-transplant, research consistently finds that survivors' average overall QOL and function within specific domains remain lower than for age-matched population norms [35]. The majority of this dysfunction seems to result from clinically meaningful impairments in 20–33% of survivors.

There are two methodologic concerns in past research that contribute some instability to QOL results across studies. One is that much of the research is cross-sectional, clustering patients with widely varying times since transplant and diverse diseases or treatments. Another is the range of assessment instruments used to evaluate QOL outcomes, which prevents ready comparison across studies. Nonetheless, outcomes are more similar than different, lending confidence to the robustness of QOL results.

Pre-transplant

Patients arrive at transplant with diverse diseases, histories of treatment, levels of physical function, and psychosocial, informational, and financial resources. The conclusion from numerous studies is that pre-transplant physical condition and psychological status predict later physical and psychologic adaptation in addition to survival [1,35,37,38]. Psychosocial predictors of outcome have received substantial attention, although data indicate that pretreatment physical QOL not surprisingly also predicts transplant outcomes [1,5,35,39]. Emotional distress predicts acute transplant symptom course, especially for pain and distress [6,40,41]. Psychosocial needs are greatest just prior to transplant if judged by the percentage of patients with clinically significant anxiety or depression at different phases of treatment and recovery and their improvement after HCT [1,5,34]. These replicated findings support guidelines calling for routine assessments of distress and psychosocial function [42,43].

Acute treatment

During the immediate post-transplant weeks, assessments find that survival is the axis around which other aspects of QOL are evaluated. In consequence, patients attribute their experiences to physical rather than psychological causes during this phase [5]. Patient focus is dominated by mouth pain, nausea, fatigue, hair loss, infection risks, adhering to treatment requirements, and efforts to maintain self-care. Research supports lower toxicity, with fewer symptoms, during acute treatment for those receiving reduced-intensity conditioning [44,45]. As would be expected, patients with acute graft-versus-host disease (GVHD) have measurable declines in QOL compared with those not experiencing acute GVHD [9,24,35,39]. A recent evaluation of QOL in the first 100 days post-transplant indicated that symptom severity during this period is significantly correlated with QOL [8]. As hospitalization has shortened and treatments for acute toxicities have improved, investigations have increasingly moved from a focus on acute QOL to recovery and long-term outcomes.

Recovery

Physical and overall QOL return to pre-transplant QOL on average in 6 months to 1 year after HCT [1,34,35]. Although data are mixed on the recovery course for allogeneic compared with autologous transplant recipients in the year after HCT, with some finding that autologous recipients have better QOL in all domains to the first year after transplant compared with allogeneic transplant recipients [8,46], others have found that any discrepancy in QOL based on transplant type disappears by 1–2 years post-transplant [1,3]. Different aspects of psychological recovery occur at different time points. Whereas depression and clinical anxiety recover on average by 1 year after HCT, distress and emotional adjustment to the impact of diagnosis and treatment can be a longer process for many survivors, taking 2–5 years, in part depending on long-term complications such as cGVHD [1,35]. Since populations receiving reduced-intensity conditioning have generally not been eligible for high-dose conditioning, it is difficult to compare QOL and function across the two groups. Nonetheless, one prospective study compared the QOL of patients receiving reduced-intensity conditioning and myeloablative HCT, and found that both groups returned to baseline QOL by 2 years after transplantation [47].

Multiple studies have reported that the following risk factors predict a slower or poorer return of function: physical health pre-transplant, depression, psychological distress, lack of social support, female sex, myeloablative conditioning, specific diagnoses, decline in body mass index, and medical complications during treatment and recovery, in particular cGVHD [1,5,6,39,48]. Older adults have more difficulties with medical recovery from high-dose treatment and are less likely to return to work or social activities; however, they have better social function after HCT [3,6,34,35,39,49]. Risk of cGVHD or other medical sequelae increases with age, and active treatment for complications is associated with poorer QOL. Overall, while medical risks vary significantly with age, QOL is noteworthy for a lack of major differences based on age independent of the higher medical risks that accompany age.

Although survivors have slower recovery in the years following transplantation if they struggle with cGVHD, or have pulmonary disease, immune compromise, or other medical complications [6,49–53], once these medical complications resolve, QOL generally improves to levels equivalent to those of patients who never had them [3,9,24,52]. Similarly, patients receiving allogeneic transplants may have more physical and emotional difficulties in the first year, but by 12 months, physical and psychological difficulties do not differ between types of transplant [1,3].

Return to work justifiably receives a good deal of attention in recovery and long-term function reports. Although 30–60% of survivors begin to return to work by the end of their first year post-transplant, return to full-time work continues to occur for patients into the third year [1,54,55]. Fortunately, by 3 years post-transplant, studies agree that 80–90% of survivors have returned to full-time work or school [1,6,55–59]. Interestingly, although the pace is slower, overall rates of return to work for HCT recipients are similar to those in patients with other cancers [59]. It appears that rates of return to work may differ to some extent by the country in which a patient lives, perhaps as a result of the different economic incentives or rehabilitation programs available [60,61].

Long-term function and late effects

Longitudinal and cross-sectional studies find that, by 5 years, a large majority of adult survivors of HCT are functioning well in their return to "normal" life in the domains of physical, psychological, social, existential, and overall subjective QOL [2,4,7,34,35,39,57,62]. However, specific deficits remain at rates higher than those of the general population. Long-term survivors report more accumulated health problems, fatigue, musculoskeletal dysfunction, more limitations in sexuality and social function, and greater infertility concern than case-matched controls [2,4,9,46,58,63–66]. Persisting cGVHD continues as the major predictor of poorer overall health and QOL [35,39,53]. Otherwise, these recent reviews conclude that strong evidence supports poorer long-term outcomes for those with worse physical functioning, emotional distress, depression, or lower social support before HCT. Women consistently report poorer psychological outcomes on average and are less likely to return to work.

Studies of adults indicate that 20–56% enter transplant with cognitive deficits that could interfere with function [67,68]. Longitudinal research has reported neurocognitive deficits up to 5 years after HCT in about 40% of allogeneic survivors compared with about 20% of non-transplanted case-matched adults, although most deficits are in the mild range [69–71]. A recent meta-analysis found no sustained changes from before to after HCT in survivors [72]. Although no single risk factor consistently predicts sustained neurocognitive deficits, cumulative risks do seem to result in poorer cognitive function [73].

Results are inconsistent in terms of whether long-term survivors have higher rates of psychological dysfunction than normative rates for the general population, although studies agree that 67–85% do well [2,4,35,39]. Survivors do report other problems, including infertility concerns, especially if they are childless, more difficulty obtaining life and health insurance coverage (although actual rates of coverage are comparable to rates in control groups), and long term financial concerns [2,57,74]. Longitudinal studies beyond 10 years are needed to determine whether aspects of QOL decrease more rapidly over time in HCT survivors relative to a naturally aging population since survivors remain vulnerable to late medical complications and mortality risks.

In summary, the physiological and psychological phases, impacts, and risk factors after HCT have been well defined. Common problems post-transplant include fatigue, musculoskeletal complications, cognitive difficulties, sexual dysfunction, infertility concerns, and efforts to manage continuing uncertainty and fear.

Pediatric QOL

Approximately 30% of children have elevated distress before and during treatment, with declining distress in the 6–12 months following HCT [75–77]. Risk factors for high levels of distress and low QOL in childhood survivors include receiving a transplant from an unrelated donor, increasing age, lower socioeconomic status, and Asian race, with African American children reporting higher QOL [78–80]. By 1 year post-transplant, both mothers and children report good QOL [76,79], and most long-term survivors lead independent, productive lives in adulthood although not without complications [81–83]. Sanders et al. found that physical functioning was poorer both for those with myeloid diseases and females, with depression more common in females, consistent with adult findings [83]. In short, research testifies to the resilience of children not unlike the resilience of adults, but not without psychological distress, need for social adaptation, cognitive limitations, and other long-term complications in a substantial minority. The restriction to overall positive outcomes is that it has not been possible yet to track many pediatric survivors past their twenties since cohorts of mature adult survivors of pediatric HCT remain small in number.

Caregivers and parents of transplant recipients

As central a role as families play both during and after HCT, research on family caregivers is only recently making headway in evaluating their QOL needs. Whether before, during treatment, or long term after HCT, QOL in caregivers is low compared with norms and their patient counterparts [84–88]. The stressful impact of supporting a loved one through HCT can last years beyond the active caregiving phase, with caregivers retaining recollection of more negative changes than survivors [84,89].

Parents of pediatric HCT patients report significant distress in the course of their child's treatment. Although some studies report that mothers of pediatric HCT patients suffer the greatest trauma in the course of their child's treatment [90], other researchers find that mothers and fathers both report significant distress symptoms [91,92]. Younger mothers and those experiencing more depression or anxiety at the time of HCT are more likely to continue to be distressed 18 months later [90]. Other caregivers manage remarkably well from early evidence, despite their levels of acute distress. Further research is needed on QOL assessment of caregivers of HCT patients, both during and after transplant.

QOL measurement

Table 33.1 lists measures commonly used to assess QOL components in HCT. Although QOL data can be rich and informative, inadequate appreciation of data collection requirements necessary for high-quality data and the importance of minimizing non-random missing data have limited the clinical value of many early QOL studies. As important as selecting the appropriate measure is the need to plan the approach to patients, retention for follow-up to maintain high response rates, quality control procedures to assure all items are completed unless intentionally skipped, and the statistical methods to account adequately for missing data whether through imputation or inclusion of unconditional analyses. When presenting results in studies with high rates of attrition through mortality or other non-random causes, it may be necessary to provide both conditional and unconditional results, i.e. report the probability of a surviving patient indicating a positive outcome (conditional analysis, often a high proportion as described in the review above) and the probability of a patient surviving *and* indicating a good outcome (unconditional analysis, which may be a much lower proportion) [93]. Wong et al., using pattern mixture modeling to represent those lost through various causes, demonstrated the increased likelihood that those with poorer QOL outcomes will be missing in conditional analyses [34]. Chapters 27 and 28 provide more information on considerations and methods in analyses of QOL outcomes.

In selecting measures of QOL in HCT recipients, the ideal is to use measures with established norms, reliability, and validity in HCT cohorts. In addition, the availability of broader population norms is valuable to permit a comparison of HCT results with those of other groups. However, the use of generic measures tested across broad populations also has a downside. There is an inherent trade-off in benefits of generic versus specific QOL measures. HCT-specific measures such as for cGVHD effects may be more sensitive, specific, and

Table 33.1 Selected QOL measures used in HCT

Relevant measures tested in HCT[a]	HCT specific	No. of items	Scores
Multidimensional QOL			
EORTC-QLQ-C30 (European Organization for Research and Treatment of Cancer Quality of Life Questionnaire – C30)	Yes	30	9 subscales, no total score
EORTC QLQ-HDC29, HCT Module		29	HCT module score
FACT-BMT (Functional Assessment of Cancer Therapy – BMT)	Yes	27 + 18	5 subscales, BMT module score, overall score
Trial Outcome Index (TOI)		37	(TOI includes 3 subscales)
SF-36 (Short Form 36 Health Survey)	No	36	8 subscales plus physical and mental components, no total score
EQ-5D	No	5 + 1	Summary index and visual analog scale health state item
PedsQL (Pediatric Quality of Life Inventory)	Yes	23	4 subscales, total score and physical summary, psychosocial summary
Transplant Module			
Versions for 3 age groups and parents		46	8 subscales
Child Health Ratings Inventories (CHRIs)	Yes	38	4 subscales, overall score,
HSCT Disease-Specific Impairment Inventory		23	HSCT inventory
Versions for 3 age groups and parents			
Symptoms			
M.D. Anderson Symptom Inventory (MDASI)-BMT	Yes	23	2 subscales, total score
Rotterdam Symptom Checklist (RSCL)	No	38	3 subscales, global score
Muscle and Joint Measure (MJM)	Yes	4–33	4 subscales, total score 4 screening items
Brief Pain Inventory (BPI)	No	10	2 subscales, severity, interference, no total
Brief Fatigue Inventory (BFI)	No	9	Total score
Fatigue Symptom Inventory (FSI)	No	14	2 subscales, total score
Pittsburgh Sleep Quality Index (PSQI)	No	18	7 subscales, global score
Mental health, emotional wellbeing			
BSI (Brief Symptom Inventory – Short Form)	No	18	3 dimensions, global severity
CESD (Center for Epidemiologic Studies – Depression)	No	20	Total score
CTXD (Cancer and Treatment Distress)	Yes	22	6 subscales, total score
HADS (Hospital Anxiety and Depression Scale)	No	14	2 scores
PHQ-9 (Patient Health Questionnaire-9, Depression Scale)	No	2–9	Total score, 2 screening items
Coping style			
Brief COPE (Coping Orientations to Problems Experienced Scale)	No	28	14 subscales in 3 groups; can select from subscales
Social function			
MOS Social Support Survey (Medical Outcomes Study)	No	19	4 Subscales, overall index
Spiritual, existential wellbeing			
FACIT-SP (Functional Assessment of Chronic Illness Therapy – Spirituality and Wellbeing Scale)	No	12	Total score
Sexuality			
SFQ (Sexual Function Questionnaire)	Yes	40	9 subscales, overall score, medical impact score

[a] Updated measure information is available from websites for the measures listed by searching for the measure name.

informative but are then only relevant to HCT-specific problems and cannot be compared with other cancer or general population norms.

In QOL measurement, a "response shift" is expected as patients' conditions change medically. Patients naturally reprioritize concerns, and self-report is, by human nature, relative to expectation rather than being tied to medical status. In the transplant process, health may decline, but many patients still report good psychological function [5,94]. Another factor that can shift response is the changing relevance of assessment dimensions. While physical symptoms may be the central focus during acute treatment, focus shifts in long-term follow-up to functional abilities and emotional adaptation [33]. This is a major reason why excellent measures in one circumstance may be of less value at a different time point.

Clinical utility of QOL data

Although the collection of QOL data is frequent in research settings, the frequency of its use in clinical settings is increasing with the FDA and national guidelines recommendations for PRO integration in clinical care [14,42,43,95,96].

One important component of usability is the definition of a "clinically meaningful difference" for a measure. Studies with large sample sizes may find high levels of significance between groups with small, clinically insignificant mean differences. Several strategies are being used to overcome the risk of spurious significant findings based on small differences between large cohorts. Accepted distribution-based strategies consider a meaningful difference to be a change in scores or a difference between groups of one-third to half a standard deviation of the observed scores [97,98]. Increasingly, Cohen's *d* is utilized with continuous data to determine the effect size, that is, the strength of differences between groups or change from small (≥0.2), or medium (≥0.5) to large (≥0.8) based on the calculation of the difference between the means of two groups divided by the standard deviation of the control or comparison group, or the pooled standard deviation of the two groups [99]. Other clinically validated strategies to determine differences between groups is to use anchor or cutoff point scores defined within specific instruments. The EORTC QLQ-C30 defines cut point differences of 5, 10, and 20 points as small, medium, and large effects, respectively [100]. A recent meta-analysis has provided interpretation guidelines for the clinical significance of mean differences on the FACT-G, with about 2 points on subscales indicating small clinically meaningful differences and 5 points on the total score indicating clinically meaningful differences [101]. Similarly, the PHQ-9 depression measure defines "mild" depression as scores of 5–9, "moderate" as

10–14, "moderately severe" as 15–19, and "severe" as 20 or above. These cut point scores translate to treatment recommendations, with 10–14 supporting consideration of treatment and 15 or above indicating that consideration of pharmacologic and/or non-pharmacologic treatment is probably warranted. The scores can be used in research to define eligibility for study entry or as a pre-set indicator of treatment efficacy.

Monitoring and discussion of QOL with HCT recipients have the potential to increase patient involvement in care, reassure patients, and improve patient satisfaction. Use of QOL assessments can improve physician–patient communication and enhance patient QOL outcomes [14,102]. Good-quality QOL data specific to a patient's type of treatment, gender, and other risk factors can aid patients in making informed treatment choices [103].

Generic measures

There are three QOL measures routinely selected when providing a "generic" assessment of cancer-related QOL outcomes: the Functional Assessment of Cancer Therapy – General (FACT), the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire C30, and the Short Form 36 Health Survey (SF-36), which is not a cancer-specific measure. One or more of these is commonly used in most cancer QOL studies. Each of these well-standardized, reliable, and valid measures has some questions in common with the others and also asks some unique questions. Each has been used effectively in HCT research. The FACT assesses physical, social/family, emotional, and functional wellbeing. The EORTC, in contrast, has a more explicit focus on functional abilities, what a patient is able to do physically, and specific symptoms, and a rather brief psychological review. There is no psychometric evidence to recommend one of these measures over the other; rather, differences in scale structure and content of the questions, and the measure best suited to the study hypotheses, are important to consider when selecting between these two cancer-specific QOL assessment tools [104,105]. The SF-36, meanwhile, is a health-related QOL measure not specific to disease [106]. Its evaluation of physical ability corresponds most closely to that of the EORTC [107]. It provides a brief assessment of psychological function, and it has minimal emphasis on physical symptoms. Given its general health orientation across populations and lack of symptom evaluation, the SF-36 is appropriate for use in the years post-transplant as issues become less HCT symptom-specific.

Research has documented that these QOL measures are correlated but do not share enough variance to be considered equivalent [107]. Investigators find that the instruments have analogous global health items (correlation $r = 0.82$), but the physical, psychological and social dimensions of the EORTC and FACT share only limited variance [104,107]. Luckett et al. provided a detailed comparison of the two measures [104].

Each of these measures has normative information on use with HCT. Each has translations into multiple languages, and all are appropriate to capture issues faced by HCT patients. These scales have particular value for their abilities to provide a normative reference for a sample or an individual patient. For comparison, Table 33.2 lists the subscales for these three QOL measures.

HCT-specific measures

Both the FACT and the EORTC have add-on modules that are specific to HCT. The FACT-BMT asks questions specific to treatment. It does not evaluate physical abilities per se. The BMT module of added questions contains items that elaborate the more common HCT symptoms and concerns [108]. The EORTC also has an HCT-specific module, the EORTC QLQ-HDC29, which measures side effects and HCT-specific issues [109].

Table 33.2 Subscales of the most widely used adult global health-related QOL measures, reliability and validity established in HCT populations

	EORTC QLQ-C30	FACT-BMT	SF-36
Subscales	Physical functioning Emotional functioning Social functioning Role functioning Cognitive functioning Global quality of life Pain Nausea/vomiting Fatigue EORTC QLQ-HDC29	Physical wellbeing Functional wellbeing Emotional wellbeing Social/family wellbeing BMT module	General health Physical function Role – physical Body pain Vitality (inverse of fatigue) Mental health Role – emotional Social function
Summary scores	Subscales only	Total score and subscales	Physical composite T-score Mental health composite T-score
Number of items	30 in core 29 added in EORTC QLQ-HDC29	27 in core 18 added in BMT module	36

Since generic measures may not have the sensitivity to capture the factors of interest in a clinical trial, measures specific to hypothesized outcomes may need to be added to a generic tool. Considering symptom and function outcomes helps to clarify this point. Patients can be assessed for general physical function with a measure such as the EORTC or SF-36, but if an intervention is targeted explicitly to reduce fatigue these scales may not have the sensitivity to capture outcome changes, although both the FACT and SF-36 have been found to be sensitive to the effects of cGVHD [16].

A construct central to cancer QOL and HCT specifically is the concept of disease- and treatment-specific distress: what are the worries, stresses, or fears specific to a patient having a transplant that contribute to how he or she is doing? Studies find that HCT-specific distress is a better predictor of pain, nausea, and stress responses during acute treatment than is depression or other general mood indicators [40,41], while depression is a better predictor of long-term outcomes [1].

Two US National Institutes of Health resources can be great assets to investigators wishing to select measures specific to a QOL construct. The Patient-Reported Outcomes Measurement Information System (PROMIS) initiative provides a database of standardized and effective PRO measures including such outcomes as fatigue, pain, emotional distress, physical function and psychosocial function. The Grid Enabled Measures (GEM) Database through the National Cancer Institute provides a web-based platform for searching for standardized measures within QOL constructs such as physical function, anxiety, depression, or social relationships. By searching in GEM, not only can an investigator identify relevant measures, but the site also offers descriptions, the targeted populations, and psychometric properties such as reliability and validity. Both of these sites are open to the public through an online search.

QOL measurement methods

Given the large number of QOL measures translated for age and language, it should be possible for the majority of patients to complete a general QOL measure appropriate to their needs in writing or online if they can read. The gold standard for QOL assessment is a patient completing written or computer assessment with a staff person who is available to answer questions and to assure that the patient is competent and responding as instructed, and also to review responses and correct skipped or double responses while the patient is present. Alternatively, most measures can be administered by mail, orally, and by telephone if necessary. Testing of QOL assessment across modalities with HCT or other cancer patients substantiates the transferability of written measures to a tablet, computer, or mobile device [110–112]. Measures are equally reliable and valid with written, mailed, and computer formats [62,110–112]. Completeness and quality of data are improved with the computer administration of measures [62,111]. Online assessments reduce both staff and patient burden by providing checks for missed items, they eliminate double responses, and they can include intentional skip patterns to reduce the number of items (e.g., a female does not have to answer questions intended only for males). In contrast, at least one error occurred in 44% of paper response forms. Patients generally prefer the electronic response version once they try it. If in-person or online assessment is not possible, mailed assessments are equally valid and reliable. If telephone or proxy responses with someone answering for the patient become necessary, responses are not as consistent or valid as with the other methods, but are generally accepted.

Pediatric QOL assessment

When selecting pediatric QOL, there are several assessments to consider. The Pediatric Quality of Life Inventory (PedsQL) includes both a patient report and a parent report evaluating QOL for pediatric cancer patients on dimensions parallel to the FACT or EORTC [113–115]. Multiple versions suited to the developmental ages of children are comparable. A companion Cancer or Transplant Module provides treatment-specific assessment of symptoms. An alternative is the Child Health Ratings Inventory (CHRIs), with its Disease-Specific Impairment Inventory-HSCT (DSII-HSCT; a transplant-specific module). Preliminary studies in HCT cohorts indicate good reliability, and moderate correlations between child and parent reports of QOL [116,117].

Proxy or parent

When a patient is unable to provide a self-report of QOL, a proxy responder or missing data may be the only choices. A proxy is usually a nurse or caregiver, or most often the parent of a young child. Research on the validity of proxy responses indicates that parents and other proxies differ in responses when compared with patient reports [77,113,117]. Proxies, including parents, tend to underestimate patients' symptoms. In contrast, they tend to rate global QOL and mental health lower than patient self-reports [77,117]. Fortunately, proxies are more accurate when they are rating concrete, observable events.

Conclusion

QOL before, during, and after HCT has been well described. Studies agree that most patients regain high levels of physical, psychological, and functional QOL by 1–3 years following transplantation. At the same time, a subset of patients will have medical problems, such as cGVHD, that impinge on long-term QOL. Even for patients who return to full-time work and normal social activities, and those who have good psychological and physical function, specific areas of difficulty often remain. Recognized long-term problems include fatigue, cognitive deficits, musculoskeletal symptoms, and sexual dysfunction for both males and females. The impact on QOL of medical late effects is receiving more attention but remains an area deserving of further investigation.

By nature, QOL shifts with the situation a patient is in, reflecting not only observable abilities, but also expectations for the immediate circumstance. As a result, very ill patients can report good QOL in some areas. In assessing QOL, many measures have been tested with adult and pediatric HCT patients and work well. A generic QOL measure provides valuable normative information that permits a comparison of scores with patients of comparable age, gender, and/or disease. At the same time, specific, focused measures such as those for fatigue, sexual function, or distress may be needed to provide adequate sensitivity when defining the nature of a target problem or to detect clinical trial differences. Fortunately, there are many effective examples of QOL assessments that have been tested with HCT patients, at all ages and phases of treatment and survivorship. Online modalities are increasingly becoming the preferred form for assessment. Finally, a remaining challenge is to determine a method that facilitates the use of QOL data in clinical decision-making.

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CHAPTER 34

Sexuality Following Hematopoietic Cell Transplantation

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Introduction

A sentinel paper on the outcomes of 107 recipients of allogeneic hematopoietic cell transplantation (HCT) was published in 1975 by Dr. E. Donnall Thomas and colleagues [1]. Nine of the individuals transplanted for acute leukemia survived at least 1 year and 16 individuals transplanted for severe aplastic anemia became long-term survivors. Outcomes of transplantation have improved significantly since that early report. These improvements are due to advances in many fields, including blood banking, tissue typing, detection and treatment of infectious complications, pharmacology, immunology, and supportive care. As outcomes have improved, the use of HCT as a therapeutic modality has increased. Current estimates of the number of transplants performed worldwide are approximately 55,000–60,000 per year with an estimated 150,000 HCT survivors five or more years post-transplant [2,3]. HCT survivors and professional organizations including the American Society of Blood and Marrow Transplantation, the Center for International Blood and Marrow Transplantation Research, the Oncology Nursing Society, the Institute of Medicine, and the National Comprehensive Cancer Network have called on healthcare providers to focus efforts on addressing long-term medical and health-related quality of life (HRQOL) issues [4–7].

Investigations into the HRQOL of HCT survivors have spanned over two decades. While the majority of HCT survivors report good to excellent HRQOL, approximately 5–20% report persistent problems that negatively affect their well-being, including fatigue, cognitive dysfunction, chronic graft-versus-host disease (GVHD), musculoskeletal symptoms, and alterations in sexual health [8]. An important HRQOL concern of HCT survivors and the focus of this chapter is changes in sexual health resulting in sexual dysfunction and dissatisfaction. It is clear that the twofold mandate of HCT is both cure of the underlying disorder and preservation of quality of life.

Sexuality is explored as a multidimensional construct with biologic, physiologic, psychologic, and social dimensions. This conceptualization of sexuality remains a salient framework for assessing alterations in sexual health. Intervention strategies for sexual dysfunctions are presented and opportunities for future research are explored.

Conceptualizing sexuality

The World Health Organization views sexuality as an integral component of the human experience, which can enrich and enhance personality, communication, and love [9]. Sexual functioning, reproductive capabilities, and body image are three distinct and interrelated aspects of sexuality [10]. Sexuality includes the image one holds of oneself as a sexual being and gender roles, the concept of what it means to be a man or a woman [11]. Sexuality is shaped by age, developmental stage, culture, beliefs, expectations, sexual preferences, past intimate relationships and experiences, and the sexual partner(s). Sexuality is expressed in appearance, attitudes, values, roles, relationships, and sexual activity [11,12]. Although the diagnosis and treatment of a life-threatening illness may alter sexuality and sexual expression, they do not eliminate the need of individuals for intimacy, affection, and emotional connection to others. The theorist Abraham Maslow in his hierarchy of human needs placed love, belonging, and intimacy immediately after physiologic needs and safety [13].

The *Diagnostic and Statistical Manual of Mental Disorders* describes sexual dysfunctions based on the phases of the human sexual response cycle. Excitement, plateau, orgasm, and resolution are the four phases of the sexual response cycle as initially described by Masters and Johnson, and later Kaplan introduced desire as a unique phase [14,15]. The corresponding disorders of these phases include hypoactive sexual desire disorder (HSDD), female arousal disorder, erectile dysfunction (ED), orgasmic disorders, and sexual pain disorders (vaginismus and dyspareunia) [16]. There are two key components to the diagnosis of sexual dysfunctions: first, a physiologic or psychologic change that negatively impacts sexual functioning, and second, the association of this change with intra- or interpersonal psychologic distress [16].

Alterations in sexual health following HCT

Sexual dysfunctions reported in male HCT survivors include HSDD, ED, delayed or absent orgasm, and ejaculatory problems. Female HCT survivors may experience HSDD, arousal disorders, orgasm disorders, dyspareunia, and menopausal symptoms secondary to premature ovarian failure (POF). Table 34.1 reviews the incidence of sexual dysfunctions, which varies across studies based on time since transplant, sample characteristics, and study instruments. Longitudinal investigations specifically focused on altered sexuality indicate that sexual dysfunctions are common and often long lasting [17–19]. In 102 HCT survivors evaluated pre-HCT and again 12 and 36 months post-HCT, both men and women experienced an increase in sexual dysfunctions over time [17]. For male HCT survivors, the

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Table 34.1 Investigations of altered sexual health following HCT

Ref.	Study details	Measurement(s)	Findings
[57]	*n* = 36 females Mean age 26 years (range 14–43 years) Mean 4 years post-HCT (range 8 months to 9 years)	1. Questionnaire regarding menopausal symptoms, sense of femininity, sexual difficulties, social activities, and coping 2. Interview for effects of hormone therapy on symptoms	61% hot flashes 36% night sweats 81% vaginal dryness *Problems in 22 sexual active women:* 73% decreased desire 64% orgasm problems 82% problems with sexual intercourse
[93]	*n* = 44 females Median age at evaluation 30 years (range 18–43 years) Median days post-HCT 357 (range 261–4628 days)	1. Physical examination 2. Laboratory studies 3. Gynecological examination 4. Interview for menopausal symptoms and sexual functioning	*Received radiation and chemotherapy:* 67% hot flashes, night sweats, insomnia, and mood changes *Received chemotherapy only:* 38% hot flashes, night sweats, insomnia, and mood changes *In 30 sexually active women:* 77% vaginal dryness 53% diminished libido and arousal 60% dyspareunia 70% diminished pleasure Gynecologic findings: tissue atropy, loss of pubic hair, small uterus, introital stenosis and atrophic vulvo-vaginitis
[48]	*n* = 37 females Mean age 30 years (range 17–46 years) Time from HCT 13–84 months	1. Gynecologic examination 2. Hormonal assessment	83% vasomotor symptoms (hot flashes, sweating, irritability) 76% vaginal dryness and dysuria 76% dyspareunia 70% sexual problems (decreased desire, altered self-image, anxiety, decreased self-confidence)
[34]	*n* = 51 men Mean age 33 years (range 19–49 years) Mean time from HCT 58 months (range 6–154 months)	1. Investigator-designed questionnaire on sexual function 2. Hospital Anxiety and Depression Survey 3. Hormonal assessment	15% diminished desire 24% ED 13% delayed ejaculation 9% dry ejaculation 20% premature ejaculation
[92]	*n* = 126 females and males Mean age 27 years (range 9 –50 years) Mean 47 months post-HCT (range 6–149 months)	1. "Faces" Scale of Life Satisfaction 2. Hormonal assessment when possible	24% ED 13% ejaculation difficulties 22% of all subjects reported diminished sexual satisfaction
[50]	*n* = 15 females Mean age 22 years (range 17–30 years) Assess pre-HCT and 3–4 months post-HCT	1. Hormonal assessment 2. Pelvic ultrasound 3. Interview	53% hot flashes 25% cystitis symptoms 25% irritability 33% mood changes 73% depression 40% diminished libido 53% dyspareunia
[47]	*n* = 74 post-pubertal female allogeneic HCT survivors Median age 30 years Mean 49 months post-HCT (range 4–118 months)	1. Gynecological examination 2. Hormonal assessment 3. Interview regarding HRT, menopausal symptoms, and sexual activity	78% hot flashes 61% genitourinary symptoms 94% of 52 sexually active women reported difficulties with intercourse Gynecologic findings: vulvo-vaginal atrophy, loss of pubic hair, decreased thickness of genital mucosa
[35]	*n* = 29 men Mean age 35 years (range 18–61 years) Mean 36 months post-HCT (range 7–97 months)	1. Psychosexual Functioning Questionnaire – male version 2. Psychosocial Adjustment to Illness Scale 3. Hormonal assessment	38% diminished desire 21% altered body image 38% ED 48% sexual dissatisfaction
[94]	*n* = 31 females and males Median age 38 years (range 16–56 years) Assessed pre- and 3 months post-HCT	1. Derogatis Interview for Sexual Functioning	*Pre-/3 months post-HCT:* 36/48% sexual dysfunction 65/52% sexual dissatisfaction 36/40% decreased desire 14/11% arousal problems 43/43% orgasm problems 25/25% ED
[95]	*n* = 64 females and males Median age 39 years (range 20–65 years) Mean 52 months post-HCT (range 19–89 months)	1. EORTC QLQ-C30 2. Leukemia-BMT specific module 3. Questionnaire on sexual functioning and fertility 4. Questionnaire on patient perception of changes in HRQOL	*Autologous/allogeneic HCT survivors:* 21/60% diminished desire 30/68% decreased sexual activity 18/47% decreased pleasure 29/53% diminished ability to engage in sexual activity
[20]	*n* = 168 females and males Median age 33 years Median time from HCT 14 months	1. Questionnaire on sexual functioning and fertility 2. EORTC QLQ-C30 3. Patient ranking of perceived changes in sexual relationships	*Autologous/allogeneic HCT survivors:* 44/51% decreased desire 48/57% decreased sexual activity 27/42% decreased pleasure 29/45% decreased sexual ability
[96]	*n* = 16 men Mean age 36 years (range 20–49 years) Median of 3 years post-HCT (range 7–96 months)	1. investigator-modified Psychosocial Adjustment to Illness scale 2. Hormonal assessment	25% diminished desire 12% intermittent ED

(*Continued*)

Table 34.1 (*Continued*)

Ref.	Study details	Measurement(s)	Findings
[77]	*n* = 30 females Mean age 37 years (range 25–49 years) Mean time with ovarian failure 16.6 months (range 6–36 months with an additional subject 4 years and another 9 years)	1. General and gynecological examinations 2. Complete blood count, liver function tests, lipid panel, hemostasis parameters, hormone levels 3. Medical history 4. Pelvic ultrasound, mammography, and computerized bone mineralometry 5. Investigator-designed questionnaire for menopausal symptoms	90% vasomotor symptoms, hot flashes, night sweats, and palpitations 54% vulvo-vaginal atrophy with dyspareunia, itching, and burning 54% mood changes with anxiety, depression, irritability, and headache 45% atrophic skin changes 42% urinary tract symptoms 26% weight gain 16% insomnia 16% memory changes Gynecologic findings: cervical and uterine atrophy, loss of pubic hair, pale mucous membranes, vaginal dryness, stenosis, and decreased vaginal elasticity
[31]	*n* = 70 Median age at time of study for men 43 years (range 24–59 years) Median age at time of study for women 45 years (range 23–59 years) Median 81 months post-HCT for men and 76 months for women (range 18–198 months for men and 17–219 months for women)	1. EORTC QLQ-C30 2. Global health status 3. Hormonal assessment	44% overall decline in QOL 56% of men and 61% of women decline in sexual relations 47% more ED than pre-HCT 38% of men absence of orgasm 38% ejaculatory dysfunction 53% vaginal dryness 19% dyspareunia and delayed or absent orgasm

incidence of ED increased from 13% to 22% and arousal problems rose from 15% to 20%. Arousal problems in women climbed from 40% at baseline to 52% by 3 years. Problems with lubrication and pain increased from 30% to 52% and from 14% to 33%, respectively. In a 5-year longitudinal assessment of sexual functioning in allogeneic HCT survivors, HSDD was noted in 44% of women and 23% of men [18]. Additional findings from this study indicated that 27% of men reported problems with ejaculation and 23% experienced ED. Of the female HCT survivors, 40% described vaginal dryness, 16% dyspareunia, and 44% difficulties with orgasm. A compelling feature of this study was the use of case-matched controls. At 5 years post-HCT, 80% of female HCT survivors had one or more sexual problems compared with 61% of the female control group and 46% of male HCT survivors reported sexual problems compared with 21% of the male control group. Another longitudinal evaluation reported ED in 33% of men pre-HCT, increasing to 50% at 1 year and then declining to 22% at 3 years [19]. The incidence of ejaculatory dysfunction remained stable across the three assessments at 22%. Men also experienced an increase in problems with orgasm from 13% before HCT to 22% at 3 years post-HCT. For women, the problems with vaginal dryness, dyspareunia, and orgasm all worsened from pre-HCT levels. The incidence of vaginal dryness increased from 49% to 72%, dyspareunia from 29% to 55% and problems with orgasm from 46% to 72%.

Findings indicate that female HCT survivors are more likely to experience sexual dysfunction than male survivors [17–20]. Important to note from these longitudinal investigations is the incidence of sexual dysfunction in both men and women pre-HCT. Although sexual dysfunctions increase in incidence post-HCT, problems clearly begin prior to transplant. These studies also highlight the concern that alterations in sexuality do not improve over time and interventions are needed early in the transplant recovery phase to maximize benefit and minimize the risk that alterations in sexuality will become chronic problems.

Etiology of altered sexual health in HCT recipients

Alterations in sexual health may be due to a number of biologic, physiologic, psychologic, and social variables and the complex and dynamic interactions among these variables. Consequently, the origins of sexual problems will rarely be isolated to a single etiology. Physiologic changes due to the cancer or treatment may result in sexual dysfunctions, as will psychologic distress. Social variables influencing sexuality include the relationship with the sexual partner(s), difficulties in establishing new intimate relationships, role changes, and the health of the sexual partner.

Biologic and physiologic variables in men

The etiology of ED, an inability to achieve or maintain an erection sufficient for satisfactory sexual intercourse, may be hormonal, vascular, neurologic, pharmacologic, psychologic or a combination of these factors [21,22]. High-dose preparatory regimens can damage the hypothalamic–pituitary–gonadal axis and thyroid leading to hormonal changes that can alter sexual functioning in men. The hypothalamus secretes gonadotropin-releasing hormone (GnRH) into the circulation, stimulating the release of gonadotropins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) from the pituitary. FSH and LH control gonadal function resulting in the production of gametes and the synthesis of the sex steroids testosterone and inhibin. The Leydig cells under the influence of LH start androgen synthesis and secretion. FSH initiates spermatogenesis, which is then maintained by testosterone [23,24]. Negative feedback to the hypothalamus and pituitary occurs when testosterone and inhibin levels rise, blocking the secretion of GnRH and the gonadotropins [25]. Both radiation and chemotherapy damage gonadal tissue, leading to decreased secretion of the sex steroids and thus a loss of the negative feedback inhibition at the level of the hypothalamus and pituitary. The result is elevated levels of FSH and LH indicative of infertility [23].

The degree of gonadal damage will depend on age, the dose of radiation and chemotherapy, and the type of chemotherapy administered [23,26,27]. Radiation directly to the testes in doses of 12–15 cGy produces irreversible azoospermia [24]. Radiation and chemotherapy, particularly alkylating agents, result in a dose-dependent depletion of the germinal epithelium lining the seminiferous tubules of the testes, and this depletion results in azoospermia, testicular atrophy, and infertility [26,28]. Features of germinal cell depletion consist of small testicular size and reduced testicular volume [26]. Endocrine values in men with germinal cell depletion include elevated FSH (25–90 mIU/mL), elevated LH (8–25 mIU/mL), low to low normal testosterone (200–700 ng/100 mL), decreased testosterone production rate (3.5 mg/day), and decreased free testosterone (8.6 ng/100 mL) [26].

Most men following high-dose preparative regimens, particularly those including total body irradiation (TBI), will become infertile as a result of damage to the hypothalamus–pituitary–gonadal axis. However, infertility following high-dose therapy is not always a certainty. In a study evaluating the hypothalamic–pituitary–gonadal axis of 31 men following a high-dose chemotherapy-only preparatory regimen, 11 men agreed to semen analysis [27]. Following allogeneic transplant, four men were azoospermic, five were oligospermic, and one had a normal sperm count. Additionally, one man following autologous HCT also had a normal sperm count.

Testosterone is strongly correlated with sexual desire and arousal [29]. Levels of testosterone in male HCT survivors may remain within normal limits as the Leydig cells are believed to be relatively resistant to the effects of chemotherapy and radiation [24,30]. In a report of gonadal function following preparative regimens consisting of high-dose chemotherapy without TBI, six men had high basal LH levels with low total testosterone whereas 10 men had high basal LH levels with normal total testosterone [27]. Another investigation reported that of 26 men who received a preparative regimen containing TBI, all had elevated FSH levels, 20 had normal LH levels, 21 had low normal testosterone levels, and five had reduced testosterone levels [31]. More extensive hormonal evaluation may reveal subtle damage to the Leydig cells, evidenced by a reduction in the amount of testosterone produced [32]. Findings from one study indicated that men receiving allogeneic HCT are at higher risk for low testosterone levels than autologous HCT recipients [27]. Men over the age of 45 years may be at increased risk of testosterone insufficiency [33]. Elevated prolactin has been noted in some men following HCT [28,34] and is associated with infertility, ED, and decreased libido [21,29,34,35]. Hypothyroidism or subclinical hypothyroidism secondary to TBI may contribute to reduced levels of free testosterone and bioavailable testosterone [28,36,37].

Cavernosal arterial insufficiency has been reported as a cause of ED, with the risk higher in those men receiving TBI than in those who received chemotherapy only [38]. Pelvic irradiation can cause penile arterial insufficiency or blood vessel adhesions contributing to ED [21,28,38–40].

Hypertension contributes to ED in healthy men [21,37] and is a common side effect of calcineurin inhibitors utilized for GVHD prophylaxis and treatment. ED and diminished intensity of orgasm may be caused by chemotherapy agents associated with peripheral neuropathies administered prior to HCT or with the HCT preparative regimen [41]. Other lifestyle factors that may contribute to ED include cigarette smoking, alcohol use, and high cholesterol levels [21,42]. Sirolimus (Rapamune), which is used for GVHD prevention and treatment, commonly causes hyperlipidemia. Chronic GVHD may cause an increase in the sensitivity of penile skin, rash, or inflammation adding to sexual dysfunction [40]. Fatigue, decreased physical stamina, loss of muscle mass, and joint problems are common following HCT and can disrupt all phases of the sexual response cycle.

Biologic and physiologic variables in women

In women, damage to the hypothalamic–pituitary–gonadal axis secondary to a high-dose preparatory regimen is well established. Damage to the ovaries by high-dose preparative regimens results in a loss of estradiol secretion. As in men, the hypothalamus secretes GnRH into the circulation, stimulating the release of the gonadotropins FSH and LH from the pituitary. FSH initiates growth and maturation of the ovarian follicles and LH controls ovulation and corpus luteum formation [23]. Negative feedback to the hypothalamus and pituitary occurs when estradiol levels rise, inhibiting the secretion of GnRH, FSH, and LH [25]. Both radiation and chemotherapy damage ovarian tissue, leading to decreased secretion of estradiol and thus a loss of the negative feedback inhibition to the hypothalamus and pituitary. In women, findings of elevated levels of FSH and LH are indicative of infertility and ovarian failure [23,27].

The degree of gonadal damage will depend on age, the dose of radiation and chemotherapy, and the type of chemotherapy administered [23,26]. Alkylating agents and radiation therapy have cytotoxic effects on both dividing and resting ovarian cells. A single high dose of TBI produces ovarian failure in all women [23]. Evaluation of ovarian function following chemotherapy-only preparative regimens found evidence of gonadal failure in all recipients of allogeneic HCT and 95% of autologous transplant recipients [27]. Serial ultrasound examinations have demonstrated that almost immediately following high-dose preparative regimens, the ovaries show evidence of structural damage, shrinkage, and loss of follicles [33]. In a group of 41 women at a mean of 1.5 years (range 0.2–9.8 years) post-HCT, ultrasound examinations showed ovarian gland atrophy, absence of follicles, and a small uterus [27]. In addition to decreased levels of estradiol and elevated levels of FSH and LH, a small number of women tested had evidence of abnormal androgen function [28]. Hypothyroidism or subclinical hypothyroidism secondary to TBI may also contribute to POF [28,36,43].

Estrogen deficiency is associated with a multitude of symptoms, including hot flashes, night sweats, insomnia, mood swings, irritability, depression, changes in cognitive function, altered appearance, reduced bone density, and cardiovascular disease [30,44,45]. In the absence of estrogen, the vaginal mucosa becomes thinner with less lubrication, leading to itching, dryness, burning sensations, an increased risk of infections, and dyspareunia [45,46]. Urogenital tissues deprived of estrogen results in dysuria, urinary frequency, incontinence, and an increased risk of urinary tract infections [46,47]. The sudden loss of estrogen secondary to a high-dose preparative regimen may result in more pronounced or severe symptoms of estrogen deprivation [45,48].

Multiple factors contribute to alterations of vaginal tissue, including the effects of POF, the preparative regimen, in particular TBI, and chronic GVHD of the vagina. Radiation may contribute to vaginal narrowing and stenosis [28]. In one report, nearly half of the 61 women studied reported vaginal chronic GVHD by 2 years following HCT [49]. Features of vaginal chronic GVHD include inflammation, ulceration, stricture formation, narrowing and obliterated introitus, and symptoms of cystitis and excoriation [49–52]. The mean onset of vaginal chronic GVHD was 10 months with a risk period of 2 years [51].

Interference in all phases of the sexual response cycle can be caused by fatigue, decreased physical functioning, loss of muscle mass, and joint problems, which are common problems for HCT survivors.

Psychologic and social variables

Psychologic distress, including depression [53–55], anxiety [53,54,56], decreased self-confidence and self-esteem [57,58], altered body image [19,33,35,57,59], and grief secondary to infertility [20,57,60], is reported by some HCT survivors and this distress can be long-lasting. Depression measured both pre-HCT and 3 years later was a significant predictor of sexual dysfunction in men and women [19]. In men, higher levels of psychologic distress pre-HCT significantly predicted for sexual dissatisfaction 3 years post-HCT [17]. Psychologic and relationship stress can contribute to ED [21]. Regardless of the biologic or physiologic causes of ED, there is an accompanying psychologic distress, including reduced self-esteem, decreased sense of masculinity, and embarrassment [22]. In women, POF is more than a physiologic transition into menopause but is associated with a number of psychologic stresses, including feelings of lost youth and femininity, altered body image, and loss of fertility [24,45,61].

Infertility may alter a woman's sense of femininity and a man's sense of virility and masculinity [11,62]. Infertility and alterations in gender roles may result in a loss of self-confidence [33,57,59,62]. Younger individuals and those couples who have not completed childbearing are likely to experience more personal and relationship distress secondary to infertility. Couples' concerns regarding infertility may not decline over time and coming to terms with infertility requires active discussion and problem solving [60]. Single individuals may be hesitant to establish new intimate relationships for fear of rejection secondary to infertility [63].

Body image changes includes those alterations apparent to others such as scars, alopecia, weight changes, skin changes due to chronic GVHD, scleroderma, joint contractures, open wounds, and Cushingoid features secondary to corticosteroids used for the prevention and treatment of GVHD. Just as importantly, body image changes include changes not apparent to others, such as the image the HCT survivor holds of him- or herself as a sexual being. Findings from one study indicated that 56% of men and 55% of women report feeling unattractive 3 years after HCT [19].

The long road from the diagnosis of a life-threatening illness through recovery following HCT is physically and psychologically challenging. During transplant and the early recovery phase, a caregiver is required who assumes significant responsibility for the care of the HCT recipient. Often this caregiver is the sexual partner. The effects of role changes on intimate relationships are not fully understood. Several publications have cited the burdens of caregiving and the significant problems faced by caregivers. A longitudinal investigation evaluating marital satisfaction indicated that the spousal caregivers experienced higher levels of depression and anxiety at 6 and 12 months post-HCT than the HCT survivor [64]. Additionally, marital satisfaction scores declined for the caregiver from pre-HCT to the 1-year assessment, whereas marital satisfaction scores for the HCT survivor remained stable. The decreased marital satisfaction scores were significantly more pronounced if the caregiver was female. In another study evaluating the HCT survivor, caregiver, and a matched control group for late effects, the HCT caregiver experienced more fatigue, sleep problems, cognitive dysfunction, and sexual problems than controls [55]. This study also reported that the incidence of depression for the HCT survivor and caregiver was ≥20% compared with an 8% incidence in the matched control group. HCT caregivers also reported less social support, marital satisfaction, and spiritual well-being than the HCT survivors or controls and were less likely to receive psychologic care.

Resuming sexual activity following treatment may be difficult for some couples. The HCT survivor may be struggling with physical recovery, psychologic distress, body image changes, fatigue, and concerns about their sexual functioning. The sexual partner may also be struggling with fatigue, psychologic distress, altered sexual health, and the increased burdens of caregiving and responsibilities at home. Psychosocial adjustment of HCT survivors is influenced by the presence or absence of social support or the perception of social support [58,65,66]. Social support and strong family relationships have been found to facilitate adjustment and an absence of social support negatively influences adaptation even years after transplant [65,67]. If a major source of social support for the HCT recipient is the intimate partner and relationship stress arises due to alterations in sexual functioning, then the recovery process may be jeopardized.

Assessment and interventions for altered sexual health

One investigation reported that 30% of HCT survivors were not informed of potential alterations in sexuality and 20% were not satisfied with the information they received on fertility and sexual functioning [31]. In a second study, half of the participants followed over 3 years indicated they had no discussion of sexuality with their healthcare providers [19]. Silence by healthcare providers remains a significant and persistent barrier to addressing sexuality. Healthcare providers cite personal discomfort, lack of education, the sensitive nature of the topic, fears of embarrassing the patient, and limited time as reasons for failure to discuss sexual health [12,39,68]. Healthcare professionals can eliminate these barriers by gaining an understanding of the impact of HCT on sexuality, utilizing standardized assessment tools, identifying educational resources for HCT survivors, and developing a referral network. Despite the sensitive nature of sexuality, when asked, HCT survivors will often discuss sexual concerns as freely as they will discuss other HRQOL issues [18].

The first intervention to address sexual health is a pre-HCT discussion of the risks of infertility, POF, symptoms of menopause, and possible alterations in sexual functioning, which is all part of informed consent. An essential component of this dialog is offering reassurance that there are effective interventions should problems in sexual functioning occur. Education is the first step in establishing expectations for life after transplant and beginning the process of adaptation [69]. Research has shown that HCT survivors experience higher levels of psychologic distress when there is a discrepancy between their expectations and their experiences [54]. Simply informing couples that decreased libido is common for many months following HCT is a simple place to start. The healthcare provider should whenever possible include the sexual partner with the aim of facilitating communication, identifying the partner's anxieties, and enlisting the partner's support. The pre-HCT conversation legitimizes sexuality as a valid area of concern and identifies the healthcare provider as a resource for the couple. Early interventions are critical based on a growing body of evidence that sexual dysfunctions following transplant do not appear to get better simply with the passage of time [17,18].

Written education materials can be valuable resource for HCT survivors and the sexual partner and should specifically address when it is safe to resume sexual activity, what precautions are needed, possible changes in sexual functioning, when to consult a healthcare professional, and a list of resources. Many organizations have websites that offer information on sexuality, including the American Cancer Society, the American Urological Association, Cancer Care, Cancer Supportive Care Programs, Fertile Hope, the Lance Armstrong Foundation, the National Cancer Institute, the Oncology Nursing Society, Sexuality Information and Education Council of the United States, and the Wellness Community [70].

Although a discussion of altered sexual health may be sufficient to address the concerns of many HCT survivors, others may be experiencing sexual dysfunctions that require a referral to a specialist.

Transplant centers should identify a group of experts for referrals that includes a urologist, endocrinologist, gynecologist, reproductive specialist, psychologist, relationship (marriage) counselor, and possibly a sex therapist. A simple approach to begin an assessment of sexual health is to ask patients if they have resumed sexual activity. The routine use of a standardized assessment tool for follow up visits will help familiarize healthcare providers with the appropriate language to use for sexuality and increase their comfort level with this sensitive topic [71].

Assessing for any psychologic distress secondary to infertility is required, so that appropriate referrals can be initiated. Pre-HCT options for attempting fertility preservation include sperm or oocyte cryopreservation, cryopreservation of testicular or ovarian tissue, hormonal suppression of ovarian function, and embryo cryopreservation, and post-HCT options include in vitro fertilization, donor oocyte or donor sperm, and embryo transfer [24,72]. Ideally, the referring hematologist or oncologist will have had discussions on fertility preservation prior to the referral for transplant as the majority of individuals will arrive at the transplant center having already received therapy that may affect fertility. Recent findings that recovery of spermatogenesis may occur even following some high-dose preparative regimens places additional obligations on the HCT team to provide adequate counseling regarding birth control and the prevention of unplanned pregnancies.

Hypoactive sexual desire disorder

Sexual desire is the least well understood and the most difficult aspect of altered sexual health to treat. Desire is strongly linked to the second phase of the sexual response cycle, arousal, hence individuals with HSDD may also experience problems with arousal. In men, testosterone is known to influence sexual desire, but the role of hormones and desire in women is not well understood. Newer models of the sexual response cycle suggest that for women, desire for sexual activity may follow the arousal phase and be motivated by the need for emotional closeness with her partner [73].

The evaluation of HSDD must include a review of physiologic and psychosocial variables. Physiologic assessment includes a physical examination focusing on fatigue and physical stamina as well as hormonal testing. Emotional distress, particularly depression, is a key factor to be evaluated in both the HCT survivor and the sexual partner. If depression is identified as a contributing factor, antidepressant therapy may be helpful; however, the selection of the antidepressant is critical. Both selective serotonin reuptake inhibitors and serotonin–norepinephrine reuptake inhibitors are associated with decreased sexual desire [74]. The risk of sexual dysfunction is up to 2.5 times greater for women taking antidepressants [74,75]. Newer antidepressants with different mechanisms of action may actually improve sexual desire [76].

The relationship with the intimate partner needs to be evaluated for stress, discordance of expectations, and adequacy of communication. One transplant center has had success in improving sexual activity using couple-based therapy focused on communication, education, and intimacy [40].

In assessing HSDD, it is important to determine if decreased sexual desire preceded or developed as a consequence of other sexual dysfunctions, as HSDD is common in both men and women experiencing other sexual dysfunctions [69]. If, for example, a man is experiencing ED or a woman is experiencing dyspareunia, both of these problems may contribute to HSDD. Therapeutic interventions will need to be targeted at treating contributing sexual dysfunctions and not specifically focused on the HSDD.

In male HCT survivors with low to low normal testosterone levels, testosterone replacement therapy (TRT) can be effective in improving sexual desire [27,38]. Testosterone therapy in women is controversial; however, some women may experience improvements in sexual desire and arousal with androgen therapy [29,39,77].

Premature ovarian failure, vaginal atrophy, and dyspareunia

Assessment of women with POF includes medical history, physical and gynecologic examination, hormonal evaluation and medication profile. The hormonal testing should include estradiol, FSH, LH, androgen level, and thyroid function tests. Many symptoms of estrogen deficiency can be ameliorated or minimized with hormone replacement therapy (HRT). HRT is effective in relieving hot flashes, improving sleep, maintaining vaginal elasticity and lubrication, and decreasing changes in the appearance of the skin and breasts. Multiple studies of female HCT survivors have reported relief of menopausal symptoms following HRT [27,47,48,57] with improvements in most symptoms noted within a short period of time [77]. Based on the effective and rapid resolution of symptoms, HRT should be offered to female HCT survivors after assessing for possible contraindications such as chronic GVHD of the liver or a history of blood clotting problems. One investigator has suggested that initiation of HRT within the first year following HCT is critical to avoiding long-standing issues of sexual dysfunction [17].

The findings of the Women's Health Initiative Study reporting that the risks of breast cancer, heart attack, stroke, and blood clots outweigh the benefits of HRT in women following physiologic menopause is a source of anxiety for some individuals [78]. The risks of HRT in female HCT survivors may not be the same as those in women following physiologic menopause. For females with POF, HRT is administered to compensate for the premature loss of endogenous hormones [24,79]. In general, women with POF should utilize HRT until the age of physiologic menopause [79].

The benefits of HRT in relieving symptoms of estrogen deficiency are clear; however, the choice of HRT is challenging and requires a committed gynecologist and additional study. Selection of the HRT should be guided by the woman's symptoms, preferences, and risk factors for complications from HRT and designed to prevent future complications such as osteoporosis [77]. One group's recommendation for women who are only recently estrogen deficient is low-dose estrogen for the first month, increased to full dose in the second month [79]. However, if she has been estrogen deficient for 12 months or longer, then low-dose estrogen replacement should be initiated with a gradual increase to maintenance doses by 6 months to avoid side effects. A recommended estrogen dose for young women with POF is the equivalent of 1.25 mg of conjugated equine estrogens combined with progestins if the uterus is present [79]. Some oral contraceptives may reduce circulating free testosterone, resulting in diminished sexual desire [29]. In women with adequate estrogen replacement and complaints of diminished desire, fatigue, and a poor sense of wellbeing, androgen therapy may be beneficial [77,79].

Non-hormonal strategies for relieving symptoms of estrogen deficiency include herbs, vitamins, yoga, acupressure, acupuncture, exercise, and diet modifications. Few of these interventions have been rigorously tested. A systematic evidence-based review found Remifemin, a formulation of the herb black cohosh, to reduce hot flashes [80]. Antidepressants can ameliorate hot flashes. Venlafaxine (Effexor) reduced hot flashes by 60% and other antidepressants that may be helpful include paroxetine

(Paxil), fluoxetine (Prozac), and citalopram (Celexa) [81,82]. Gabapentin (Neurotin), clonidine hydrochloride (Catapres), and bellergal (Bellergal-S) have also been reported to decrease hot flashes [82]. The North American Menopause Society is an excellent web-based resource for women.

Female HCT survivors with vaginal atrophy, narrowing, and dyspareunia may benefit from the use of vaginal lubricants and dilators. Vaginal lubricants should be water soluble to avoid the risk of infections. Vaseline is not water soluble and therefore should not be used. K-Y Jelly (Johnson and Johnson, New Brunswick, NJ, USA), vitamin E, Replens (LDS Consumer Products, Cedar Rapids, IA, USA), and Astroglide (Biofilm Inc., Vista, CA, USA) provide lubrication for sexual intercourse. Astroglide has been reported by some users to last longer during sexual activity. Additionally, Replens can be used to restore moisture to vaginal tissues. One applicator of Replens can be inserted into the vagina three to four times per week at bedtime to restore moisture. These products can be found at most drug stores or via the Internet.

Dilators can be obtained from a gynecologist or through the radiation therapy department. Women should lubricate and then insert the dilator into the vagina until she experiences slight discomfort. She should leave the dilator in place for 10–15 minutes three to four times per week. As the vaginal tissues begin to stretch, the depth of penetration and size of the dilator can be gradually increased. The level of commitment for pursuing these measures in a consistent manner will optimize results. Pelvic floor strengthening exercises guided by a pelvic physical therapist may reduce vaginal pain. Women experiencing dyspareunia should assume a position during sexual intercourse that allows her to control both the rate and depth of penetration, enabling her to stop if she becomes uncomfortable. Women worried about lubrication and their response to sexual stimulation can be encouraged to masturbate, as a way to explore their response without simultaneously being concerned about their partner's needs [83].

Evaluation of the medication profile and adjustment of medications may improve sexual functioning, as many medications adversely affect sexual functioning, including anti-androgens, sedatives, antidepressants, and stimulants [84].

A comprehensive program for the prevention of chronic GVHD of the vagina incorporating patient education, topical estrogen, early initiation of HRT, vaginal dilatation in the absence of sexual activity, and regular gynecological examination minimized the development of severe GVHD of the vagina in a series of 61 women [49]. The investigators also outlined treatment for established chronic GVHD of the vagina, which included topical steroids, topical cyclosporine, and vaginal dilatation in women who had evidence of vaginal narrowing. Fifteen of the 28 women had complete resolution of symptoms, eight noted improvement and in five the symptoms remained stable. None of the women required surgery, which is in contrast to other reports were surgical interventions were necessary [51].

Erectile dysfunction

The assessment of ED requires a medical history and physical examination, a review of the medication profile, knowledge of coexisting health problems and lifestyle factors, and hormonal testing, which includes total testosterone, bioavailable testosterone, sex hormone-binding globulin, FSH, LH, prolactin, and thyroid function [21,38]. A comprehensive evaluation of the functional and structural capacity of the penis is required for men with ED [85].

TRT often restores sexual desire and arousal and may also improve erectile function [29,38]. In one report, TRT restored testosterone levels to normal after 2 years of therapy, allowing discontinuation of the hormone replacement [27]. The men in this study reported improved sexual functioning, sexual desire, and wellbeing. TRT is available as an intramuscular injection, transdermal patches, gel and oral forms. Testosterone enanthate (Delatestryl) and testosterone cypionate (Depo-Testosteron) with a recommended dose of 100–200 mg intramuscularly every 2–4 weeks are two options [21,85]. TRT is contraindicated in men with a history of prostate cancer and routine monitoring of prostate-specific antigen levels should be performed at 6-month intervals [86]. TRT may contribute to coronary artery disease by its effect of lowering high-density lipoproteins [85]. Hyperprolactinemia observed in some male HCT survivors is associated with decreased desire and ED and can be effectively treated with bromocriptine mesylate (Parlodel) [87].

Performance anxiety can lead to ED and subsequently HSDD. Suggesting masturbation may improve a man's confidence in his erectile function without simultaneous concerns about his partner's needs [83]. Erectile function will be improved by staying very focused on sensations during sexual activity to minimize negative thoughts and performance concerns and prolonging foreplay will ensure maximum arousal.

Many classes of medications, including antihypertensives, anticholinergics, antihistamines, cardiac medications, antidepressants, alcohol and other recreational drugs can contribute to ED. A review of the medication profile is a component of the assessment for the etiology of ED. Reducing doses or trying alternative medications may result in improved sexual functioning.

The phosphodiesterase inhibitors sildenafil (Viagra), vardenafil (Levitra) and tadalafil (Cialis) have made an important contribution to the effective treatment of ED. Phosphodiesterase inhibitors do not increase sexual desire but improve penile response to sexual stimulation. Sildenafil (50–100 mg) and vardenafil (10 mg) should be taken approximately 1 hour prior to sexual activity. Tadalafil (10–20 mg) has a longer half-life and is generally taken 30 minutes to 12 hours prior to sexual activity. In 2008, tadalafil (2.5–5 mg) was approved for once-daily dosing for men anticipating more frequent sexual activity [88]. The phosphodiesterase inhibitors are generally safe and well tolerated; however, the concurrent use of nitrates is an absolute contraindication [22]. A number of other strategies for the treatment of ED include intracavernosal injections, transurethral therapy, vacuum devices, and surgical interventions, but in general these strategies are not as well accepted [39].

One group of investigators hypothesized that a combination of TRT with sildenafil would improve erectile functioning in men following HCT [89]. The sample consisted of eight men aged 22–58 years and 2–24 months post-HCT with evidence of hypogonadism, ED, decreased libido, ejaculatory disorders, and documented cavernosal arterial insufficiency. The men were treated with a combination of TRT given monthly for 6 months and sildenafil taken once or twice per week. Results indicated that all eight men had improvements in erectile function allowing satisfactory sexual intercourse, seven men had resolution of ejaculatory dysfunction and all had improvements in both total and free testosterone levels. The authors concluded that this combination therapy is a safe and effective therapy for ED in HCT survivors and should be evaluated in a larger clinical trial.

Areas for future research

The interventions presented for the treatment of sexual dysfunctions in HCT survivors are based on clinical expertise and the literature of sexual dysfunctions in other cancer survivor populations and in healthy individuals. There remains a paucity of research focused on interventions to restore sexual health in HCT survivors. Designing and testing an education program to help HCT survivors and their spouses/partners develop realistic expectations of life after HCT including alterations in sexual health may bring expectations and outcomes closer together and thereby decrease psychologic distress. Investigations are needed to develop insights into the effects on intimate relationships when the spouse/partner is the caregiver and interventions to decrease the psychologic distress of caregiving need testing. Exploration of the biologic, physiologic, and psychosocial variables that underlie alterations in sexual health needs to continue with the aim of designing treatment interventions. Identification of variables that can predict which HCT survivors are at risk for alterations in sexual health is necessary. The effects of reduced intensity conditioning regimens on fertility and sexual health have not been fully explored. Testing the use of TRT plus a phosphodiesterase inhibitor in a larger sample of men suffering from ED post-HCT would be valuable. Future research must include a diverse population of HCT survivors that includes the elderly, adolescents, same-sex couples, and diverse ethnic groups. Development of validated instruments that can be easily incorporated into time-limited clinic appointments will advance the treatment of sexual dysfunctions. As infertility may not be a certainty, resources should be devoted to systematic follow-up of pregnancy outcomes.

Conclusion

Sexuality is a broad concept encompassing much more that sexual activity and remains an important aspect of being human, regardless of the diagnosis and treatment of life-threatening illness. The understanding of alterations in sexual health following HCT is not complete. It is clear that the etiology of sexual dysfunctions for both male and female HCT survivors has biologic, physiologic, and psychosocial components and complex and dynamic interactions among these variables.

Research findings have been presented suggesting that there are effective interventions for the treatment of POF symptoms, HSDD, vaginal chronic GVHD, and ED. Although additional investigations are needed, these results are clinically relevant now and can be utilized to address this critical aspect of HRQOL. After a pre-HCT discussion of sexuality, the next step is to include an assessment of sexual health as part of routine care following HCT. It has been shown that an open discussion of sexuality with a healthcare professional can be therapeutic and address many areas of concern. Developing a referral network of other healthcare professionals, including gynecologists, urologists, reproductive specialists and mental health professionals, enables the HCT team to assist HCT survivors with alterations in sexual health.

Although the focus of this chapter has been sexual dysfunctions, it must be stated that many HCT survivors report satisfying sexual relationships after transplant [90–92]. The challenge for the HCT team is to identify those HCT survivors who are experiencing sexual dysfunctions and intervene. If the transplant team can improve the HRQOL of HCT survivors by addressing issues of sexual health, one of the two mandates of transplantation will be met.

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CHAPTER 35

Hematopoietic Cell Transplantation: The Patient's Perspective

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> *I still find it almost bizarre that I would be the one to get so sick. I was very physically active. I rode my bike 2000 miles a year, swam about 100 miles and was active in my children's lives. Leukemia happens to people you read about in the newspaper during their appeals for donors or money for transplants. It doesn't strike enormously healthy, happy and vital 39-year-old family men.*
>
> HCT survivor Mike Eckhardt, 1995

Preparing for transplant

Like Mike, most people have never contemplated the notion that they may some day have an illness that requires treatment with hematopoietic cell transplantation (HCT). Regardless of whether a patient is newly diagnosed or previously learned of the illness and has exhausted other therapies, the prospect of HCT is traumatic for both the patient and his or her loved ones.

Having lived through this experience myself in 1989, after being diagnosed with acute myelogenous leukemia, I know first hand how difficult it can be to process information under duress, make life or death decisions, and cope emotionally with the HCT experience. In 1990, I created Blood & Marrow Transplant Information Network (BMT InfoNet; http://bmtinfonet.org/services/support) to help other families navigate the HCT experience. Through lay-language books about HCT, survivorship symposia, and peer counseling, BMT InfoNet services more than 10,000 patients and their loved ones each year. Their experiences helped inform this chapter.

Patients are overwhelmed with fear: fear that the transplant will not cure; fear that the transplant might kill; fear of pain; and fear that the side effects of treatment will be intolerable. Many are also angry and/or depressed over the loss of control over their wellbeing. A disease now controls their body, and the patient must rely on a team of strangers to save his or her life. Preparing a patient for the rigors of HCT against this backdrop of fear, anger, and/or depression is challenging. Some patients are so overwhelmed emotionally that they hear and process little of what a physician tells them. They can process only discrete packets of information, are overwhelmed by details, and prefer to leave much of the decision-making in the hands of the physician.

At the other end of the spectrum are the patients who attempt to regain control over their body by accumulating as much information as possible about their disease and treatment options. They want complete, detailed information about the risks and benefits of treatment. They will search external sources, such as the Internet, for information. They want to be fully engaged as partners in their care.

Since patients' styles of coping with troubling medical information vary greatly, it is important to ascertain in advance how much information a patient wants, and how best to deliver it. Level of education, cultural biases about discussing illness and death, the degree of trust that a patient has in the medical establishment, and individual coping styles will define the best method of discussing treatment options with a patient. Asking how much detail the patient wants when discussing medical treatments, and whether he or she prefers to hear it all at once or in small segments, enables a physician to tailor the delivery of information to best suit each individual patient.

Despite these differences, there are certain universal elements of communication that are helpful to bear in mind when preparing a patient for transplant.

Put the risks into perspective

When a patient meets with the transplant team, a long list of potential treatment-related complications is described. For many, this is the first time they have heard a detailed discussion of the risks associated with HCT. The prospect of developing these complications is frightening for patients, and many leave the consult with the transplant team more depressed than when they arrived.

> It was like a whirlwind, a dream. One day our child was a normal 15-year-old boy who would live to be 80. The next day we were staring at blackboard diagrams about transplants, and hearing doctors tell us our son might die. It wasn't real. We didn't understand. All we could do was hug each other and cry. (Lorraine Boldt, mother of transplant survivor)

Patients need help putting the risks associated with HCT into perspective. Grouping the complications into (a) those that always occur, (b) those that often occur, and (c) those that seldom occur provides patients with a manageable framework for understanding relative risks and coping emotionally with the prospect of complications. Knowing that the risk of developing severe liver damage is small, while the risk of developing mucositis is high, for example, conveys a far more accurate picture of what a patient should expect. Without such a framework, patients may imagine the risk of developing very serious complications to be greater than it is.

It is also important to distinguish between those side effects that are temporary and those that may persist long term. Most people

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believe they can deal with a problem for a discrete period of time. Knowing in advance what the normal range of duration is for each complication enables patients to cope better with the difficulty when it arises. Without such information, patients can become distressed if a complication persists, and may become concerned that it signals relapse, disease progression, or another serious problem.

It is equally important to discuss how complications will be managed. Although a detailed discussion of each therapy is not necessary, patients should know that treatment for each complication exists. This is particularly important when discussing painful complications, since some patients fear pain more than death. Many patients do not automatically assume that pain medication will be available to relieve discomfort. This must be explicitly stated. Leave out this discussion and a patient can assume that he or she will suffer tremendous pain, or that the complication is not treatable, and this can increase the emotional load.

Communication in lay language

Although many patients are well educated, most are unfamiliar with the medical terms used routinely by transplant personnel. Terms such as "CBC," "aspirate," "Hickman," "bilirubin," "TPN," and even "stem cells" are often foreign to people without medical training. Even more commonly used words such as "prognosis" and "remission" may be meaningless to patients who have never used these terms. Most patient advocacy groups, and also the general print media, strive to use language that can be understood by a person with a fifth- or sixth-grade education. Given that HCT patients are under a great deal of emotional stress and may not be able to process information as easily as the average person, medical procedures and terms should be described as simply as possible. In the absence of a clear explanation, patients may misinterpret the message being conveyed by the physician.

Using precise language also improves a patient's understanding of what he or she is about to experience. For example, when describing a painful procedure, using words such as "hurt" or "pain" does not adequately prepare a patient for the sensation he or she will feel. Terms such as "pressure," "stinging sensation," or "dull ache" convey a more precise idea of the sensation. Precise language will help reassure patients that these sensations are normal when they occur.

Repetition and reinforcement are important

The volume of information that HCT patients receive is overwhelming, and few completely digest the information during the first presentation. Patients who are emotionally overwhelmed may shut out complicated or frightening information, and later recall only part or nothing of what was said.

It's important to repeat information at several points during treatment. A patient who was unable to absorb critical information when it was initially presented may be better prepared to absorb it later after the initial shock about the diagnosis and the difficult treatment he or she is about to undergo wanes. Some patients have found video- or audiotaping the initial consultation with the transplant physician to be helpful. Such a record enables them to double-check their understanding of what was said later when reflecting upon the information. It also helps them catch important details they may have missed during the initial presentation. Suggesting this option to patients can provide them with a valuable tool, and can reassure them that the physician wants them to be as informed as possible about the impending treatment. Suggesting that patients bring a support person to the consultation whose sole purpose is to take notes can also be helpful. Proactively suggesting this in addition to taping the conversation will help those who might be too distraught to think of these tools on their own.

Encouraging patients to ask questions can also enhance understanding. Some patients come from a cultural background where questioning a physician is considered rude or disrespectful. Inviting them to ask questions to confirm their understanding can give them "permission" to do so. Inviting questions means more than simply saying, "Do you have any questions?" Inviting questions in a way that elicits more than a yes/no answer may allow patients to formulate and state their concerns better. For example, a physician might say, "Some patients don't understand what I mean when I say they will become neutropenic. Do you understand what I mean by neutropenic, or should I explain it more clearly?" Such a question normalizes a confusion the patient may have and affirms the physician's responsibility to make the explanation as clear as possible.

Patients who have questions during treatment sometimes do not feel that they have an adequate opportunity to present them to the physician. Creating an atmosphere that encourages questions from patients is essential. A physician who appears busy or distracted can discourage patient questions without meaning to do so, leaving the patient feeling anxious and uninformed. Sitting down when entering a patient's hospital room, for example, signals to the patient that the physician has time to focus on the patient's concerns. Consciously making an effort to engage a patient in conversation, rather than simply delivering news, can create an atmosphere that elicits patient concerns. Suggesting that the patient maintain a notebook of questions that arise when the physician is not present can also help patients organize their thoughts.

Different learning styles

It is well accepted by educators that individuals have different styles of processing information and learning. A person who is primarily a *visual learner* does best when charts or pictures accompany the explanation. Seeing the person with whom he or she is talking, rather than just hearing an explanation, enhances understanding. An untidy environment can interfere with this patient's ability to absorb information.

Auditory learners, on the other hand, do best with verbal explanations. They are more likely to use telephone support systems, such as help-lines, than pore over charts or diagrams that explain a problem. They are easily distracted by sounds.

Kinesthetic learners learn by doing. They like hands-on explanations and can be distracted by movement.

When educating transplant patients, accommodating the variety of learning styles is important. A person who is primarily a visual learner can miss key information if the education plan relies solely on verbal explanations. Patients who are primarily auditory learners can have difficulty absorbing print information. Kinesthetic learners may benefit from a tour of the facilities where procedures will be performed, with an opportunity to handle equipment that will be used during transplant.

Although it may be difficult to ascertain each individual's learning style, providing the patient with a menu of learning tools will maximize the likelihood that he or she will absorb important information. Augmenting verbal explanations of procedures with written publications or audiovisual aids produced by organizations such as BMT InfoNet or the National Marrow Donor Program (NMDP) (www.marrow.org) enables a patient to choose the information vehicle that best suits his or her needs.

Discussing pain control is important

Many patients fear pain even more than death. However, although all transplant teams inform patients about potential complications, some neglect to discuss the steps that will be taken to manage pain. They assume that patients know that pain medication will be administered as needed. Many patients, however, have had little experience with hospitalization and painful medical procedures, and do not automatically assume that pain relief will be available. Others have undergone painful medical procedures without adequate pain control and have little reason to assume that pain will be managed more effectively by the transplant team.

Explicitly telling patients that pain control medications will be used to prevent and control pain, and educating them about when to ask for pain relief, can ease their anxiety. Many patients try to be stoic, asking for pain medication only after pain becomes intense. Explaining that pain is easier to manage before it becomes intolerable, normalizing the use of pain medications in the transplant setting, and encouraging patients to request relief before pain becomes unbearable can minimize the discomfort and anxiety that a patient experiences. If a patient-controlled analgesia device will be available, letting the patient know that he or she will have some control over the administration of pain medication can make the prospect of pain more manageable.

It is equally important to warn patients and caregivers in advance about possible side effects of pain medications. Most are unprepared for the change in mental status that accompanies the use of some medication, and both patients and caregivers can become alarmed when this occurs. Patients can see or hear things that are not real and talk nonsensically. Caregivers who observe this without understanding that this is a side effect of the medication, and patients who later realize they have been hallucinating, can become emotionally distraught. Assuring both patient and caregiver that these drug reactions are within the range of normal and are temporary can alleviate unnecessary anxiety.

Fear of addiction discourages some patients from asking for pain relief. Patients, and also many physicians, do not realize that drug addiction is a psychologic phenomenon, whereas drug dependence is a physical phenomenon. Although a patient may develop a physical dependence on a drug, he or she can be weaned from it by tapering the dosage. An addiction, however, requires psychologic interventions to address the underlying cause of the addiction, in addition to tapering or discontinuation of the drug. Patients who have never had a drug addiction are unlikely to develop an addiction to pain medications administered as part of HCT [1].

Discuss psychologic difficulties

An important area of patient education that is often overlooked is a discussion of the psychologic difficulties that patients and family members encounter during transplant. Patients may be told that the transplant will be a “difficult” or “stressful” experience, but these words fail to describe or normalize the type and depth of emotions the patient and his or her family are likely to experience.

If not given this information, a patient becoming angry or depressed during treatment and recovery may assume that there is something wrong – that he or she is a weakling or is not coping as well with the stress as most patients. After all, the patient reasons, if these emotional difficulties were common among HCT patients, the medical team would have said so in advance, just as they warned of the other medical complications. Some become angry with themselves for not coping well, which exacerbates their emotional distress.

As difficult as the transplant experience is for patients, it can be even more difficult for the family member/caregiver who is keenly aware of the patient’s progress or lack thereof. The person upon whom the caregiver normally relies for support – the partner – may be the patient, who is ill equipped to deal with anyone’s emotional load besides his or her own. Usual support networks, such as family members or friends, may fail to comprehend the severity of the situation and be unprepared to address the caregiver’s needs.

It is important that the healthcare team be explicit with both the patient and caregiver about the range of emotions they may experience during and after transplantation. Anger about the disease, resentment over the loss of control of the patient’s wellbeing, fear about medical procedures or possible treatment failure, and depression about the slow pace of progress are typical emotions that patients and caregivers face. The patient may express distress by lashing out at the medical team or a loved one. Assuring both patient and caregiver that these emotions are normal, and explicitly telling them what types of assistance are available to help them cope, maximize the likelihood that they will seek and accept help, should they need it.

Patients need to understand that HCT is an extraordinary experience and that they can expect to need more than their everyday coping tools to manage the stress. Normalizing the use of antianxiety or antidepressant medications in this circumstance, and assuring the patient that, in the absence of a history of substance abuse, drug dependence is not likely, can alleviate patients’ concerns about using these tools.

For some patients, the acute care period is the time of greatest stress. For others, the post-transplant period poses the greatest psychologic challenge. The support system provided by the hospital and medical personnel has been curtailed, the busy daily schedule of tests, doctor visits, and other hospital routines is now replaced by long days of isolation and inactivity, and the challenge of resuming a normal lifestyle can be daunting.

> When I left the hospital, I thought the tough part was over. I’ve never been so wrong. The inpatient part was the easiest part of the bone marrow transplant process. I was focused and fired up… All the worldly things, such as my role as a husband and father, were secondary to winning the inpatient battle. Other routine things such as house payments, medical bill, career, and church weren’t even on my mind. Nurses and doctors took care of me. It wasn’t easy, but I felt I was making significant progress toward beating my disease.
>
> The clarity of purpose and sense of progress were lost when I came home. Instead of feeling like a successful patient, I felt like a failed person. All those worldly things, such as my roles as husband and father that I had ignored in the hospital, came roaring back. They were once again important and I felt woefully inadequate in those roles.
>
> The steroids and cyclosporine made me extremely emotional and irrational. I would cry because I had too much milk on my cereal. I couldn’t sleep (steroids), couldn’t shower (Hickman), couldn’t read (no concentration), couldn’t drink coffee in the morning (nausea), couldn’t exercise (no strength) and couldn’t get close to my children (might get an infection). Even taking my medicine was confusing and overwhelming. I spent time worrying about things I couldn’t control. I was convinced I was going to run out of money, lose my house, my dog, etc. There was no measured progress anymore. I felt I was regressing. I was in an emotional rut. [2]

Emotional difficulties can surface weeks or months after transplant. Unexpected rehospitalization for a complication can cause distress. Some experience symptoms similar to post-traumatic stress syndrome: a sight or smell may trigger an unpleasant memory of the transplant experience, leaving the patient shaken.

Returning to work, school, or a social world that has moved on during transplantation can make a patient feel isolated. Fear of relapse causes many patients to avoid long-term planning. It can take many months before a patient is able to get through a single day without thinking about the transplant.

A study has confirmed what many caregivers report: the intensity and duration of transplant-related distress are in some ways greater for caregivers than for patients long term [3]. The caregiver must deal not only with his or her own fears about the patient's health, but also with those of other family members. The caregiver may be thrust into new roles, assuming responsibilities once handled by the recovering spouse. These tasks, coupled with his or her own emotional turmoil, can create significant distress. Some who handled the acute phase of transplant well find themselves emotionally drained and unable to function fully many months later:

> In some ways, it was like we were no longer married. We were never alone. Our whole relationship – everything – was based on illness, medical procedures, hospital, and family. Trying to resume a normal relationship was not possible. Family was continually around – always. I understood, but some days I didn't want to share my husband. I wanted him all to myself.
>
> I had to take over all of his responsibilities – paying bills, yard work, etc. Instead of being an equal partner, it was like having another child to worry about. I felt like I'd lost the man I married and just wanted him back.

Psychosocial support networks for patients and their families

A number of support networks are available for families facing the emotional challenges associated with transplant. BMT InfoNet has a Caring Connections Program that links HCT patients and family members with survivors who can provide emotional support. Patients and survivors are paired according to diagnosis, type of transplant, and other factors the patient may deem important such as gender, transplant center, religion, etc. Survivors are screened to ensure they provide emotional support, rather than medical information, and the pairs communicate by telephone or email. This service is particularly helpful for patients who do not have access to email, or prefer not to communicate by that means. Patients can access this service at www.bmtinfonet.org/patient.html.

Many patients and family members find "list-servs" on the Internet helpful. A list-serv is an electronic mailing list that enables a person to discuss issues with a large number of peers online, and to offer and get support. The list-serv is managed by an individual who typically screens postings before emailing them to the entire list, although the screening is generally limited to weeding out commercial solicitations and hostile emails. Members can opt to receive individual emails from other members after they have been posted, or a daily digest of emails that enables them to determine, by the subject heading, whether any of the issues discussed in the posts are of interest to them. Patients can respond to a posting on the list-serv by emailing the entire list or by privately emailing the individual who posted it.

Discussions on HCT-related list-servs are lively, and many patients find the list-serv an invaluable place to vent their feelings and concerns with others who understand and will empathize. A great strength of list-servs is that they can validate a person's feelings, and normalize his or her experience. A downside is the potential for sharing medically inaccurate or inappropriate information, although patients can easily verify the information with their own physician. More often, valuable information about medical interventions that have worked with other patients is shared which the patient can discuss with his or her physician.

The Association of Online Cancer Resources (ACOR) (www.acor.org) maintains a list-serv for HCT patients (BMT-Talk), one for patients with graft-versus-host disease (GVHD), and list-servs organized by diagnosis. Anyone can subscribe via the ACOR website by following the links under Mailing Lists.

A real-time online chat room also exists for HCT patients and caregivers. BMT-Support (http://bmtinfonet.org/resources) meets several times weekly. There are separate chat rooms for patients and for caregivers. Participants log in at the specified time, and the live online chat is moderated by a survivor with nursing training.

Disease-specific organizations, such as the Leukemia & Lymphoma Society and the International Myeloma Foundation, offer local or online support programs for patients. Although some transplant patients find these helpful, others find discussions with people who have not undergone a transplant unsatisfying. A list of organizations that provide disease-specific information and support can be found on BMT InfoNet's website at www.bmtinfonet.org/resource.

General support groups for caregivers are offered by the Well Spouse Foundation (www.wellspouse.org) and by general cancer support organizations such as Cancer Support Community (www.cancersupportcommunity.org), the Wellness Community (www.thewellnesscommunity.org), and local cancer support centers.

Young adult patients often find resources targeted to their age group helpful. The Ulman Cancer Fund for Young Adults (www.ulmanfund.org) provides a peer support program similar to BMT InfoNet's Caring Connections Program. Young adults can be linked with others whose life has been affected by transplant and read their stories online. The fund also provides educational scholarships to young adults diagnosed with cancer.

Some patients or their family members require individual counseling to help deal with their emotional distress. Although transplant centers may provide psychiatric help and counseling while the patient is undergoing transplant, that support system is often unavailable when patients return home. It can be difficult to find a local mental health professional who is experienced in dealing with cancer- or transplant-related issues. The American Psychosocial Oncology Society (www.apos-society.org) offers a help-line for patients and families seeking a local specialist who is trained in working with cancer patients and can address their needs.

Quality of life post-transplant

From the patient's perspective, successful HCT does not simply mean that the patient survives. It also means that he or she resumes a reasonably normal life after transplant. Significant survivorship challenges are often not discussed during the acute phase of treatment, and even at discharge patients may not be adequately prepared for the psychologic stress that ensues. They may also not be equipped with coping strategies for medical difficulties such as depression, infertility, cognitive problems, chronic fatigue, and sexual difficulties that can occur after HCT.

In 2006, BMT InfoNet invited HCT survivors and their family members to complete an online survey to determine issues of concern to survivors post-transplant. Invitations were issued by email to persons on the *Blood and Marrow Transplant Newsletter* mailing list, and via the home page of BMT InfoNet's website.

A total of 580 persons completed the survey. Respondents identified themselves as:

- an HCT survivor – 68%;
- the parent of a survivor – 12%;
- the spouse/significant other of survivor – 11%;
- the child of a survivor – 1%;
- the sibling of a survivor – 1%;
- other (caregivers or transplant center staff) – 8%.

Some 40% had an autologous transplant, 34% had an allogeneic transplant with a related donor, and 26% had an allogeneic transplant with an unrelated donor.

Respondents reported their last date of transplant as follows:

- 2001–2006 – 55%;
- 1996–2000 – 25%;
- 1990–1995 – 17%;
- pre-1990 – 3%.

When asked which issues, based on their experience, were most important to transplant survivors, respondents checked the following options (more than one answer being allowed):

- emotional/psychologic health of transplant survivor – 72%;
- detecting and preventing long-term complications – 71%;
- fatigue – 63%;
- emotional/psychologic health of the survivor's family – 50%;
- chronic GVHD – 49%;
- learning and memory problem – 46%;
- health insurance – 44%;
- educating local physicians about transplant survivors' needs – 43%;
- secondary cancers – 41%;
- sexuality after transplant – 37%.

In response to an open-ended question, survivors expressed frustration over the lack of resources to deal with the psychologic burden after transplant, including significant depression. They also expressed concern with problems that interfered with their ability to resume work or school and function effectively, such as cognitive deficits or fatigue. Input from this survey was used to develop a 1-day survivorship symposium for HCT survivors in Oakbrook, IL, in April 2007. Presentations by experts on fatigue, psychosocial problems post-transplant, cognitive issues, chronic GVHD, sexuality, infertility, insurance, and employment issues were featured. About 350 survivors and caregivers from throughout the United States attended this educational event, which also featured psychosocial support groups where survivors could network with each other.

For most, this was the first opportunity post-transplant to hear presentations on issues that were significantly impacting their daily life, such as psychosocial difficulties, cognitive issues, and insurance issues, and to discuss them with other survivors. Not only were their experiences validated, but also they learned tools for managing some of the problems. Evaluations were overwhelmingly positive, and more forums of this type were requested.

Many who attended the session on infertility remarked that they had been poorly equipped by their transplant program for this side effect of treatment. Not only had prophylactic measures, such as sperm banking, been ignored in some cases, but few resources were offered them when it was confirmed that they were infertile, either because of prior treatment or because of the transplant. One attendee reported that she had not been advised that infertility was a potential complication of transplant. For some patients, the prospect of infertility post-transplant is as upsetting as the diagnosis itself. Disappointment over the prospect of infertility is not limited to patients who have never had children but extends to some individuals who already have children and would like to expand their family.

Patients' emotional distress can be lessened if they are advised that it is sometimes possible to conceive a child post-transplant. Men may be able to bank a sufficient quantity of viable sperm before treatment to allow attempts at pregnancy post-transplant, provided that prior therapy has not led to aspermia. Some women may be able to conceive a child with assisted reproduction techniques and a donor oocyte. Although these steps do not guarantee a successful pregnancy, the technology is improving each year, and it comforts many patients to know that there is at least a chance of conceiving or carrying a child to term.

Even if a woman does not become infertile immediately after transplantation, the chemotherapy or radiation administered as part of the HCT can reduce the number of viable oocytes and cause her to become prematurely menopausal later on. Therefore, women who wish to conceive a child post-transplant should be counseled that their window of opportunity may have shortened, and plans for pregnancy should be pursued as early as practical [4].

Many transplant survivors experience significant, long-term changes in sexuality following treatment. Problems including lack of libido, inability to perform, and pain are reported by persons with both normal and abnormal hormone levels post-transplant. As one man stated, "It seems like my brain has been cut off from the rest of my body. I can conjure up all sorts of juicy sexual images in my brain, but can't get the rest of my body to perform."

Changes in sexuality can seriously stress a relationship, particularly if the survivor's partner believes that the problem is purely psychologic rather than physical. Although fatigue and psychologic trauma do, indeed, affect sexuality, it is important that patients and their partners know that physical changes occur following transplant that may also affect the survivor's sexuality. Patients should be advised that they may need to develop new approaches to intimacy after transplant to enable both partners to have a satisfying sexual relationship. Patients should be encouraged to report sexual difficulties and be provided with referrals to experts who may be able to help resolve some of the problems.

A significant issue that is often overlooked in discussions with patients is changes in cognitive abilities they may experience, both short and long term. Much has been written about learning difficulties observed in children following HCT, but little note has been taken of the cognitive changes experienced by some adult HCT survivors. Problems reported by survivors include poor concentration, memory problems, stuttering, difficulty in spelling, inability to perform jobs that were previously mastered, and difficulty in learning new tasks. Many survivors have been able to overcome the problem by changing the way in which they manage information, such as making lists, writing down all appointments, keeping notes, and so forth. For others, the solution is not so simple, and the problem may interfere with their ability to perform a job or learn a new skill.

One survivor who was a sculptor said, "Before my HCT, conceptualizing the design and drawing it was the most difficult part of my work. When it came to the actual sculpting, I could do it without even thinking. However, after my HCT, I could barely concentrate on the sculpting. Conceptualizing and drawing the design became impossible. No one has been able to explain why this happened, or suggest what I can do to relieve the problem."

One woman who underwent high-dose chemotherapy and autologous HCT was delighted that her company held her computer operator job for her while she underwent treatment. However, when she returned to work 4 months later, she found that the nature of the job had changed and she needed extensive training to perform the new tasks. After 2 weeks of training, she feared she would be fired. As she explained, "When I'm at work and in training, all the explanations make perfect sense. But then I come home, fix dinner, and clean up. When I sit down to review the day's lesson I'm totally confused. By the following morning, I've completely forgotten everything I learned the previous day."

Although most patients do not develop cognitive problems as severe as those experienced by the two persons described above, patients should be advised that changes in cognitive abilities sometimes occur. If cognitive problems do develop, survivors will be less frustrated if they know that their problem is not unique. Advance warning about this potential problem can also relieve tension in the home. Tired and distraught family members will understand that the survivor's new forgetfulness is not simply laziness, but a consequence of the treatment.

Chronic GVHD

Survivors with chronic GVHD face a unique set of challenges post-transplant that can severely impact their quality of life. Preliminary results of a 2007 survey conducted by BMT InfoNet of more than 300 transplant survivors who have or had chronic GVHD found that chronic GVHD interfered with the daily activities of nearly two-thirds of patients and nearly 40% of other family members; 41% reported financial difficulties as a result of chronic GVHD, and nearly one-third reported marital difficulties; 57% of survivors with chronic GVHD reported sadness or depression, and 41% said the mental health of other family members had also been negatively impacted.

Survivors with significant chronic GVHD often feel isolated. There are few HCT support groups available for patients after transplant, and disease-specific support groups seldom include a significant number of survivors who have been through transplant, let alone those who have experienced chronic GVHD. Improvements in treatment are rare, and opportunities to network and learn from other survivors are limited.

For those with Internet access, a list-serv on ACOR provides a forum for patients with chronic GVHD. List members discuss their symptoms, therapies they have tried to manage them, and clinical trials in which they have participated. Often, a newly diagnosed person or a list member who has become overwhelmed with the difficulties presented by chronic GVHD shares their distress with list-mates, and the outpouring of emotional support is impressive.

When the patients with chronic GVHD surveyed by BMT InfoNet were asked what resources would be helpful, 98% said a central website with chronic GVHD information, 93% said a print newsletter about chronic GVHD, and more than 8% said a DVD with information about chronic GVHD, a local meeting with transplant doctors to discuss chronic GVHD, or a local support group would help. BMT InfoNet is exploring the feasibility of hosting a chronic GVHD website as a centralized resource for patients.

Other resources for patients and survivors

As described earlier, a number of resources are currently available for families dealing with the prospect of HCT in addition to survivors.

BMT InfoNet

BMT InfoNet provides online resources for patients during all phases of treatment:

- *Transplant center directory* (http://bmtinfonet.org/transplantcenters): an online directory of transplant programs in the United States and Canada providing detailed information on each program, including the medical director and key medical personnel; whether or not the center is accredited by the Foundation for Accreditation of Cellular Therapy and/or is an affiliate of the NMDP; the number of transplants performed, by type, in each of the three previous years; patient age and donor match criteria; and contact information. Patients can search the database by name of center, state, diagnosis, and age of patient (pediatric or adult). Data posted are provided directly to BMT InfoNet from each transplant center and are updated annually.
- *BMT InfoNet books* (http://bmtinfonet.org/products/books): access to patient handbooks about HCT and being a caregiver, and a calendar featuring transplant survivors, including one patient guide about HCT in Spanish.
- *Survivorship forum DVD*: videotaped presentations and slides from BMT InfoNet's Celebrating a Second Chance at Life Survivorship Conference.
- *Caring Connections Program* (http://bmtinfonet.org/services/support): a moderated peer support program linking patients with survivors who can provide emotional support.
- *Resource directory* (http://bmtinfonet.org/resources): a list of organizations that provide disease-specific information and financial support to HCT patients.
- *E-newsletter*: an electronic bulletin with information of interest to patients.
- *Help-line* (http://bmtinfonet.org/services/contactus): a service enabling patients to pose questions to a panel of medical experts.
- *Survivorship forums*: regional conferences to educate patients and survivors.
- *Attorney referral service*: for patients having difficulty securing insurance approval for their transplant or related treatment.
- *Directory of drugs used during transplant* (http://bmtinfonet.org/drugs): describes the purpose of the drug in the transplant setting, and potential side effects grouped by likelihood of occurrence.

In addition, patients in the United States can telephone toll free 888-597-7674 for personalized help with any of the above services.

Be the Match/National Marrow Donor Program

Be the Match provides resources for patients undergoing an HCT with an unrelated donor, some of which are also appropriate for patients transplanted with a related donor or undergoing an autologous transplant:

- Information about the donor search and HCT process.
- *Transplant center directory*: this details the cost of HCT with an unrelated donor at transplant programs in the United States, and also medical personnel at each center, the number of transplants performed for specific diseases, raw and risk-adjusted survival rates, and contact information.
- *DVDs*: for special-interest groups including young adults, pediatric patients, and older patients.
- *List of cord blood banks*: including contact information and criteria for donating cord blood.
- *Financial guide*: a workbook to help patients organize their finances.
- *Survivorship newsletter*: discussing some of the issues that survivors face from 3 months to 2 years after transplant.

Some of the online information is available in Spanish and other languages. Be the Match's Office of Patient Advocacy (OPA) provides telephone counseling for patients undergoing HCT with an unrelated donor, including help with tracking donor searches and research on disease- and treatment-related topics.

National BMT-Link (www.nbmtlink.org)

Offers a guide for HCT caregivers, a DVD entitled *The New Normal*, and a peer support program for HCT patients. Periodic teleconferences and an in-person support group for Michigan residents are also offered.

Center for International Blood and Marrow Transplant Research

The Center provides consensus guidelines on long-term follow-up care for patients after HCT (http://www.cibmtr.org/ReferenceCenter/Patient/Guidelines/pages/index.aspx). Recommendations are outlined for 6-month, 1-year, and subsequent annual check-ups. A separate version is available for patients and physicians. The information can be downloaded, or hard copies can be ordered.

The Children's Oncology Group

The Children's Oncology Group (www.survivorshipguidelines.org) provides long-term follow-up guidelines for pediatric cancer survivors.

National Institutes of Health

Sponsored Clinical Trials (http://clinicaltrials.gov) lists federally supported clinical trials with separate descriptions available for healthcare professionals and the lay public. Included are enrollment criteria, contact information for principal investigators, and a brief description of the trial's objectives.

The *Clinical Center* (http://clinicalcenter.nih.gov) in Bethesda, MD, provides free clinical care to patients who qualify for one of their clinical trials. Protocols for some HCT patients are available.

Other resources

- The *Children's Hospital Oakland Research Institute* (www.chori.org) offers free cord blood banking for families who have a child with a disease that is treatable by HCT.
- *Private HLA typing* (www.bonemarrowtest.com) to determine whether a person is a potential donor for a family member is sometimes requested by a physician without providing guidance to the potential donor on how to be tested. Some DNA laboratories provide this service only through a contract with a medical facility. Kashi Clinical Laboratories will arrange the service directly with the potential donor and provides necessary testing supplies and results via mail.
- *Disease-specific information and fundraising* or financial assistance for HCT patients is provided by a number of organizations. A list of such groups is maintained by BMT InfoNet (http://bmtinfonet.org/resources).
- The *ACOR* (www.acor.org) provides information about various diagnoses and treatment options, in addition to list-servs where patients and survivors can connect. The HCT group (BMT-Talk) and GVHD group are both very active and supportive lists.
- *BMT-Support* (www.bmtsupport.org) offers a real-time online chat room each week for HCT patients and caregivers. The chat is moderated by an HCT survivor with a nursing background.

Conclusion

Undergoing HCT is a very difficult experience both physically and emotionally. Working with a compassionate medical team that is attuned not only to the patient's physical needs, but also to his or her emotional needs, is a blessing that all patients will deeply appreciate. Being fully armed with information about long-term quality of life issues will help make the HCT patient's transition from being a successful patient to a successful survivor much easier.

Transplant physicians need not be the sole source of information for patients and survivors. Directing patients to resources outside the transplant program's area of expertise, such as experts in fertility, sexuality, cognitive retraining, fatigue management, and psychosocial support, will expand a patient's support network and enhance the likelihood that the survivor and his or her family will live well, even in spite of ongoing medical concerns.

Although HCT creates enormous challenges for patients and their loved ones, many survivors find personal growth in the experience. When asked what he would advise patients about to go through transplant, Steve Dugan, a 4-year survivor of an allogeneic HCT, offered this advice:

> **Expect** to be amazed by how much strength you find in yourself.
> **Expect** the shock of what's happening to you to continue for some time. Stolid acceptance of your situation is just not realistic.
> **Expect** to feel very alone and, at times, even detached from your surroundings.
> **Expect** to be shown, by the person closest to you, what love really means – maybe for the first time in your life. For me, that was, and always will be, my wife and best friend, Joyce. In Greg Allman's words: "When life's game gets so hard, I hold the highest card – the Queen of Hearts." That's Joyce!
> **Expect** there to be days when you say to yourself, "I just can't do this any more."
> **Expect** to make outrageous promises to God in exchange for clemency.
> **Expect** to find a whole new group of heroes – nurses.
> **Expect** your real friends to show up. Be prepared for surprises – both good and bad.
> **Expect** to be truly shaken – and, at times, depressed, by your physical appearance. Just remember: the mirror reflects what you look like – not who you are.
> **Expect** to catch yourself sometimes staring at your children, not wanting to miss a single breath they take.

But most importantly:

> **Expect** and believe that some day soon you will look at this whole episode of your life – in the rearview mirror.

Kathleen Jones, who underwent two HCTs after being diagnosed with chronic myeloid leukemia, summarizes a perspective shared by many survivors:

> The bone marrow transplants affected my life in every way. I am thankful to wake up every morning. I have learned compassion and empathy for those unable to be normal. I have learned not to spend time worrying about little unimportant things. I have learned to appreciate sunrises and sunsets. I have learned what it means when others are kind. I have learned the value of my family in my life, and just how precious life is, and how much I want to live it.

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CHAPTER 36

Hematopoietic Cell Procurement, Processing, and Transplantation: Standards, Accreditation, and Regulation

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Introduction

Hematopoietic cell transplantation (HCT) is established therapy for many serious congenital and acquired diseases of hematopoietic origin, including many high-risk malignancies. Hematopoietic cells (HCs) obtained from bone marrow, peripheral blood, and placental and umbilical cord blood cells are common graft sources derived from autologous, related, or unrelated donors. HCs are also used for functions other than as progenitor cells, such as post-transplant donor lymphocyte infusion [1]. Recent advances have expanded the range of patients who can successfully be treated using cord blood transplantation [2,3]. Cellular therapies have also expanded beyond hematopoietic and immunologic reconstitution to regenerative cellular interventions and new therapies using mesenchymal stem cells, dendritic cells, and gene-modified and other cells [4]. Rapid evolution of such therapies has heightened concern about the safety, purity, potency, and efficacy of these cellular products.

Governmental regulation

Governmental oversight of cellular therapies in the United States is based upon the Public Health Services (PHS) Act, the Federal Food, Drug, and Cosmetic Act (FFDC), and the Stem Cell Therapeutic and Research Acts of 2005 and 2010. Under the authority of the PHS and FFDC Acts, the Food and Drug Administration (FDA) is empowered to enact binding legislation related to cellular therapy products. Under the Stem Cell Acts, the Health Resources and Services Administration (HRSA) of the U.S. Department of Health and Human Services (HHS) oversees the several components of the C.W. Bill Young Transplantation Program and the National Cord Blood Inventory. Worldwide, blood and marrow stem cells are frequently transported across international borders to reach intended recipients. Each individual country has its own national and regional regulations. The FDA collaborates with foreign regulatory authorities to harmonize regulatory requirements and minimize the regulatory burden.

Role of the FDA

In 1997, the FDA published its unified and tiered approach to the regulation of traditional and new human cells, tissues, and cellular and tissue-based products (HCT/Ps) intended to provide regulatory uniformity and only the amount of regulation necessary to protect the public health [5]. Products expected to be at a higher risk for disease transmission or for contamination during collection or processing, such as allogeneic cells or cells highly manipulated ex vivo, are subject to more regulation than those products with less risk, such as autologous or minimally manipulated products. These FDA regulations are based on five public health and regulatory concerns: (1) prevention of transmission of communicable disease; (2) assurance that necessary processing controls exist to prevent contamination and to preserve the integrity and function of cells and tissues; (3) assurance of clinical safety and effectiveness; (4) assurance of necessary product labeling, including permissible promotion for proper product use; and (5) establishment of a mechanism for the FDA to communicate with the cell and tissue industry. A Tissue Reference Group, comprised of representatives of the Center for Biologics Evaluation and Research and the Center for Devices and Radiological Health, was established to provide a single reference point within the FDA for all regulatory questions about HCT/Ps. Recent decisions of the Tissue Reference Group have defined the appropriate regulatory pathway for specific products.

FDA regulations for cellular therapies are defined in Part 1271 of Chapter 21 of the Code of Federal Regulations (21 CFR 1271) that became effective May 25, 2005 [6–8]. This regulation establishes a uniform system for registration of manufacturers of HCT/Ps including HCs, delineates donor eligibility criteria, and describes current good tissue practices to prevent the introduction, transmission, and spread of communicable diseases [7,8]. HCT/Ps that meet the criteria listed below are regulated solely under the PHS Act, Section 361.

1. The product is minimally manipulated.
2. The product is intended for homologous use only, as reflected by the labeling, advertising or other indications of the manufacturer's objective intent.
3. The product does not involve combination of the cells or tissue with another article (excepting water, crystalloid, or sterilizing, preserving or storage agent).

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4 The product does not have a systemic effect and is not dependent upon the metabolic activity of living cells for its primary function (unless autologous, first- or second-degree relative donor or for reproductive use) [6].

Other HCT/Ps, including unrelated donor allogeneic hematopoietic stem/progenitor cells, are regulated additionally as "biologic products" under PHS Act Section 351, current Good Manufacturing Practices, Quality Systems Regulations, and other portions of CFR, Chapter 21. These products are more than minimally manipulated (undergo processing that alters the biological characteristics of the cells), are used for other than their normal function, are combined with non-tissue components, and/or have a systemic effect (including unrelated donor HC) or are dependent upon the metabolic activity of living cells (excluding those for autologous or first- or second-degree related recipients) [5].

Under 21 CFR 1271, any establishment that participates in the manufacture of HCT/Ps must register with the FDA initially and annually, and maintain an updated list of HCT/Ps at least every 6 months.

Subpart C requires HCT/P facilities to screen and test cell and tissue donors for risk factors for, and clinical evidence of, relevant communicable disease agents or disease. Donor screening is the review of relevant medical records for indications of past or present infection and for risk factors for a relevant communicable disease. Donor testing is performing laboratory tests on a specimen, generally a blood specimen, collected from the donor to determine if she or he has been exposed to or infected with a relevant communicable disease or its agent. Testing must be performed by a laboratory certified to perform such testing on human specimens under the Clinical Laboratory Improvement Amendments of 1988 (42 U.S.C. 263a) and 42 CFR Part 493 (or equivalent) using appropriate FDA-licensed, approved, or cleared donor screening tests in accordance with manufacturer's instructions. Criteria for a relevant communicable disease are: (1) the disease agent is potentially transmissible by the HCT/P; (2) the disease agent or disease is sufficiently prevalent to affect the potential donor population; (3) the disease is potentially life-threatening or extremely serious; and (4) appropriate screening measures and/or testing assays are available. For allogeneic donors of HCs (including viable leukocyte-rich products), relevant communicable diseases and agents as defined in 21 CFR 1271 include at least: human immunodeficiency virus type 1 and type 2, human T-lymphotrophic virus type I and type II, hepatitis B virus, hepatitis C virus, *Treponema pallidum*, and human transmissible spongiform encephalopathy, including Creutzfeldt–Jakob disease [9–11]. Additional diseases/disease agents subsequently identified as relevant communicable diseases requiring donor screening are: West Nile virus, sepsis, vaccinia, and *Trypanosoma cruzi* (Chagas disease agent) [12–14]. An interorganizational Cellular Therapies Task Force has developed a Donor History Questionnaire, User Instructions, and supporting informational materials to screen hematopoietic progenitor cell (HPC) donors for communicable disease risk factors in accordance with these requirements. These materials are updated regularly and are available on organizational websites.

The current good tissue practice (cGTP) regulations are intended to ensure that products do not contain relevant communicable disease agents, are not contaminated or become contaminated during manufacturing, and that the function and integrity of products are not impaired through improper processing [6]. These GTP regulations include the requirements for an adequate organizational structure, sufficient personnel, and the establishment and maintenance of a comprehensive Quality Management Program. The Quality Program is responsible: (1) to ensure procedures related to core GTP requirements are established and maintained and personnel are appropriately trained in these requirements; (2) to ensure compliance with standard operating procedures requirements; (3) to receive, investigate, evaluate, and document complaints; (4) to share information related to potential contamination of HCT/Ps or disease transmission; (5) to ensure that HCT/P deviations are investigated and trended, and corrective and preventive actions are taken; and (6) to periodically audit activities related to core requirements. Core GTPs include adequate facilities; equipment, supplies, and reagents; environmental control and monitoring; proper processing, including process change and process validation; proper labeling and labeling controls; storage at proper temperatures in a manner to prevent mix-ups, including quarantine storage; and procedures for receipt, predistribution shipment, and distribution of HCT/Ps. Facilities must also report adverse reactions and product deviations and maintain procedures for tracking the product from recipient to donor and from donor to recipient or ultimate disposition.

Subpart F of 21 CFR 1271 describes the Inspection and Enforcement provisions. FDA conducts onsite inspections at its discretion with or without prior notification to determine compliance with applicable regulations. Facilities found to be out of compliance with regulations are ordinarily required to document compliance; FDA may also issue an order for retention, recall, or destruction of an HCT/P, or cessation of manufacturing.

FDA periodically issues guidance documents under the provisions of Good Guidance Practices, delineated in the Code of Federal Regulations to describe the Agency's interpretation of a regulatory issue, or to clarify specific issues which have been misunderstood [15,16]. Several of these documents are of particular relevance [9,11,17,18]. Draft guidance may be published, wherein interested persons are invited to comment on the current Agency thinking related to issues in the manufacture of cellular therapy products [19].

Cord blood cells

FDA regulations apply to all unrelated cord blood transplants performed in the United States, regardless of the country of origin of the cord blood unit. Umbilical cord blood cells are HCT/Ps as defined in 21 CFR 1271.3(d), and these regulations, which encompass registration and listing, donor eligibility, and good tissue practices, apply. In addition, as of October 20, 2011, unrelated donor, minimally manipulated cord blood is regulated as a biologic drug, requiring a biologics license application (BLA) for products intended for hematopoietic reconstitution for specified indications. The licensure process invokes additional regulations [20]. Licensed cord blood units meet all specifications in the licensure guidance and in the license application, including but not limited to description and characterization of the cord blood unit (safety, purity, potency, and identity), facility description, and validated manufacturing methods (including collection, processing, storage, selection, shipping and handling). To date, a few cord blood banks have achieved licensure; other applications are in progress.

Continued availability of unlicensed cord blood units is essential to ensure diversity of HLA phenotypes and access to transplantation for individuals with less common HLA types. Unlicensed cord blood units are available for transplantation under an approved Investigational New Drug (IND) application. The IND may be held by a cord blood bank, by a physician or transplant center, or by a national or international registry involved in coordinating the distribution of cord blood units, or by another sponsor [21]. Cord blood units obtained from ineligible donors and distributed via the IND mechanism require documentation of urgent medical need as defined in 21 CFR 1271.60. The National Marrow Donor Program holds an IND for the distribution of unlicensed, minimally manipulated, unrelated allogeneic cord blood units intended for hematopoietic reconstitution for specified indications. Unlicensed cord blood units may also be available through a separate treatment IND for patients with other serious conditions who lack therapeutic alternatives.

State regulation

Governmental regulation of HC therapy at the state level is fragmented. Some states have adopted mechanisms of qualifying HCT programs and facilities, such as certificate of need process or licensure. Some states, such as New York, have adopted specific regulations for processing of and storage facilities for HCT/Ps [22,23]. Other states require accreditation by a professional organization. Currently, Massachusetts and Maryland require accreditation by the Foundation for the Accreditation of Cellular Therapy (FACT) to perform HCT within those states. Many US states have little specific regulation.

Stem cell Acts

The Stem Cell Therapeutic and Research Act of 2005 and the Stem Cell Therapeutic and Research Reauthorization Act of 2010 authorize the C.W. Bill Young Cell Transplantation Program and the National Cord Blood Inventory, managed by the Health Resources and Services Administration (HRSA). The overall goal of the Stem Cell Acts is to increase access to unrelated donor transplantation and improve transplant outcomes. The Advisory Council on Blood Stem Cell Transplantation provides expert recommendations to the Secretary of Human and Health Services on advances in stem cell transplantation and the activities of both the Program and the National Cord Blood Inventory (NCBI), including the designation of the entities to provide accreditation for participating cord blood banks.

There are four major components to the C.W. Bill Young Transplantation Program. Three components are managed by the National Marrow Donor Program (NMDP): The Office of Patient Advocacy/Single Point of Access and the Coordinating Centers for Bone Marrow and Cord Blood. The fourth component, the Stem Cell Therapeutic Outcomes Database (SCTOD), is operated by the Center for International Blood and Marrow Transplant Research (CIBMTR), and requires reporting of all related and unrelated donor allogeneic HCT performed in the United States or performed using products procured from the United States. Created in 2004, CIBMTR is a partnership between the research arm of the NMDP and the International Bone Marrow Transplant Registry. Its expertise is in collecting and managing data, working with transplant centers and regulatory agencies, histocompatibility and bioinformatics, and in creating and managing research sample repositories. The SCTOD allows analysis of diagnoses for which transplant may be an option, novel therapeutic uses of HCs, transplant preparative regimens and procedures, transplant center-specific patient outcomes, patient access to HCT, quality of life, and donor registry and cord blood inventory size and adequacy.

The National Cord Blood Inventory (NCBI) provides some funding to selected cord blood banks for the collection and storage of at least 150,000 cord blood units to be available to treat patients who may need an unrelated donor graft. Goals of the NCBI are to encourage cord blood donation, particularly among racially or ethnically diverse populations, to collect and store units in designated cord blood banks, to ensure appropriate consent and confidentiality, and to provide cord blood unit data to the SCTOD. Thirteen US cord blood banks currently participate in this effort.

Voluntary professional accreditation

Foundation for the Accreditation of Cellular Therapy (FACT)

Historical background

FACT is non-profit organization affiliated with the University of Nebraska Medical Center, Omaha, Nebraska that was cofounded in 1995 by the International Society for Cellular Therapy (ISCT) and the American Society for Blood and Marrow Transplantation (ASBMT) to promote quality patient care and laboratory practice in HCT through the establishment of comprehensive professional standards and implementation of its voluntary accreditation program for the procurement, processing, and transplantation of HC products [24,25]. FACT was initially founded as the Foundation for the Accreditation of Hematopoietic Cell Therapy (FAHCT). The name was changed in December 2001 to encompass, in addition to HC products and therapies, the new and exciting therapies using mesenchymal stem cells, dendritic cells, targeted lymphocytes, genetically modified cells, pancreatic islets, and others.

ISCT was formed in 1992 as a professional society of scientists and physicians working in HC manipulation. Its membership includes most of the major HCT programs worldwide. The Regulatory Affairs Committee of ISCT developed the first draft of Standards for Hematopoietic Cell Collection and Processing in 1994. The other parent society of FACT, ASBMT was formed in 1993 as a professional society of physicians and investigators involved in the clinical conduct of HCT. The ASBMT Clinical Affairs Committee developed the first Clinical Standards for Hematopoietic Cell Transplantation. Believing that quality care can only be achieved if both clinical and laboratory issues are addressed, these laboratory and clinical standards were merged to form the basis for the first edition of FAHCT's *Standards for Hematopoietic Progenitor Cell Collection, Processing and Transplantation*, published in 1996 [26]. These Standards were unique in breadth and depth, applicable to all phases of the collection, processing, storage, and administration of hematopoietic progenitor cells (HPCs) regardless of tissue source (bone marrow, umbilical cord blood, peripheral blood, or other tissue source); and to "therapeutic cells," nucleated cells collected for use other than as HPCs. Standards define an infrastructure required for the safe and efficacious collection, processing, storage, and use of HCs; define the minimum education and experience necessary for staff; and require an ongoing assessment of patient outcome, including neutrophil and platelet engraftment and 100-day and one-year morbidity and mortality.

A companion accreditation manual was published to provide guidance to applicant facilities and personnel and to FACT inspectors [27]. Based upon this first edition of standards, the FACT accreditation program was initiated in 1996. The first on-site inspections were performed in 1997; and the first US HCT accreditations were awarded in March 1998.

In 1997, representatives of FACT, ISCT, and ASBMT collaborated with members of The International NetCord Foundation, an independent association of cord blood banks, to draft additional standards to cover the complexity of cord blood banking. The first edition of NetCord–FACT *International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection, and Release* was developed by consensus of international experts in the field, initially published in June 2000, and revised in 2002 [28]. NetCord–FACT Standards superseded all cord blood standards in FAHCT *Standards for Hematopoietic Progenitor Cell Collection, Processing, and Transplantation*, excepting those clinical standards related to the transplantation of cord blood cells.

These comprehensive standards apply to both unrelated donor cord blood units and units collected and stored for a specific family or designated recipient. A key concept is that the accredited cord blood bank is responsible for all phases of donor recruitment, screening, and testing, and for product collection, transport, processing, storage, and release for clinical use [29]. These Standards form the basis for the voluntary accreditation of cord blood banks worldwide. The first cord blood banks achieved FACT–NetCord accreditation in 2004.

FACT representatives worked with colleagues from the European Group for Blood and Marrow Transplantation (EBMT) and ISCT-Europe to establish the Joint Accreditation Committee of ISCT-Europe and EBMT (JACIE) [30]. JACIE adopted the first edition of the FAHCT Standards in 1999 [31]. The second edition of the Standards was jointly reviewed by FACT and JACIE; subsequent editions have been jointly developed and entitled *FACT–JACIE International Standards for Cellular Therapy Product Collection, Processing, and Administration* [32]. The primary aim of JACIE is to improve the quality of HCT in Europe through its accreditation and education programs, and to work toward international harmonization of standards and regulations.

Based on the FACT model, the JACIE accreditation program was fully implemented in January 2004 with initial support from the European Union under the Public Health Programme (2003–2008). During this process, JACIE inspectors and staff found that almost all centers were functioning at a high level of excellence, with the majority having only minor deficiencies noted at the on-site inspection. When formally surveyed, these centers reported that implementation of JACIE accreditation required a significant investment of time and resources, but all believed that the result was a demonstrable improvement in the accredited program [33]. Recently, the impact of the JACIE quality management system was assessed among the 421 transplant teams in Europe [34,35]. Patient outcome after allogeneic transplant was systematically better when the transplantation center was at a more advanced phase of JACIE accreditation, independent of the year of transplantation and other risk factors. Improvement was assessed by relapse-free survival, comparing each center against itself during four periods of time: pre-application for accreditation, preparation for accreditation, application, and accredited. This approach was possible because centers in Europe did not embark on the JACIE accreditation process at the same time. A similar trend toward improved survival was also seen among autologous transplant recipients. Additional information and documents are available on the JACIE website (www.jacie.org).

Standards

All FACT Standards are developed by consensus of world-renowned experts active in cellular therapy. Development includes discussion with representatives of regulatory bodies and legal review. Standards are based on established evidence from the medical literature whenever possible, incorporate sound recommendations that foster quality medical and laboratory practice; and have maintained their unparalleled depth and breadth through subsequent revisions. All Standards require compliance with applicable law, but may exceed minimum regulatory requirements.

An international oversight committee is responsible for each edition of Standards. An overall Standards Committee Chairperson is appointed by the FACT Board of Directors for a term to encompass the development and publication of one edition of each set of standards, cellular therapy and cord blood banking. Each edition is published for comment by members of the cellular therapy community and the public. Each comment is reviewed, and incorporated as appropriate.

FACT–JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration

Cellular Therapy Standards, now in the sixth edition, are designed to provide minimum guidelines for facilities and individuals performing HCT and related cellular therapies [32]. Central to the Standards is the requirement that all clinical, collection, and processing facilities develop and maintain a comprehensive Quality Management Plan that includes at least the following components: defined organizational structure; personnel requirements including qualifications and training; process and procedure development and document control; agreements; outcome analysis and product efficacy; audits; management of errors, accidents, complaints, and adverse events, including management of products with positive microbial culture results; product tracking; and, where appropriate, validation and/or qualification of critical procedures, reagents, supplies, equipment, and facilities [36,37]. The current edition also includes many of the regulatory requirements from the United States FDA and the Directives of the European Union [6,38–40]. Cellular therapy product proper names are defined as the official terminology of *ISBT 128* [41,42].

Standards for each participating service or facility in the cellular therapy program require minimum procedure volumes and leadership experience, adequate facilities, specific content and format for standard operating procedures, and specific personnel. Participating services are required to maintain active communication with each other. Histocompatibility testing must be performed according to currently accepted standards and protocols by a laboratory accredited by the American Society for Histocompatibility and Immunogenetics, the European Federation for Immunogenetics, or an accrediting body providing equivalent services. All other laboratory testing must be performed by laboratories appropriately licensed and/or accredited for the specific assays.

Clinical standards define a blood and marrow transplant program as an integrated medical team housed in a defined location, with a Clinical Program Director(s) and common staff training programs, protocols, and quality management systems that use HC collection and processing facilities that meet FACT–JACIE Standards. Clinical standards enumerate support and consultative staff; cover donor evaluation, selection, eligibility, and consents; provide minimum guidelines for cellular therapy product administration, including the preparative regimen of high-dose therapy; describe the appropriate management of clinical research and Institutional Review Board-approved protocols; require maintenance of complete and accurate records; and require outcome analysis of both individual patients and aggregate data.

Cell collection standards define elements common to both bone marrow- and apheresis-derived peripheral blood HCs, and detail those requirements unique to each cell source. Comprehensive laboratory standards detail requirements for personnel; process controls; inventory management; validation and qualification of facilities, supplies, reagents and equipment; labels and labeling; storage; transport; and records. The intent is to establish that the laboratory is operated in a responsible and responsive manner, and that deviations in processes or products are documented, investigated, and appropriately communicated to the clinical team. Laboratory and collection personnel are expected to follow clinical outcome as one measure of product safety and efficacy.

Notable changes in the sixth edition of the HPC Standards include,

1. Change in the title to add Hematopoietic, to define the scope of these requirements, particularly for the increasing number of accredited facilities that also support non-hematopoietic cellular therapies. FACT has published a separate set of Standards (the "Common Standards for Cellular Therapy") applicable to non-hematopoietic indications; however, the current Hematopoietic Standards are all-inclusive. Facilities pursuing accreditation for hematopoietic services in addition to other specialties may consult these Hematopoietic Standards as a single reference.
2. Revision in the definition of a single clinical program to remove the requirement for geographic proximity, but retain the intent that all clinical sites within a single program demonstrate regular interaction. Advances in technology have enabled clinical sites across greater distances to cohesively interact.
3. Increased emphasis on outcomes and outcome analysis. Outcome of HPC therapy must include individual product / patient data and aggregate data for each type of product or recipient type. For HPC products intended for hematopoietic reconstitution, review of outcomes must include acute and chronic graft versus host disease and central venous catheter infection, in addition to engraftment and overall and treatment-related mortality at 100 days and one year as required in the fifth edition. Further, the Clinical Program should achieve one-year survival outcome within or above the expected range when compared to published data. For US programs, this is intended to utilize CIBMTR Transplant Center-Specific Outcome Data at a minimum. If the expected one-year survival outcome is not achieved, the accredited Clinical Program will be required to submit a corrective action plan that includes investigation of the causes for less than expected survival and process improvement aimed at correction of those issues. Programs are expected to also assess other relevant outcome measures.
4. Definition of minimum amount of annual continuing education as ten hours per year for physicians, advanced practice professionals, pharmacists, and quality manager. Appropriate subject matter is also defined.
5. Addition of specific requirements for pharmacists and physicians-in-training who participate in clinical care.
6. Programs must be actively implementing ISBT 128 technology in addition to use of standard terminology. Previously, a plan to implement ISBT 128 was required.
7. Specific procedures are required for administration of ABO-incompatible products, including indications for red cell and/or plasma reduction. Additionally, this topic is a new requirement for attending physician education and competency.
8. For cord blood units, programs must have defined procedures to verify unit identity.
9. Informed consent and donor evaluation by a health care provider who is not the primary provider for the recipient is now required rather than recommended [43–45].
10. Pregnancy test rather than only assessment is required.
11. Clinical programs must have a policy for anti-HLA antibody testing in mismatched donors.
12. Histocompatibility laboratory accreditation broadened slightly, to allow demonstrable equivalence.
13. Clinical programs must utilize appropriately accredited laboratories for chimerism testing.
14. Numerous changes were made in quality management requirements, including strengthening the requirements for document control, annual reporting, and deviation investigation and resolution.

The Accreditation Manual has been updated to utilize increasingly universal language and to include governmental regulations of the European Union, the Australian Therapeutics Goods Administration, and others. FACT–JACIE Standards and the accompanying accreditation guidance manual are available in print and online at the FACT website (www.factwebsite.org).

The Accreditation Manual has been updated to utilize increasingly universal language and to include governmental regulations of the European Union, the Australian Therapeutics Goods Administration, and others. Guidance material is organized to include the standard, followed by Explanation, Evidence (documents that may be requested or provided as documentation of compliance), and Examples (specific processes that would be considered to be compliant with the Standards). FACT–JACIE Standards and the accompanying accreditation guidance manual are available in print and online at the FACT website (www.factwebsite.org).

NetCord–FACT International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection, and Release

These Standards are intended for the field of cord blood banking, in which a cord blood bank is defined as an integrated team responsible for the collection, processing, testing, banking, selection, and release of cord blood units, either for unrelated donor use or restricted for use by a specific donor family [29]. There are some differences between Standards for family units and Standards for unrelated donor units, and in the methodologies employed to meet these Standards, particularly in the relationships among the bank, the cord blood unit collector, the donor, and the collection facility. However, in either case, Standards are inclusive of all processes from donor recruitment through distribution for administration. Most processing standards are similar due to the importance of adequate numbers and viability of cells.

Cord Blood Bank Standards require that each cord blood bank establish and maintain a comprehensive quality management program. The bank staff is required to follow clinical outcomes from each unit released for transplant in sufficient detail to ensure that the procedures in use continuously provide a safe and effective product, and to assess aggregate data as part of a stability program designed to determine the appropriate expiration date for cryopreserved units. Comprehensive processing, storage, and labeling standards are consistent with *ISBT 128* terminology and labeling requirements [41,42].

An Accreditation Manual accompanies the Cord Blood Standards and provides Explanation, Evidence (documents that may be requested or provided as documentation of compliance), and Examples (specific processes that would be considered to be compliant with the standards), similar to the Cellular Therapy Accreditation Manual [46]. Standards and guidance manual are available in print and online at the FACT website (www.factwebsite.org).

Accreditation process

The goal of the voluntary inspection and accreditation is to improve the quality of care at each transplant center in the expectation that these improvements will lead to improvement in patient outcomes. Multiple levels of review and oversight ensure accreditation is consistently awarded based upon documented compliance with the published international standards as assessed through submitted documents and a rigorous and comprehensive on-site inspection. The process is conducted in a collegial and collaborative manner among peers, and is designed to accommodate various practices that are compliant with the Standards. All inspectors are volunteers

Table 36.1 Foundation for the Accreditation of Cellular Therapy (FACT) Inspector Qualifications

Hematopoietic Cell Therapy Inspector
- Individual member of ISCT, ASBMT, or American Society for Apheresis (ASFA)
- Affiliated with a FACT-accredited program
- Meets all educational and experience requirements
- Attended a FACT training course; passed written exam over current Standards and accreditation processes, and successfully completed inspection as a trainee
- Documented competency on FACTWeb
- Maintains current statements of compliance with confidentiality, duality of interest, and other policies
- Participates in at least one FACT continuing education event annually

Clinical Program Inspector
- Is a licensed physician
- Has at least 2 years' experience in hematopoietic cell transplantation

Apheresis Collection Inspector
- Has a relevant doctoral, nursing, or biological science degree
- Has completed formal training in apheresis or has at least 1 years' experience in peripheral blood progenitor cell collection by apheresis as a director, medical director, physician, supervisor, or assistant supervisor

Cell Processing Facility Inspector
- Has relevant doctoral or biological science degree
- Has at least 2 years' experience as director, medical director, or supervisor of a cell processing laboratory

Processing Facility with More than Minimal Manipulation Inspector
- Processing Facility Inspector qualifications plus
- Has experience processing hematopoietic-derived cells with more than minimal manipulation under IND

Cord Blood Bank Inspector
- Individual member of ISCT, ASBMT, ASFA, ISCT-Europe, or NetCord
- Affiliated with a FACT or JACIE accredited hematopoietic cell therapy program or FACT–NetCord accredited or applicant cord blood bank
- Meets all educational and experience requirements
- Has attended FACT–NetCord training course, passed a written exam over the current CBB Standards and processes, and successfully completed inspection as a trainee
- Maintains current statements of compliance with confidentiality, duality of interest, and other agreements
- Participates in at least one FACT continuing education event annually

Cord Blood Collection Inspector
- Has a relevant doctoral, nursing, or biological science degree
- Has at least 1 years' experience as a collection supervisor in a cord blood bank, or is an active FACT or JACIE clinical or collection inspector

Cord Blood Laboratory Inspector
- Has a relevant doctoral or biological science degree
- Has at least 1 years' experience as director, medical director, or supervisor of a cord blood bank laboratory or hematopoietic progenitor cell processing laboratory

active in the HC therapy field, and meet the minimum qualifications as listed in Table 36.1.

FACT accreditation in HCT is focused on the clinical program, which must perform the minimum number of transplants per year, and must use cell collection and cell processing facilities that also meet FACT–JACIE Standards. A collection and/or processing service may be an integral part of a clinical program, or may be accredited to provide these services by contract to one or more clinical programs. No specific structure is required; however, each collection or processing facility must have a minimal level of experience prior to accreditation. Mechanisms have been defined for new facilities to be initiated while accreditation is maintained.

In response to inquiries, FACT now offers a specific accreditation for collection facilities that perform marrow and/or peripheral blood cellular therapy product collection by apheresis for products under IND or licensure, intended for further manufacture. FACT supports responsible research in compliance with applicable laws and regulations, and offers its accreditation to those facilities collecting cell products for and distributing to entities following appropriate regulatory pathways. Facilities accredited under this pathway must fully disclose what processes are accredited by FACT in order to properly inform the public of the scope of their accreditation.

In addition, FACT now offers accreditation for cellular therapy product processing with more than minimal manipulation in an effort to increase quality practices throughout cellular therapy research. By encouraging facilities to meet minimum requirements for FACT accreditation, FACT contributes to a major goal of bringing promising therapies to patients. It is understood that some of FACT–JACIE Standards for processing are specific to HCs, and that there are inherent differences between research products and standard of care HCs. FACT–JACIE Standards can provide direction on meeting requirements of GMP and GTP regulations, and FACT accreditation provides external validation of regulatory compliance. Typically, these products require an IND application for clinical use. Where apparent discrepancies between FACT Standards and IND requirements exist, the quality requirements for planned deviations provide a path forward. Inspectors for these programs are knowledgeable and experienced with the intricacies of INDs, and are motivated to advance the quality of research and not to restrict it. While some of these cellular therapy products may be used in the traditional transplant program and/or manufactured in the traditional cellular therapy laboratory, others will be utilized in areas of regenerative medicine and/or immunology and may require separate staffs and facilities for their manufacturing procedures. The outcome analysis of these newer products is often based in safety, with recommendation to attempt to assess efficacy as applicable. This is an important extension of standards and accreditation for the future.

Though international in scope, the FACT inspection and accreditation process is conducted in English and requires applicant organizations to translate some, but not all, documents. On-site translation, demonstration of compliance, and translated flow charts are some of the tools used in the on-site inspection, which is built upon trust that each participant endeavors to comply with Standards and achieve quality for patients.

The essential elements of FACT accreditation are an initial eligibility application, submission of required documents, an on-site inspection, review by an Accreditation Committee, correction of deficiencies, and fulfillment of all other requirements. The accreditation process has recently been converted to an electronic, web-based system, the FACTWeb Accreditation Portal. The FACTWeb portal was developed by the FACTWeb Oversight Committee comprised of Board members, volunteer inspectors, and representatives of each type of accredited organization. This oversight committee verified that all user needs were considered, that applicants and inspectors were appropriately trained, and that the process remains congruent with FACT's objectives. Following the initial launch of the portal in 2012 with implementation of the fifth edition of FACT–JACIE Standards, the committee turned its attention to continuous improvement in design, accessibility, and clarity.

The process for initial FACT accreditation of HCT is detailed below.

1 *Eligibility application.* A Program or Facility Director will register interest in accreditation for one or more of the transplant-related services using the online eligibility application. Required information includes demographics and a description of the services for which accreditation is sought. The FACT Accreditation Office staff will review this information, determine if the program or facility is eligible for accreditation, and will generate the customized compliance application specific to the applicant.
2 *Compliance application.* The compliance application is based on services provided by the applicant. Generally questions that do not apply to the specific program should not be present. Applicants will indicate compliance with each Standard, and upload documentation as required. Guidance related to each standard is readily available on the application to assist the applicant in completing

the application and uploading required documents. The compliance application also serves as a checklist for the inspector at the on-site inspection. Use of checklist documentation ensures that each program is measured against the Standards, and that the inspection will be complete. Completion and submission of the compliance application is expected within 12 months of its receipt for initial accreditation.

3 *Request for information.* Request for information (RFI) is a functionality used by the FACT coordinators and inspectors to communicate in real time with each other and/or the applicant to clarify information or request additional documentation.

4 *On-site inspection preparation.* The submitted materials are reviewed by a designated FACT Accreditation Coordinator. Observations are communicated electronically to the applicant, allowing time for additional submissions or corrections. The applicant indicates potential dates for the on-site inspection when all key personnel will be available.

The inspection team is selected from the available inspectorate based upon the size and complexity of the applicant program, assuring that members of the team have the training and experience necessary to assess all HCT activities. Inspectors may be replaced if the inspector or applicant perceives a potential conflict of interest. When the assignment has been confirmed, each member of the inspection team is given access to the applicant's on-line compliance application and all uploaded documents that serve to verify compliance with the Standards. The inspection team has 3–4 weeks to review the application and related documents, request additional information, and meet by teleconference to prepare for the inspection.

5 *On-site inspection.* The on-site inspection should be completed in 1 day, starting with introductions and ending with a summation of major observations, but not a final determination of accreditation status. Observations made at the on-site inspection are recorded electronically on the compliance application. A deficiency is any observation that does not comply with a mandatory requirement, stated in the Standards as "shall." A variance from recommendation is the failure to be in compliance with a standard stated as "should."

Within 2 weeks of the on-site inspection, each inspection team member will complete his/her report online, and the Team Leader will complete and submit the final report. Submitted reports are reviewed by the FACT Accreditation Coordinator, edited as required, and submitted to the Accreditation Committee.

6 *Accreditation Committee review.* Each applicant program is presented to the FACT Cellular Therapy Accreditation Committee by the Accreditation Coordinator who has worked with the applicant and the on-site inspection team. Each of the on-site inspectors is invited to join the Accreditation Committee call when a relevant program is being discussed to provide additional information as needed, and to learn the process and interpretations from seasoned inspectors and reviewers. The Accreditation Committee is chaired by the FACT Medical Director, and consists of the chairperson of the Standards Committee, active inspectors representing all three of the major areas (clinical, collection, and laboratory), and representatives of the FACT Board of Directors. The possible outcomes of Accreditation Committee review are listed in Table 36.2. The Board of Directors retains ultimate responsibility for these outcomes, handles any complaints or appeals, hears and decides contentious issues, and makes any precedent-setting decisions. The accreditation outcome is communicated via the FACTWeb portal to the Program Director and other designated personnel. This notification includes instructions for submitting responses or additional information necessary to complete the process and achieve accreditation. The Accreditation Committee members also contribute suggestions to improve the process.

Table 36.2 Foundation for the Accreditation of Cellular Therapy (FACT) potential accreditation outcomes

No deficiencies or variances	Full accreditation for 3 years granted as of the date of the Accreditation Committee meeting
Few or scattered deficiencies	Accreditation granted upon documentation of correction of deficiencies, with or without review of corrections by the Accreditation Committee
Significant systemic deficiencies; numerous repeat deficiencies	Requires documentation of correction of all deficiencies, then focused re-inspection of all or parts of a program. Accreditation is granted after successful completion of the re-inspection
Failure to meet eligibility requirements or to respond appropriately with documentation of correction of deficiencies	Non-accreditation. If circumstances change, resubmission of eligibility application required to restart the process

7 *Accreditation.* Accreditation is awarded after any deficiencies have been corrected and these corrections documented. The Accreditation Committee may review this documentation if needed. Accreditation is valid for 3 years. Accredited programs are announced in the newsletters of the ISCT and ASBMT, and listed on the FACT website.

8 *Annual report.* Each accredited program and facility reports annually on the number of autologous and allogeneic HCT patients treated in the previous 12 months, and on any significant changes in personnel, location, or complexity of service. In addition, programs may be asked to document compliance with specific Standards that are new or have been problematic.

9 *Accreditation renewal.* Programs are expected to have completed the renewal process prior to the expiration of the prior accreditation. One year prior to expiration of accreditation, each accredited program will receive notification to begin the application for renewal, which is essentially identical to initial accreditation. The applicable standards will be the edition that is current on the day of the on-site inspection.

10 *Cord blood banking.* The accreditation process for cord blood banks is identical to that for cellular therapy programs, except that the on-site inspection generally requires at least 2 full days. There is a separate Cord Blood Bank Accreditation Committee; and the Boards of Directors of FACT and NetCord maintain accountability for these accreditation decisions.

Currently, over 200 programs in North America, Australia, New Zealand, South America, and Asia have achieved and maintained accreditation. For North America, this represents an estimated 90% of eligible programs. Special recognition has been given to the over 90 programs continuously accredited for 10 or more years. Over 45 cord blood banks have been accredited worldwide. There are over 160 active FACT inspectors. The initial inspection summaries of the 145 programs or facilities inspected under the first edition of the FACT Standards (1996) were reviewed to determine the most frequently cited deficiencies and variances from recommendation. The results from the first 76 programs have been published [47]. Similar to the results observed by JACIE, the results of recent on-site FACT inspections demonstrate that most programs are functioning at a high level of quality and have addressed most of the Standards. Deficiencies observed

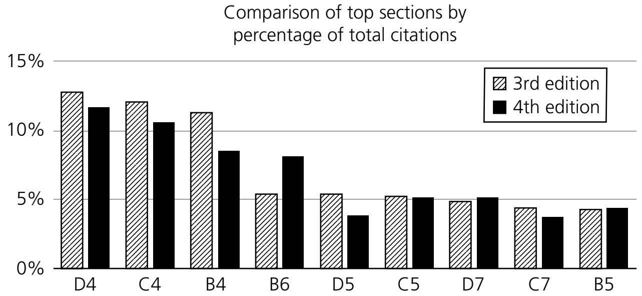

Figure 36.1 The top issues cited as deficiencies during FACT inspections based on the third and fourth editions of Standards, shown as percentage of the total. The Standards sections are: B4 – Clinical Quality Management; C4 – Collection Quality Management; D4 – Laboratory Processing Quality Management; B6 – Donor Selection, Evaluation, and Management; B5 – Clinical Policies and Procedures; C5 – Collection Policies and Procedures; D5 – Laboratory Processing Policies and Procedures; C7 – Collection Labeling; D7 – Laboratory Processing Labeling.

generally represent failure to completely address a Standard or to update practice based upon a new requirement in a new edition of Standards.

The most commonly observed deficiencies related to the third and fourth editions of FACT Standards are shown in Figure 36.1. The total numbers of deficiencies were decreased between the third and fourth editions, despite the increase in the total number of standards; however, the areas in which deficiencies are noted have remained constant. The top issues are Quality Management, Policies and Procedures; Donor Selection, Evaluation, and Management; and Labeling, cited across all areas of the HCT program. Deficiencies represented a failure to perform specific required activities, or failure to describe the ongoing quality activities in the QM plan. Standard operating procedures deficiencies included deficiencies both in format and content. Potential explanations for the continued frequency of these deficiencies include a large number of standards; new requirements in each edition of Standards, complex processes that include many concepts to be complete; and lack of attention to labeling process while completion of the label is simpler. In addition, inspectors have improved, both in their understanding of standards requirements and in the quality they expect to see in the applicant program.

Significance of FACT accreditation

FACT accreditation helps a cancer program attain its ranking among America's Best Hospitals, published by *U.S. News & World Report* [48]. Since April 2007, FACT accreditation for allogeneic HCT has been awarded one point toward best hospital status. FACT accreditation for autologous HCT only is awarded one half-point. In addition, *U.S. News & World Report* includes FACT accreditation as a factor in the selection of the top 10 America's Best Children's Hospitals [48].

FACT–JACIE Standards have achieved international acceptance, as best demonstrated by the joint authorship and committee membership in Standards development. In Australia, the Therapeutic GoodsAdministration has accepted the collection and laboratory standards as the regulation for the field. In the United States, cooperative groups require institutions entering patients on HCT trials to have FACT accreditation. Most insurance companies require HCT programs to disclose FACT accreditation status as part of the application for Center of Excellence designation.

Beginning in 2010, cellular therapy programs that have achieved 10 years of continuous accreditation are surveyed about the impact of FACT accreditation on their program and associated challenges. To date, respondents have unanimously agreed that FACT accreditation has positively impacted the quality within their programs. Peer perception, attitude of the affiliated institutions towards the program, insurance reimbursements, number of transplants performed, and participation in clinical trials were also identified as having been positively impacted. Challenges identified, including time and resources required to maintain accreditation, keeping current with the standards, and reviewing, revising, and organizing documents, provide valuable feedback to FACT in development of future educational opportunities, tips, and tools to assist programs in compliance. Peer to peer advising and a consulting service are newly launched initiatives based on the needs identified by applicant and accredited programs.

AABB

The AABB (formerly the American Association of Blood Banks) is an international professional society representing approximately 8000 individuals and 2000 institutions involved in blood banking, transfusion medicine, and cellular therapies [49]. The association includes physicians, nurses, medical technologists, administrators, scientists, and other healthcare providers committed to patient safety and optimizing donor care through its programs of voluntary standards, accreditation, and education. The AABB has a long history of standard-setting in blood and blood component collection, processing, storage, and transfusion, having published its first edition of *Standards for a Transfusion Service* in 1958. Since that time, AABB has expanded its repertoire of standards to include immunohematology reference laboratories, perioperative autologous blood collection and administration, relationship testing, molecular testing for red cell, platelet, and neutrophil antigens, and cellular therapy product services, including hematopoietic progenitor cells, somatic cells, and cord blood cells. In 2009, AABB formally established its Cellular Therapy Section within the association to provide leadership to the organization and its members; and in 2012, it launched its Center for Cellular Therapy to further serve cellular therapy professionals, to foster patient and donor safety, and to encourage responsible innovation in cellular therapy and regenerative medicine [49].

Standards

Originally published as separate volumes, AABB now publishes a single set of cellular therapy standards that encompass hematopoietic cells, somatic cells, and cord blood services [50]. AABB Standards are developed by expert volunteers; are revised biennially, and are based upon published literature, accepted good medical practice, principles of good manufacturing practices and quality assurance, applicable regulations, and the standards of other organizations. Internationally accepted quality management systems requirements are accompanied by technical requirements appropriate to the specific discipline. Although very different in structure, the content of the AABB Standards for Cellular Therapy Services is fundamentally consistent with the FACT–JACIE International Cell Therapy Standards.

Accreditation: The current edition of Standards and the Accreditation Information Manual that accompanies it form the basis for AABB accreditation. Accreditation is based upon submission of demographic information and an on-site inspection, coordinated by a paid, full-time quality assessor, and executed by volunteer assessors with relevant subject matter expertise who have been trained in auditing and assessing techniques. On-site assessments are loosely scheduled to occur within a specific 3-month period prior to the expiration of the prior accreditation; however, the exact dates are unannounced. The on-site assessors determine compliance or non-compliance with Standards, and leave a written report of non-conformities with the applicant organization at the end of the assessment. Accreditation is offered worldwide. Facilities may apply for variance if desired, especially due to US regulatory requirements that may not be applicable in other parts of the world. Accreditation is valid for 2 years.

Separate from its standard-setting and accreditation activities, AABB offers a consulting service in cellular therapy, educational and training materials for individual or institutional use; numerous publications, and an annual meeting. AABB participates in several inter-organizational and international activities that address prevention of transmission of infectious diseases, labeling, and development of the field of cellular therapy worldwide. With the growth of clinics and physicians using adult autologous cell therapy for a host of indications outside the traditional hematopoietic stem cell transplant setting, AABB announced in 2012, a collaboration with the International Cellular Medicine Society (ICMS) to develop standards and offer accreditation globally for these therapies [51].

National Marrow Donor Program

The National Marrow Donor Program (NMDP) was founded in 1987 as a cooperative effort of the AABB, the American Red Cross, the Council of Community Blood Centers, and the United States Navy to facilitate volunteer unrelated donor marrow transplantation. It comprises a network of cooperating facilities, including transplant centers, donor centers, marrow collection centers, apheresis centers, cord blood banks, recruitment groups, cooperative donor registries, contract laboratories, and cell and serum repositories. The Health Resources and Services Administration has regulatory authority over NMDP.

Since its inception, NMDP has established standards for membership participation. A Standards Committee composed of experts in various aspects of HCT is in place to provide continuous review and revision of these standards. NMDP Standards cover cells obtained from marrow, peripheral blood, and umbilical cord blood. The Standards include standards for participating centers and groups, personnel qualifications, required support services, policy and procedure requirements, confidentiality, recruitment of the unrelated donor, donor medical and laboratory screening and testing, informed consent, donation and transplant process, a few collection, packaging, labeling, and processing standards, and records requirements. Standards for laboratory processing are less detailed than those of FACT–JACIE, NetCord–FACT or AABB, since most of the product processing is performed by laboratories integral to the recipient's transplant program. Participating cord blood banks are also required to be otherwise accredited.

Other Standards

WBMT: The Worldwide Network for Blood & Marrow Transplantation is a collaborative research and educational non-governmental body consisting of the world's foremost blood and marrow stem cell transplant organizations that exists to promote excellence in this field. Through its accreditation committee, WBMT has created a cross-walk comparing various voluntary standards; and begun development of minimal core standards in hematopoietic cell transplantation for application in developing countries.

WMDA: The World Marrow Donor Association is a global association whose mission is to foster the international availability of high-quality stem cell products and to promote the interests of stem cell donors. The WMDA has voluntary standards and an accreditation program for donor registries. WMDA Standards promote the quality of procedures necessary to obtain, in the shortest possible time, an appropriate quality and quantity of hematopoietic stem cells from the best unrelated donor, while also protecting the anonymity and well-being of the volunteer donors. Since 2003, 20 of the 71 stem cell donor registries have become accredited by WMDA, accounting for approximately 75% of hematopoietic stem cell donors and cord blood units listed in the database of Bone Marrow Donors Worldwide.

Future directions

In an era when regulation and voluntary professional standards coexist, and when changes in healthcare delivery will place increasing importance on defining and meeting validated quality metrics, the field of blood and marrow transplantation has pioneered an accreditation system that could be adapted by other specialties. It is important that the professional organizations continue to lead the field by setting those quality standards demonstrated to be important in patient outcome, to provide the educational materials necessary for programs to meet and exceed regulations, and to continue communication with regulatory bodies to ensure clear understandings and enable appropriate regulation. Biannual liaison meetings between representatives of the Center for Biologics Evaluation and Research, the Office of Cellular, Tissue and Gene Therapies, and the professional societies representing active professionals in the cellular therapy community continue as a venue for the communication by industry of issues of concern.

Regenerative medicine applications of numerous cellular therapy products are expanding rapidly. Both autologous and allogeneic products are in clinical trials; and arguments continue to be made that at least some of these products should not require regulation [52]. The next years will define which of these products will prove useful, which if any will be designated as practice of medicine, and which will be regulated as biologic products.

To address needs in these emerging and evolving areas that include cellular therapies, FACT has recently published FACT Common Standards for Cellular Therapies [53]. These Standards represent the basic fundamentals of cellular therapy that can be applied to any cell source or therapeutic application, and are intended to be used throughout product development and clinical trials. The aim is improve outcomes in patients suffering from a variety of diseases and conditions. Ideally, various discipline-specific or product-specific standards will be added in the future with the input, collaboration, and acceptance of those experts actively involved in the specific field and their clinical and laboratory programs who volunteer to contribute and comply with them.

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CHAPTER 37

Bone Marrow and Peripheral Blood Cell Donors and Donor Registries

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Introduction

Hematopoietic cell (HC) donors provide bone marrow or peripheral blood progenitor (hematopoietic) cells (PBPCs) for more than 20,000 allogeneic HC transplant (HCT) recipients annually. In the United States, annual HCT using unrelated donors exceed those using related donors, but not to the extent expected given that at least 70% of HCT candidates lack a suitably matched family donor. This suggests that a significant fraction of the potential patient population is currently not being served. As unrelated donor registries grow and international collaboration increases, more patients will have the opportunity for transplantation. Even under the most optimistic scenarios, however, alternative approaches will also be necessary. Umbilical cord blood transplantation (see Chapter 39) and haploidentical transplantation (see Chapter 45) are strategies that will play increasingly important roles in meeting the needs of patients.

This chapter discusses the evaluation of HC donors, the risks and side effects of donation, and the logistics of HC collections.

Products from HC donors

Bone marrow was once the only graft source for allogeneic HCT [1–4], but since 1995, the role of PBPC has been clarified and its use increased dramatically. Data from the Center for International Blood and Marrow Transplant Research (CIBMTR) show that in the period 2006–2010, 80% of adult allogeneic transplant recipients received PBPC grafts [5]. National Marrow Donor Program (NMDP) statistics confirm dramatic growth in the use of PBPCs for unrelated donor transplantation (Figure 37.1).

PBPCs are now the most common choice for both autologous and allogeneic transplantation worldwide. Under normal physiologic conditions, a small number of $CD34^+$ HCs circulate in the peripheral blood, but their concentration is too low to enable efficient collection of an adequate dose for transplantation. However, in the mid-1980s, it was demonstrated that HCs could be mobilized from the bone marrow into the blood following chemotherapy and/or administration of hematopoietic growth factors such as rhG-CSF (filgrastim, Neupogen®) and rhGM-CSF (sargramostim, Leukine®). Around the same time, advances in apheresis instrumentation and collection techniques occurred such that an adequate number of mobilized HCs ($1.0–5.0 \times 10^6$ CD 34^+ cells/kg of patient weight) could be collected from the patient (in the case of autologous transplantation) or an allogeneic donor in one to three daily large-volume apheresis procedures.

Donors of allogeneic bone marrow and PBPC

Donors of bone marrow and PBPCs for allogeneic transplantation are either blood relatives of the transplant recipients or unrelated, anonymous volunteers. Related allogeneic HC donors are usually first-degree relatives of their recipients. Most are full siblings because of the much higher likelihood of complete HLA identity. The family of genes responsible for encoding the proteins of HLA A, B, C, and DR are co-located in a short region on chromosome 6, and as such are usually inherited *en bloc* as a single haplotype. Thus, there are only four combinations possible with the two haplotypes of each parent. Between two siblings, the likelihood of two identical HLA haplotypes, a single identical haplotype, and no identical haplotypes is, therefore, 25%, 50%, and 25%, respectively. A parent and their offspring are usually only half-matched (haploidentical) except in the uncommon situation where both parents happen to each possess an identical or nearly identical haplotype. Given that the average size of a modern family is just over two offspring, when one child needs an allogeneic transplant, the likelihood of an HLA-identical sibling match is 25–30%. The likelihood of a haploidentical match (full-sibling, half-sib, parent or child) is much higher, and successful strategies using such donors continue to evolve (see Chapter 45).

Unrelated donors and unrelated donor registries

Most potential transplant candidates do not have a fully HLA-matched family donor, a limitation that became apparent early in the history of allogeneic transplantation. By the early 1970s, however, case reports began to appear documenting the feasibility of HCT from HLA-matched unrelated donors [6]. Such donors were usually gleaned from the files of HLA-typed platelet donors at community blood centers or large hospitals. In 1974 in the UK, the Anthony Nolan Registry was established and dedicated to recruiting and maintaining a file of HLA-typed volunteer bone marrow donors. Within a few years, similar efforts were established in countries throughout Europe, the Americas, and Asia.

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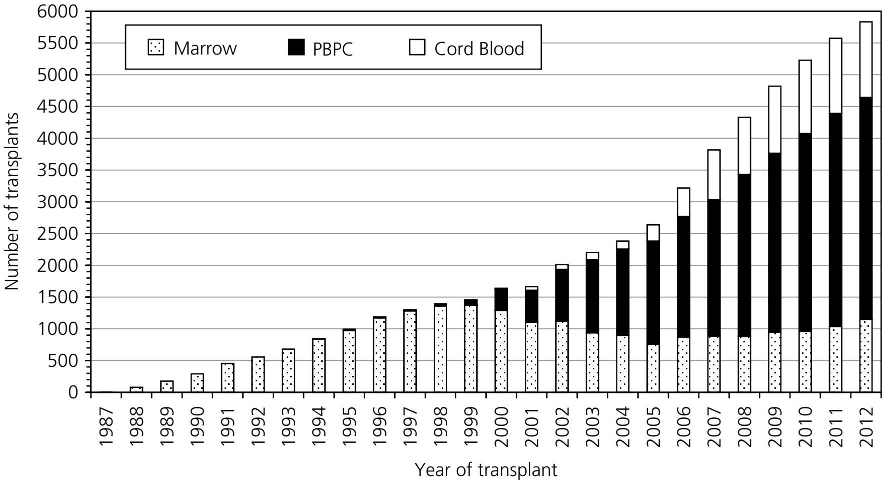

Figure 37.1 Annual transplants from unrelated donors facilitated by the National Marrow Donor Program since its inception. Annual figures include products collected outside the United States and imported, as well as those collected within the USA and exported. Prior to July 1999, only bone marrow was used for initial transplantation, but PBPC were available in the setting of re-transplantation. Stippled bars = bone marrow; solid bars = PBPC; and open bars = cord blood units.

Today, most of the world's unrelated donor registries collaborate under the auspices of the World Marrow Donor Association (WMDA). The WMDA was organized in 1988 to promote international cooperation and the exchange of HC products [7,8]. The association has established standards and provides guidance on the organization and operation of unrelated donor registries [9].

The WMDA annually surveys unrelated donor registries around the world [10]. Seventy-three registries responded to the 2012 survey. Together, these registries listed more than 22,300,000 potential unrelated HC donors. Approximately 89% of these donors have been tissue typed for HLA A, B, and DR, while the remainder has been typed only for HLA A and B. Most of the registries serve one or more transplant centers in their country; together the registries reported 1399 transplant centers with potential access to unrelated donors. The Japan Marrow Donor Program reported the largest number of transplant centers with 222.

The WMDA respondents reported collecting 15,114 unrelated donor marrow and PBPC products in 2012. In 6927 instances (46%) the collected products were exported to another nation. Germany performed 6265 collections in 2012, which was the most of any nation and represented 41% of the world total. Germany exported 4384 (70%) of the products collected, mostly within the EU and to North America. The United States was the largest consumer of HC products with 3763 (25% of the world total) of which 1485 (39%) were imported. Worldwide, PBPC and bone marrow collections numbered 10,988 (73%) and 4126 (27%), respectively.

Much of the world's available inventory of unrelated adult donors and cord blood units can be accessed through Bone Marrow Donors Worldwide (BMDW). HLA data from 67 registries and 46 cord blood banks representing more than 20.7 million adult donors and 560,000 cord blood units were listed by BMDW at the end of 2012. BMDW provides a highly important resource, but is limited by incomplete participation and lack of comprehensive search and fulfillment functionality.

The largest registry in the world is the NMDP with more than 6.7 million United States registrants followed by the German National Bone Marrow Donor Registry (ZKRD) with more than 4.8 million. The NMDP adds about 500,000 new donors annually. The vast majority of these are recruited at community donor drives, which are held at numerous locations including blood centers, college campuses, community centers, sporting events, and homes of worship. More than 1000 drives are held each month. New recruits are screened to confirm good health and the absence of serious transmittable diseases, for example HIV.

Once a donor is recruited to a registry, it may be months or years before that donor's identification code appears on a patient search. At that time the donor may or may not be available for further testing and evaluation. Reasons for this include obsolete contact information, changes in the donor's health, or changing donor priorities. In the WMDA survey of registries, among 113,422 total donor requests for blood samples, 44,489 (39%) were canceled for donor reasons [10]. Donor availability in the United States is somewhat worse than the world average; in 2012, 44% of nearly 36,000 requests for donor blood samples were cancelled for donor reasons.

Donor eligibility and qualification

Prior to HC donation, donors must be evaluated to ensure that: (1) they can safely donate, (2) their HC will be safe for the recipient, and (3) they understand fully what they are being asked to do [11].

Regulatory requirements

In 1997, the US Food and Drug Administration (FDA) proposed an approach to regulation of human cells and tissues designed to minimize the risk of contamination and maintain the integrity and function of cell- and tissue-therapy products, including PBPC and umbilical cord blood HC (bone marrow for transplantation remains exempt from these regulations because of Congressional legislation) [12]. Subsequently, in 2004, the Food and Drug Administration published three final rules in the US Code of Federal Regulations that describe, among other things, the requirements for registration of establishments that "manufacture" HC, for determination of donor eligibility, and for compliance with Good Tissue Practices [13]. The FDA determined that minimally manipulated PBPCs and umbilical cord blood HC, collected from *related donors*, would be regulated under Section 361 of the Public Health Service Act. These regulations are intended to reduce the risk of transmission of relevant communicable diseases, either acquired from the donor or introduced into the products during manufacturing. For HC products from *unrelated donors*, however, the FDA felt there was a need for additional manufacturing controls, and chose, therefore, to regulate these products under Section 351 of the Public Health Service Act. Section 351 sets additional requirements for Good Manufacturing Practices, which must be followed to ensure the safety, potency, and efficacy of HC products from unrelated donors. Where Section 351 is silent, for example donor eligibility, Section 361 requirements apply. The FDA regulatory framework is not unique; similar regulatory frameworks have arisen in the European Union, Canada, Australia, and elsewhere.

A uniform feature of every regulatory system is establishment of controls to prevent transmission of infectious agents by HC products. There are numerous infectious diseases that if present in the HC donor would pose definite or theoretical risk to the transplant recipient. It is clear that HC can transmit many of the same infections that are transmissible by blood transfusion including hepatitis B (HBV), hepatitis C (HCV), and human immunodeficiency virus (HIV). In addition, some congenital or acquired conditions in the donor, such as genetic defects and immune system abnormalities, may be transmissible to the recipient. Minimizing the risk of disease transmission requires combining the information obtained from a targeted behavioral/medical history, a search for the physical signs of disease, and the results of laboratory testing for specific pathogens [14,15].

HC donors, similar to blood donors, complete a written questionnaire that has been specifically designed to elicit medical history and identify behaviors or activities that may increase the risk of infectious disease transmission [14,15]. Questions regarding high-risk behaviors, non-prescription drug use and skin-breaching procedures, such as tattooing and piercing, are included, as well as questions to assess residence in regions where exposure to malaria or the agent of bovine spongiform encephalopathy may occur. The donor screening questionnaire is intended to detect information that may place the transplant recipient at increased risk for transplantation-transmissible diseases. Testing of the donor's blood cannot substitute for direct questioning because the donor might have a "window-period" infection, the blood tests may be falsely negative or, equally important, the testing does not include all potentially transmissible diseases. Indeed, suitable screening blood tests for some disorders, such as Creutzfeldt–Jakob disease and variant Creutzfeldt–Jakob disease, do not currently exist. Positive responses on a proper donor-screening questionnaire may lead to donor disqualification or the donor may be determined to be ineligible by FDA criteria. Donors may also become ineligible as a result of physical examination findings or testing for relevant transmissible infectious diseases as described later.

In the United States, FDA regulations provide a mechanism for collecting HC products from ineligible donors [14,15]. In this case, the transplant physician may determine that there exists a state of "urgent medical need," which means that a suitable alternative donor or graft source is not readily available and the risks of alternative therapies are greater than the risk of proceeding with the ineligible donor. If the transplant physician elects to proceed with an ineligible donor, the declaration of urgent medical need must be documented and appropriate paperwork and labeling must accompany the PBPC product. It should be emphasized that donor eligibility from a regulatory standpoint is distinct from donor suitability. A donor may be eligible, but have medical conditions, as described later, that make him or her unsuitable for donation.

The donor physical examination, discussed in greater detail later, should detect behavioral and experiential stigmata, such as recent tattoos, piercings, illicit drug use, and so on, as well as signs of significant conditions, including specifically sepsis and vaccinia [14]. In addition, a sample of the donor's blood must be tested within 30 days prior to donation for at least the following infectious diseases: HIV 1/2, HBV, HCV, *Treponema pallidum*, human T-cell lymphotrophic virus I and II, West Nile virus, and cytomegalovirus (CMV). For infusion in the United States, testing must be performed in a certified laboratory, which uses test kits specifically licensed or approved for donor screening and which follows the test kit manufacturer's instructions. Testing that is performed with an unlicensed test kit or a test kit that is licensed for a different purpose (e.g., diagnosis or monitoring) is a violation of FDA regulations.

Testing of HC donors for Chagas' disease is not currently required, although FDA-licensed kits are available for this purpose. Testing for prior infections with varicella-zoster virus, Epstein–Barr virus, and toxoplasmosis, while not required, may also be desirable. The results of regulated and non-regulated infectious disease testing must be available and reviewed prior to the initiation of preparative conditioning therapy for the recipient [14–16]. Donors with a confirmed positive test for HIV must not donate. As already described, donors with prior exposure to HBV and HCV may be used when there are no suitable alternatives and the potential gain outweighs the risk. Issues related to hepatitis are fully discussed in Chapter 95.

Medical suitability

HC donation is not without risk. As described later, adverse events are uniformly encountered, but these are generally minor and short-lived. Serious adverse events, including death, may, however, occur. It is essential that prior to donation each donor is assessed to ensure the absence of conditions that might increase donor risk to unacceptable levels. To ensure objectivity and preservation of the donor's best interests, this assessment should be performed by a physician who is not directly involved in the care of the proposed recipient. O'Donnell et al. conducted a survey of transplant programs in the United States to assess current donor care practices [17]. This survey demonstrated frequent overlap in provider care transplant recipients and their related donors.

History and physical

The donor receives a medical history and physical examination appropriate for the anticipated donation procedure. The history, which addresses risk to the donor, as opposed to the screening history described earlier, should focus on matters relevant for the anticipated donation, including the psychologic issues discussed later. For all donors, these would include a review of known health problems, a listing of medication and allergies, and review of the family history. Marrow donors should be questioned about prior surgical procedures and types of anesthesia received. Marrow donors should also have a careful review of systems directed toward neurologic, respiratory, cardiovascular, and musculoskeletal problems. PBPC donors should be questioned about prior whole blood or apheresis donations. The review of systems for PBPC donors should include a careful cardiovascular and neurologic review as well as specific questions about a history of venous access problems, autoimmune diseases, and splenic disorders.

The donor's physical examination should focus upon the neurologic, respiratory, and cardiovascular systems. In addition, marrow donors need an assessment of the oral airways and an evaluation of access to the iliac crests. Donors with a history of musculoskeletal symptoms need a careful examination of the spine and lower extremities. The examination of PBPC donors should additionally include evaluation of venous access and an abdominal examination for splenomegaly.

Laboratory and procedural evaluations of donors

The laboratory evaluation of all HC donors typically includes the following: complete blood count with white blood cell differential, ABO/rhesus grouping, serum electrolytes, glucose, alanine aminotransferase (ALT), bilirubin, creatinine or blood urea nitrogen (BUN), total serum protein and albumin, and prothrombin time and partial thromboplastin time [11]. Some physicians will also recommend evaluations of immunoglobulin levels and a screen for monoclonal proteins in the blood. Additional evaluations of donors may include urinalysis, chest X-ray, and electrocardiogram.

HC donors whose intended recipients suffer from inherited conditions, such as hemoglobinopathies or inborn errors of metabolism, may require specific testing to rule out carrier states that could affect transplant outcome. Donors with sickle cell trait and thalassemia minor can serve as donors in successful bone marrow transplants. As discussed later, however, donors with S-beta thalassemia, SC or other complex sickle hemoglobinopathies should not receive rhG-CSF.

A pregnancy assessment must be performed for female donors with childbearing potential [11]. Pregnancy is considered a contraindication to marrow donation; however, successful marrow collection from pregnant donors has been reported [18]. Pregnant women cannot be PBPC donors as hematopoietic growth factor administration is contraindicated.

Ethical and psychosocial issues

Psychologic aspects of marrow donation

The psychologic condition of the donor should be assessed. In particular, what are the donor's motivations for considering the HC donation? Is the donor acting out of a genuine desire to help, or are there other motives involved – perhaps an unrealistic expectation of reward or personal gain?

Switzer et al. [19] interviewed 343 unrelated marrow donors prior to donation, then immediately, and 1 year post-donation. They determined that donor motives could be classified into six categories and that an individual donor might express motives in more than one category. Donor motives varied between men and women and were associated with reported levels of pre-donation ambivalence and with both psychologic and physical difficulties post-donation [19].

Compared to unrelated donors, related donors may have different motivations and may be subject to increased emotional and physical stress [20]. Discussions of the psychologic issues affecting donors post-donation are presented in Chapter 32.

Occasionally donors may be subjected to coercion. Switzer et al. [19] have reported that unrelated donors who feel they were pressured, either encouraged or discouraged about donation, are less likely to have a positive donation experience.

Donor consent for HC donation

HC donors must provide written consent prior to donation [11]. The model of informed consent for research is appropriate for the HC donor even in the absence of planned research.

HC donors as research subjects

It is important to determine circumstances that make the HC donor a bona fide research subject. This can be complicated because much of the research involving HC donors also involves the HCT recipient. Several tests may be applied to help determine whether a particular activity constitutes research involving the HC donor. These include the following: (1) Is there collection of individually identifiable information or product for the purpose of research?; (2) Is there collection of information or product in support of an FDA IND application or an FDA Investigational Device Exemption (IDE)?; and (3) Is there an interaction with a donor that would not be occurring in the absence of the research? Affirmative responses to any of these questions serve to define research [21].

Remuneration of donors

Donor registries reimburse the unrelated donor for any out-of-pocket expenses related to their donation. Remuneration, however, that is, direct payment of cash or other valuable consideration, has been thoughtfully avoided. The mission of the registries is service to patients in need of blood or marrow transplantation, and the entire pipeline works to meet that need beginning with the humane, altruistic volunteer donor. Some have speculated that more people would donate if they were offered cash payment or other valuable consideration. However, such remuneration of the donor disrupts the pipeline, suddenly creating two persons in need, the recipient who is still in need of HCT and the donor who needs to be paid for inducement; for the latter, the mere prospect of saving someone's life is not sufficient motivation.

Standards of the WMDA and Guiding Principles of the World Health Organization both prohibit direct payment to volunteer donors [22,23]. The practice of the world's donor registries in service to their patients has been strict adherence to these principles. In the US, this practice was recently challenged by a legal decision from the 9th Circuit Court of Appeals [24]. The Court held that although the National Organ Transplantation Act [25] prohibits direct payment to donors of bone marrow and "subparts thereof," the Act does not prohibit payment to PBPC donors. The Court held that PBPCs do not represent a subpart of the bone marrow, but rather a subpart of blood. The exact mechanism by which PBPCs reconstitute the entire hematopoietic system without being a subpart of the bone marrow was not addressed in the Court's opinion. Thus far, donor registries in the United States, including the NMDP, have continued their policies of non-remuneration for donors. The issues of remuneration for HSC donors have been carefully described by Boo et al. [26].

Donation procedures

Bone marrow

Marrow is typically collected from the posterior iliac crests of the donor under general or regional anesthesia. The posterior superior iliac spine is the primary landmark for initiating the marrow collection. Marrow is aspirated through large bore needles into glass or plastic syringes that have been rinsed with a heparin-containing solution.

Typically, the iliac crest is entered through a skin puncture or a small skin incision. Once the cortical bone has been penetrated, a small amount of marrow (5–10 mL) is aspirated by applying vigorous suction. The syringe is removed and its contents are immediately transferred to an anticoagulant solution. Commonly, the aspiration needle is then advanced several millimeters and the process repeated. Several aspirations may be obtained from a single bone puncture as the needle is repeatedly advanced. Through a single skin entry site, the bone may be entered multiple times. A typical collection may involve 200–300 marrow aspirations obtained through a few or several skin punctures or incisions. Occasionally, marrow is also collected from the anterior iliac crests.

The aspiration of marrow produces an admixture of marrow cells from the bone cavity and capillary blood. The ratio of marrow to blood appears to depend upon the technique employed. Short, vigorous aspirations are generally thought to produce a higher concentration of marrow cells relative to peripheral blood. Even though the dilution of marrow cells with peripheral blood is variable, the adequacy of marrow collections is determined by the nucleated cell count of the mixture. For many years, the target cell dose for allogeneic marrow collection was $>2.0 \times 10^8$ nucleated cells/kg recipient body weight. Sierra et al. [27] evaluated the effect of marrow cell dose in 174 unrelated donor bone marrow transplant patients with high-risk acute leukemia. Their analysis showed a beneficial effect of marrow cell doses above the study group median (3.65×10^8 nucleated cells/kg). As a result of this analysis and similar studies, many centers now routinely seek marrow cell doses of 4×10^8 nucleated cells/kg.

Peripheral blood HCs

The PBPC collection site is usually a blood center or the apheresis unit of a hospital. Donors typically remain as outpatients throughout the mobilization and collection process. Filgrastim is currently the most commonly used mobilizing agent although lenograstim (not available in the USA) yields similar numbers of $CD34^+$ cells [28]. Although also not yet licensed in the USA for normal donors, the CXCR4 antagonist AMD 3100 effectively mobilizes PBPCs in autologous and allogeneic donors [29]. The rate of engraftment with cells from related donors appears similar for the filgrastim- and AMD 3100-mobilized PBPCs [29].

Filgrastim is usually administered at a daily dose of 10–16 μg/kg donor weight, which may be given as a single subcutaneous injection or divided into twice-daily injections. PBPCs are collected using a continuous-flow cell separation device. Collections begin after the fourth or fifth dose of filgrastim.

There is wide variability between donors in the number of $CD34^+$ cells that will be mobilized and collected following stimulation with filgrastim. The exact dose of $CD34^+$ cells appropriate for allogeneic transplantation has also not been established, but the usual target is $4–6 \times 10^6$ $CD34^+$ cells/kg recipient weight [30,31]. For the majority of adult recipients, it appears that one or two 12 L aphereses, or a single large volume procedure, will provide sufficient HCs for successful engraftment.

Adverse events associated with hematopoietic cell donation

An adverse event is any untoward medical occurrence. Serious adverse events are those adverse events that pose a threat to the individual's life or functioning. Serious adverse events include at a minimum adverse events that are: (1) fatal, (2) immediately life threatening, (3) require or prolong hospitalization, (4) result in a significant or persistent disability, or (5) represent a congenital anomaly. Both marrow and PBPC donations are associated with adverse events. Fortunately, serious adverse events are not common. In the NMDP experience, hospitalization is the most common reason for an adverse event to be rated as serious. Unexpected adverse events, which may be either serious or not, are those that are not described in a protocol, consent form, Investigator's brochure, or the medical literature.

Pulsipher et al. recently reported on the NMDP's collection of adverse events from nearly 10,000 bone marrow and PBPC donors [32]. Data were prospectively collected on 2726 bone marrow and 6768 PBPC donors between 2004 and 2009. Time points for data collection were at baseline, during donation procedure, and following donation. After donation, donors were contacted within 2 days and then weekly until reporting full recovery; all donors were also contacted at 1 month, 6 months, and annually after donation. All collections were performed at NMDP-approved sites. PBPC were collected according to an NMDP-sponsored research protocol, which operated under an Investigational New Drug application filed with the US Food and Drug Administration. Characteristics of bone marrow and PCSC donors were similar and overall 61% of donors were male. Eighty-six percent of bone marrow donors received general anesthesia with the rest having epidural or spinal anesthesia. Seventy-seven percent of PBPC donors donated with a single apheresis procedure. Anemia was more common after bone marrow donation, but only 0.5% of bone marrow donors received homologous blood transfusions. Thrombocytopenia was more likely following PBPC donation with platelet counts <150,000/mcL occurring in more than 50% of donors and counts <100,000/mcL in 26%. All blood counts returned to near baseline levels within 1 month and remained so in annual follow-up out to 3 years.

Bone pain occurred in more than 80% of donors irrespective of donation type. The character of bone pain was similar in the two groups with the vast majority experiencing mild to moderate discomfort. The timing of bone pain was obviously different with PBPC donors experiencing most pain during filgrastim administration and prior to donation, while bone marrow donors experienced pain after the donation. Bone marrow donors were more likely to report persisting pain at both 1 week and 1 month post-donation. Other side effects, such as fatigue, insomnia, and constitutional symptoms were similar in the two groups irrespective of the donation type. Women were more likely to experience pain and other symptoms than were men. Older donors were more likely to report pain and other symptoms 1 week post-donation.

In the Pulsipher report, 21% of women required central venous catheter placement for PBPC collection as opposed to just 4% of men [32]. Women were also more likely than men to require unplanned hospitalization in association with either type of donation.

Consistent with the Pulsipher study, Rowley et al. [33] reported the experiences of 69 sibling donors who participated in a randomized comparison of bone marrow and PBPC transplantation. The frequency and intensity of symptoms between the two groups of donors was no different.

Additional adverse events specifically associated with marrow donation

Minor complications occur in 6–20% of marrow donations [32,34,35]. These include such events as hypotension, syncope, severe post-spinal headache, excess pain, and minor infections. By definition, these complications resolve within a few to several days of onset.

Laboratory and radiologic abnormalities also accompany marrow donation. Anemia is the most significant laboratory finding. Marrow harvesting also causes significant, but transient, elevations in serum alkaline phosphatase and ostoecalcin [36]. X-rays, CT scans and radionuclide scans disclose abnormalities in the pelvis and sacrum that may persist for weeks or months [37].

Serious adverse events following marrow donation

The frequency of serious adverse events following marrow donation is estimated at 0.1–0.3% [38]. A review of data collected between 1969 and 1983 on 2248 allogeneic marrow donations reported to the International Bone Marrow Transplant Registry (IBMTR) and 1160 additional donations occurring in Seattle revealed nine total incidents (0.27%) [39]. These included one case of aspiration pneumonia, two cases of deep vein thrombosis (including one occurrence of pulmonary embolism), three serious bacterial infections (including two with bacteremia), two instances of serious cardiac arrhythmia, and one case of cerebral infarction.

The medical literature also contains reports of serious infections following marrow collection [34,39]. Infectious osteomyelitis appears to be a relatively rare complication following marrow collection [40,41].

Hemorrhage associated with the collection can create severe pain from compression of soft tissues. Sciatic pain lasting up to 18 months has been reported in a related marrow donor [34]. Prolonged and significant pain may occur following marrow collection, leading to permanent disabilities. Fractures of the iliac crests have also been reported [34].

Marrow donation and the risk of death

Death has occurred among normal marrow donors. A review of 7857 marrow collections reported to the CIBMTR revealed two deaths [42]. Table 37.1 lists 11 documented deaths occurring within a few days of HC donation [43–46]. As is evident in Table 37.1, two deaths actually occurred prior to scheduled marrow donations. Whether the stress of impending donation somehow contributed to these events is unknown. Similarly, it is difficult to know whether death in the days immediately following HC donation is causally related.

Additional adverse events associated specifically with PBPC donation

Bone pain is the most prominent adverse event among PBPC donors occurring in 25–86% of donors receiving filgrastim [47,48]. Bone pain likely results from altered bone metabolism, which is reflected by increased bone-derived alkaline phosphatase and decreased serum osteocalcin. The pain is typically diffuse but most prominent in the spine, hips or pelvis, and ribs [48]. Headache is also common. The discomfort from bone pain improves with mild analgesic therapy, such as acetaminophen or a non-steroidal anti-inflammatory drug (NSAID). Other symptoms seen with filgrastim include nausea and vomiting (~10%), myalgia (~20%), fatigue (~15%), insomnia (~10%), and injection site reactions (rare). Symptoms are probably less frequent at filgrastim doses below 10 μg/kg daily. Pain symptoms resolve promptly with discontinuation of filgrastim, and are rarely sufficient to cause premature cessation, or even dose reduction [32,48].

HC mobilization with filgrastim also causes numerous alterations in serum chemistries and blood cell counts. Lactate dehydrogenase, AP and ALT increase two- to fourfold after five daily doses of filgrastim. The white blood cell (WBC) count and in particular the absolute neutrophil count (ANC) increase dramatically during filgrastim therapy [32,48]. These increases are dose-dependent. At daily filgrastim doses of 10 μg/kg or greater, the total white count may reach 70–80 × 10^9/L by day 5. Eighty to 90% of the white cells will be neutrophils or band forms. It is generally recommended that the filgrastim dose be reduced if the WBC exceeds 70–75 × 10^9/L.

The apheresis procedure used in PBPC collection is also a source of adverse events. Anticoagulation with acid-citrate-dextrose (ACD) solution may elicit symptoms of hypocalcemia – perioral numbness, paresthesias, and carpopedal spasms. These symptoms may be ameliorated with oral calcium supplementation, but are more effectively managed with an intravenous infusion of calcium during apheresis. Reducing the blood flow rate may also improve symptoms of hypocalcemia, but at the expense of prolonging the collection procedure. Another approach that has been used is to supplement the anticoagulation with heparin to diminish the amount of ACD required [49].

Thrombocytopenia is the most consistent and significant finding with PBPC apheresis [32,48]. The pre-apheresis platelet count reproducibly declines between 20 and 30% with each collection and does not begin to recover until 3–4 days after the last collection. Following two procedures, it is common to encounter total platelet counts below 100 × 10^9/L.

Table 37.1 Deaths occurring in association with hematopoietic cell donation

Age	Sex	HSC source	Proximity to donation	Cause of death	Ref.
40	M	marrow	prior	cardiac arrest	[81]
46	M	marrow	prior	cardiac arrest	*
57	F	marrow	immediate	v. fibrillation	*
35	M	marrow	immediate	respiratory arrest	*
35	F	marrow	immediate	myocardial infarction	*
35	M	marrow	after	pulmonary embolism	*
47	F	PBPC	immediate	sickle crisis	[83]
57	F	PBPC	after	stroke	[84]
62	F	PBPC	after	cardiac arrest	*
6	M	PBPC	after	cerebral hemorrhage	*
19	F	PBPC	before	hemopneumothorax	*

*Personal communication to the authors.

Serious adverse events following peripheral blood hematopoietic cell donation

Filgrastim administration may precipitate severe sickle crisis in persons with sickle cell anemia or complex sickle cell hemoglobinopathies [43,50]. Indeed, a 47-year-old woman with Hb SC disease, who had never had any symptoms from her condition, suffered a fatal sickle crisis during filgrastim mobilization of PBPC intended for her sister with CML [43]. It remains to be clarified whether persons with sickle trait (Hb AS) are at any increased risk from filgrastim. Kang et al. [51] safely mobilized and collected PBPC from nine donors with sickle trait. These donors did experience higher symptom scores during mobilization than did eight simultaneous control donors, but there were no symptoms suggestive of painful sickle crisis.

There have been several reports of spontaneous splenic rupture apparently related in some way to extramedullary hematopoiesis [52–55]. In three cases the spleen was surgically removed, but conservative management was effective in two cases. Platzbecker et al. [56] performed ultrasound evaluations of spleen size before and after rhG-CSF mobilization in 91 healthy PBPC donors. Although no adverse splenic events occurred in this group, significant increases in spleen length and width were routinely documented. No donor factors or clinical findings (change in WBC, increase in alkaline phosphatase, etc.) correlated with the degree of splenic enlargement. Similar findings were reported in independent studies by Stroncek et al. [57], which also demonstrated that splenic enlargement may persist for up to 10 days in some donors. Based on these studies, ultrasound does not appear to be useful for predicting the risk of hemorrhage into the spleen.

Growth factor is probably contraindicated in donors with a history of autoimmune disorders [58–60]. Flares of rheumatoid arthritis and ankylosing spondylitis have been reported in non-PBPC donors following therapy with filgrastim or sargramostim [58,60]. A variety of eye inflammatory responses have been reported among allogeneic PBPC donors. These have included marginal keratitis, episcleritis, and iritis occurring during therapy with filgrastim [59,61].

PBPC donation and the risk of death

As with bone marrow donation, death at the time of, or in close proximity to, PBPC donation has been reported [43,44]. Table 37.1 lists five deaths among PBPC donors. It is unclear whether the risk of death associated with PBPC donation differs from that of bone marrow donation.

Long-term donor follow-up

The long-term safety profile of growth factor therapy in normal individuals has not been established. Several investigators have performed serial evaluations on the peripheral blood of normal PBPC donors using a variety of techniques to evaluate chromosomal content, DNA stability, replication timing, and gene expression profiling [62–66]. In every instance, except one, filgrastim-induced alterations during HC mobilization have been transient; Nagler et al. [64] reported persistent aneuploidy of chromosome 17 in the lymphocytes of PBPC donors 6–9 months after mobilization. A recent study by Hirsch et al. could not replicate these findings and disclosed no evidence of filgrastim-induced aneuploidy or replication asynchrony [66].

There have been several reports providing 1–5 years follow-up on limited numbers of PBPC donors [30,48,67–70]. In no instance have serious adverse events attributable to filgrastim been identified. Pulsipher et al. reported on 2408 NMDP donors with a median follow-up of 49 months (range 0 to 99 months) [48]. Among these donors, no cases of acute leukemia were seen and a single case of chronic lymphocytic leukemia was reported. Anderlini et al. [71] recently reported on the follow-up of 343 PBPC donors of whom 281 (82%) were able to be interviewed. With a median follow-up of 39 months (range 7 to 80), there were no instances of leukemia or other hematopoietic adverse events.

PBPC versus marrow: which is easier for donors?

Switzer et al. [72] evaluated 70 unrelated bone marrow donors who subsequently provided a second HC product (25 PBPC and 45 bone marrow). Donors whose second product was PBPC reported that, in comparison to their prior bone marrow donation, PBPC donation was less difficult physically, required less time, and was more convenient. In the NMDP experience, PBPC donors recover from donation more quickly than bone marrow donors [32].

In randomized trials of PBPC versus bone marrow among sibling donors, the distinction between the two procedures is less clear. Similar levels of anxiety and pain are reported, and time to complete recovery may also be similar [73,74]. When differences are observed, however, in acute symptoms, fatigue, time to recovery or late complications, these tend to favor PBPC donation [74–76].

Special considerations

Infants and children as HC donors

Children may safely donate marrow [34,77,78]. They are probably more likely than are adults to receive allogeneic blood transfusion, but serious complications among children donors have been rare [34,78].

Sanders et al. [78] reported on 23 marrow donors under the age of two. Harvested volumes were between 11.5 and 19.3 mL/kg donor weight. No serious complications were encountered, but 22 of the 23 infants required allogeneic blood.

In a survey of pediatric marrow transplant physicians, only seven of 56 responders were unwilling to collect marrow from infant donors 0–6 months old [77]. There was little agreement, however, on the management of large volume collections. Of 52 respondents, six would limit collections to 25% or less of the donor's blood volume, whereas 24 placed the limit at 50% or higher. Twenty-two respondents preferred to manage large volume collections in two stages.

Children appear similar to adults with respect to their response to filgrastim and PBPC collection [79]. They tolerate filgrastim similarly and provide high yields of CD34$^+$ cells. Central venous access may be required more often for successful collection from children [79]. A report described PBPC collections in five children aged 4–13 years [49]. The children each received filgrastim at a dose of 6 μg/kg every 12 hours for 3 or 4 days. One or two leukapheresis procedures were performed. No child required a central venous line and no immediate complications were encountered. Pulsipher et al. [80] recently reviewed the clinical, ethical, and research issues surrounding administration of filgrastim to children.

Children who are being evaluated as potential HC donors deserve special attention. They may have very limited understanding of their family member's illness and of the donation process. Their fears and concerns are often complex and accompanied by significant ambivalence [81]. With hapoloidentical transplantation protocols, children may also be considered as donors for parents, which raises added concerns about understanding, stress, and informed consent.

Consent for children presents special concerns. In most instances, parents are expected to consent for their children, which may create conflict of interest situations. When they are later asked, children have reported that they were forced to donate by families or physicians or, short of being forced, that they felt they had no real choice in the matter [81].

A survey of pediatric transplant physicians in the United States confirmed that most felt the role of consent appropriately rested with the parents [77]. In some locales, it remains the standard practice to appoint legal guardians to determine whether HC donation is in the child's best interest.

Subsequent donations following an initial HC donation

A person can donate hematopoietic cells repeatedly. Most second donations of bone marrow or PBPC are intended for the original transplant recipient and used for treatment of graft failure, poor graft function, or disease recurrence. Buckner et al. [34] reporting on 1160 marrow donors identified 99 who donated marrow twice, usually within a 2-month period. There was a modest decline in the average total nucleated cells collected with the second donation. Stroncek et al. [82] reported on 16 second donation donors at the University of Minnesota. There was a trend toward lower total cells and lower cell concentrations in the second collections. When fewer than 90 days separated the collections, the decrement in cell concentration was statistically significant [82]. Donors whose second donation occurred within 60 days of the first were frequently transfused with allogeneic blood (5 of 8 within 60 days vs. 0 of 8 later than 60 days).

Consecutive PBPC collections are also feasible. Stroncek et al. [70] collected PBPC products 1 year apart in 19 healthy volunteers.

There were no differences in premobilization blood counts, the response to filgrastim stimulation, or the CD34$^+$ cell yield between the first and second collections. Similar results were reported by Anderlini et al. [30] who described 13 allogeneic PBPC donors providing a second donation at a median of 5 months (range 1 to 13). No differences were identified in preapheresis WBC counts of the donors or in the yield of CD34$^+$ HC. In more recent studies, de la Rubia et al. [83] noted a decline in the total CD34$^+$ cell yield following a second PBPC mobilization and collection. Donor symptoms were no different between the two donations. Similar results were noted by Platzbecker et al. [84] who also found that female sex and a low peripheral blood CD34$^+$ cell count during the first mobilization predicted for a low CD34$^+$ yield with the second donation.

The WMDA has published consensus guidelines for subsequent donations by unrelated donors [85]. These guidelines establish recommended intervals between donations and limits on the number of donations.

Growth factor-mobilized bone marrow

In efforts to accelerate the engraftment rate for bone marrow transplantation, investigators have explored filgrastim administration prior to bone marrow collection [86–88]. These reports, a combination of single arm trials and comparison studies, suggest indeed that rhG-CSF pretreatment of the marrow donor may accelerate recovery of neutrophils and platelets. Morton et al. [89] reported a randomized comparison between PBPC and filgrastim-primed bone marrow. Engraftment of neutrophils and platelets was not different between the two groups, but patients receiving filgrastim-primed bone marrow had significantly less steroid-refractory acute graft-versus-host disease (GVHD), less chronic GVHD, and fewer days of immunosuppressive therapy. Taken together, these preliminary observations suggest that it may be possible to gain some of the advantages of PBPC without the added risk for increased acute and chronic GVHD.

These studies, however, have important implications for HC donors because many of the most significant and non-overlapping adverse events of PBPC and marrow donation are combined in a single procedure. While reduced volumes of marrow collection may be possible [86], this may not fully offset the added risks of filgrastim administration. This would seem to be an area where carefully designed, prospective clinical trials are needed.

Summary

Allogeneic HC donation is safe. Serious adverse events are uncommon and death is exceedingly rare. Nevertheless, all donors must be carefully evaluated and fully informed prior to HC donation. Emerging regulatory requirements must be anticipated and met. The two common procedures for obtaining an HC graft, bone marrow and PBPC collection, differ in their acute and longer term impacts upon the donors. The safety data for both procedures is emerging and confirms long-term preservation of a functional, benign hematopoietic system.

It is highly likely that annual numbers of allogeneic HCTs will continue to grow. Based on estimates of current need, the numbers of related and unrelated allogeneic HCTs will easily double within the next several years.

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CHAPTER 38

The Role of the Transplant Program in a Nuclear Accident or Terrorism

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Introduction

The irony of this chapter should not be lost on the reader. After all, it was the nuclear detonations in Hiroshima and Nagasaki that led to the clinical observations that the bone marrow was very sensitive to the effects of radiation. Many of the victims of the nuclear detonations presented days later with pancytopenia. This bone marrow sensitivity led to the initial murine and then canine studies confirming that the bone marrow was the organ responsible for making all the blood elements and that bone marrow shielding or the transfer of bone marrow from another individual could rescue the animal from a lethal dose of radiation. Moreover, transplantation biology with the description of the major histocompatibility complex (MHC) was a direct result from these initial studies. This observation was then confirmed in humans with various maladies affecting the bone marrow or immune system who received total body irradiation followed by an autologous or allogeneic bone marrow graft. Thus the field of hematopoietic cell transplantation (HCT) was born. Our field has brought a combination of very exciting basic science, translational medicine, and clinical care to our patients leading to significantly better outcomes in many disease states. Yet, it seems we are coming around full circle with the increased threat of a nuclear detonation brought by a terrorist attack or a natural disaster.

Release of radioactivity, either intended or not, is an undeniable possibility and potentially catastrophic [1]. The recent tsunami and the Fukushima reactor meltdown is a stark reminder of the potential risk. If the winds had not been favorable blowing radioactivity out to sea, the effects could have been much worse [2]. Reports of nuclear proliferation in nations unfriendly to the United States and the Western world and the poisoning of a Russian dissident with polonium-210 are further reminders of the terrorist threat [3]. In addition, more than 400 radiologic accidents have occurred since 1944, resulting in over 3000 significant exposures [4]. Approximately 10 million "sealed sources" of radioactive material (e.g., cesium-137, cobalt-60) are used for medical, industrial, agricultural, and research purposes worldwide [5]. Over 600 of these have been lost or stolen since 1995 and less than half were eventually recovered.

Detonation of a nuclear device would cause unprecedented loss of life and injuries as well as economic, political, and social disruption. Many victims exposed to significant doses of radiation will develop bone marrow suppression. Thus, hematologists and oncologists, especially those with HCT experience, are uniquely suited to help evaluate and manage radiation exposure victims [6]. Depending on the scale of the event, there may be a national call for surge capacity. Hematologists, oncologists, and hematopoietic cell transplantation (HCT) specialists across the country could be asked to balance the needs of their local patient populations with requests to accept patient transfers or even travel to other institutions. Therefore, we must collectively prepare for this contingency. Since a nuclear detonation would be the most severe form of damage, the rest of the chapter deals with this contingency planning but the infrastructure suggested would be able to respond to a radiologic dispersal device (e.g., a "dirty bomb" or a radiologic source under a subway seat or in a park) or a nuclear power plant accident.

A public prepared to protect itself from fallout and an effective medical response could save tens of thousands of lives. Since 2001, all levels of government, academic institutions, and professional organizations have done significant work to enhance our ability to prepare for and respond to a nuclear detonation. In the public health and medical arena, notable achievements are several different publications and websites including The Planning Guidance for Response to a Nuclear Detonation [7], the multipaper series from the Nuclear Detonation Scarce Resources Working Group [8–23], health and medical educational material available from the Centers for Disease Control and Prevention (CDC) [24] and just-in-time material including algorithm-based management available on the Radiation Emergency Medical Management (REMM) website [25]. The Federal Emergency Management Agency (FEMA) along with interagency partners is developing improvised nuclear device (IND) national strategy and implementation plans.

Preparedness and planning for a nuclear detonation

Several types of events, either accidental or intentional, could result in radiation exposure (Table 38.1). Intentional events can be caused by radiologic exposure devices (REDs), radiologic dispersion devices (RDDs), and improvised nuclear devices (INDs). An RED is a radioactive source placed surreptitiously in a public space or other location. An RDD spreads radioactive material over a wide area, either using a conventional explosive (i.e., a so-called "dirty bomb") or via other means (e.g., by tainting the food or air supply). Although these devices would likely produce comparatively few fatalities, large numbers of people could be exposed to small doses, engendering widespread public confusion and anxiety. Victims of events involving RDDs or REDs would most likely be treated at centers near the incident, except in specific cases that require highly specialized care for

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Table 38.1 The spectrum of potential events involving radioactive material. Numbers of deaths are rough estimates

Categories	Description	Number of deaths
Radioactive source accident	Loss or theft of a radiologic source (e.g., Goiana, Thailand)	0–10s
Nuclear reactor accident	Release of radioactive gas or material (e.g., Chernobyl)	0–100s
Radiologic dispersal device	Device or scheme for dispersing radioactive isotope (e.g., dirty bomb[a] or radioactive material in the food supply)	0–1000s
Radiologic exposure device	Radioactive material intended to expose people in the vicinity (e.g., cesium source placed on a train)	0–100s
Improvised nuclear device	Incorporates radioactive material intended to produce a nuclear explosion	1000s to >1,000,000

[a] Only a small fraction of deaths would be expected to result directly from radiation exposure.

burns or marrow suppression. It is unlikely that individual hematologists in other regions of the country would be involved.

In contrast, an improvised nuclear device (IND) using fissionable material such as plutonium or uranium would have devastating consequences and require the utilization of all available resources. A nuclear detonation produces both physical damage (trauma and burns) and radiation exposure. While the blast, heat and radiation zones overlap close to the epicenter, there can be major zones where there is physical injury without radiation (upwind glass breakage injuries) and others where there is radiation with limited or no physical damage to the infrastructure (fallout areas). The response to a nuclear detonation is organized around three physical damage zones (severe, moderate, and light); a dangerous fallout zone; and a variable fallout zone, designated (>10 mR/hr), in which response activities can occur but rescue time may be limited. The Radiation TRiage, TReatment, and TRansport (**RTR**) system overlays a functional response on these response zones (Figure 38.1) [26].

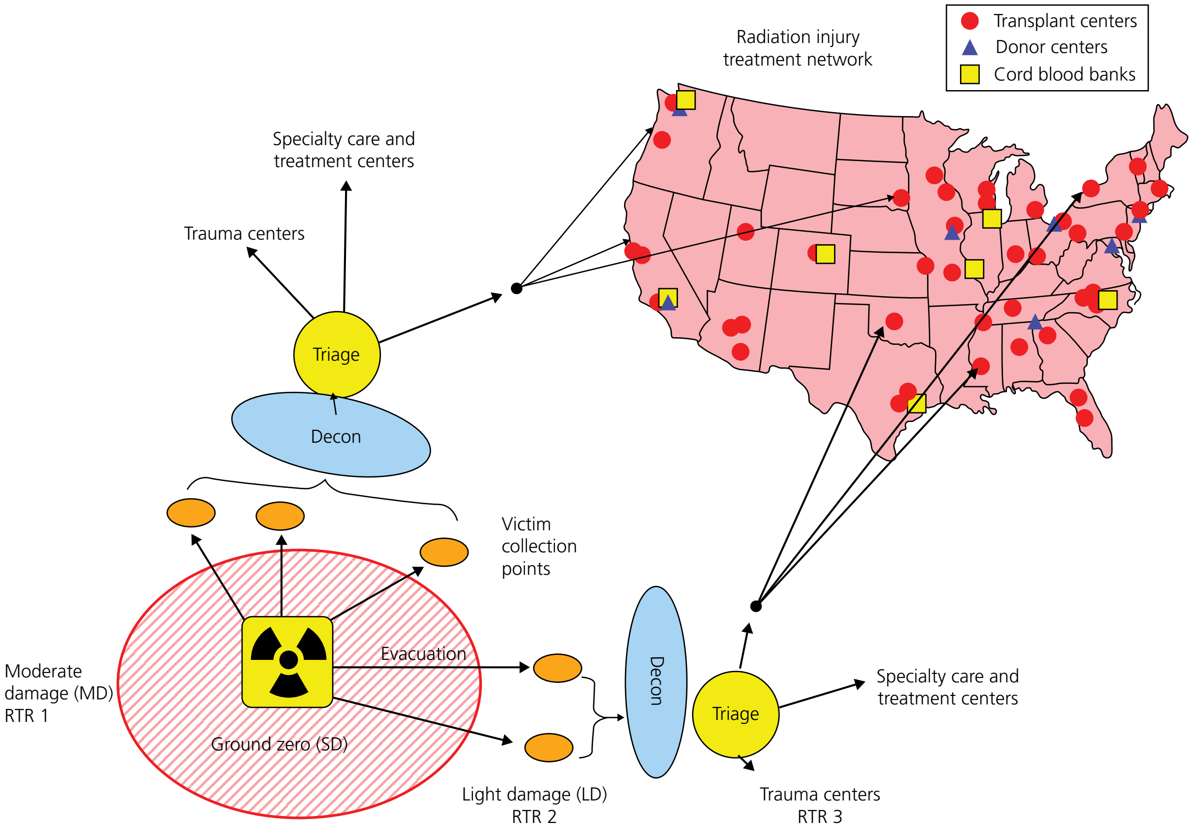

Figure 38.1 Schematic for triage and response to a large-scale radiation event developed by the Office of the Assistant Secretary for Preparedness and Response. Triage centers are located in concentric rings around the affected area, providing initial stabilization and decontamination (RTR 1–3), more extensive medical care (MC), and rapid screening of unexposed or minimally exposed individuals at assembly centers (AC). Patients who require further care are evacuated to referral centers in unaffected regions.

Damage Zones:

- Severe Damage (SD) zone: extensive infrastructure damage and few survivors
- Moderate Damage (MD) zone: probably not readily passable due to physical damage but can be cleared, with variable to no levels of radiation, which, if present, decrease fairly rapidly over time the first hours to day
- Light Damage (LD) zone: passable, most damage is glass breakage, with little or no radiation other than where fallout areas overlap the LD zone.

Functional Response System: the RTR system helps organize the response [6,7]. This system includes several types of sites:

Spontaneously forming RTR sites (casualty collection points):

1. RTR 1: at or near major physical damage with significant radiation present early on radiation present
2. RTR 2: limited physical damage and varying radiation present that declines over the first few hours to a day, likely near the dangerous fallout (DF) zone, and
3. RTR 3: minimal damage and no radiation.

It is estimated that approximately 40,000 hospital beds are available at any given time nationwide, a capacity far lower than the projected number of potentially salvageable casualties. Thus, medical centers around the country may be asked to accept patients from the disaster area, both victims of the event and local residents who require care for chronic or intercurrent medical conditions. These efforts would initially be coordinated as part of the National Disaster Medical System (NDMS), a federally coordinated system of public and private hospitals that augment the nation's medical response. As observed in the aftermath of Hurricane Katrina, healthcare needs for displaced populations can easily overwhelm the infrastructure in regions immediately surrounding a disaster area. For patients who require intensive medical care or those with cancer, hematologic disease or other chronic diseases, this disruption can be particularly dangerous.

Responding to the consequences of a nuclear incident: concepts, information and resources

Response is based on the assumption that resource availability will vary by proximity to the incident. Moreover, response will be an iterative process that will change over time. Hospitals closest to the detonation will be quickly overwhelmed by a surge of casualties and will rapidly exhaust supplies. The plan, therefore, is to distribute patients as quickly as possible for medical assistance farther away from the affected area where it will be easier to replenish supplies and staff. Key strategies for managing medical care in scarce resource settings are summarized in Table 38.2.

Developing a quantitative understanding of the local capacity for non-traditional emergency response assets (private practices, skilled nursing, commercial labs, etc.) and coordinating them is crucial. Planning for proactive resource support from the surrounding region, tribal, state, and Federal entities and the use of regional staging areas in case of a catastrophic incident will greatly shorten the time to resource availability to the end users. Assembly and evacuation center planning by healthcare facilities and the community at large will considerably mitigate bottlenecks and confusion in the hours and days after an incident.

Local planning is most effective when it is integrated across the tiers of response including state and Federal systems. Effective integrated planning is facilitated through the use of planning and response tools such as the Health and Human Services (HHS) resource mapping tool, MedMap [27]. MedMap facilitates sharing situational awareness with the local/regional responders and can show locations of healthcare facilities in relation to other overlays and demographic information. Designated local and state government partners can request access to MedMap through their HHS/ASPR Regional Emergency Coordinators.

Table 38.2 Strategies for scarce resource situations

- **Prepare** – stock disaster supplies and increase par levels on commonly needed items such as tetanus vaccines, laceration trays, narcotic analgesics, dressings, etc.;
- **Substitute** – use a clinically equivalent item or staff person [12];
- **Adapt** – use items or technologies to provide sufficient care (use transport ventilators or anesthesia machines instead of full-featured ventilators), use staff with similar or congruent skill-sets (specialty surgeons assisting with trauma surgeries), or adapt locations of care (performing surgical procedures outside of the OR environment);
- **Conserve** – use less of a resource by lowering dosage or changing utilization practices;
- **Re-use** – after appropriate disinfection/sterilization, re-use supplies;
- **Re-allocate** – prioritize a therapy in scarce supply so that it is only given to those with a higher chance of benefit or greater need.

Medical management of acute responses to radiation

People exposed to high levels of radiation (usually >1–2 Gy over a short period of time may develop acute radiation syndrome (ARS). Symptoms vary according to the dose but include vomiting, diarrhea, headache, dizziness, weakness, bleeding, and redness of the skin. In a large mass casualty setting, efficient triage of irradiated casualties is essential to identify those who have received clinically significant but not invariably lethal doses of radiation estimated at 2–10 Gy of whole body exposure. These are the victims that need specialized and sometimes urgent care. In resource-scarce settings, symptomatic care is given if possible and life-sustaining measures should be withheld from casualties with non-survivable trauma, thermal burns, and/or radiation exposures. Extensive triage algorithms that emphasize fairness were recently published to guide the selection of appropriate candidates for life-sustaining care in resource-limited settings in order to maximize survivability for the overall population [28] (See Table 38.3 for a list of websites containing additional information on approaches to medical triage, assessment and management after radiation exposure).

The current management of ARS does not substantially differ from the management of pancytopenia in other settings, such as after treatment with myelosuppressive chemotherapy. Although there are no cytokines currently licensed for treatment of acute radiation exposure, administering myeloid cytokines to appropriately selected victims offers two potential benefits after a mass casualty radiation incident. It can reduce morbidity and mortality resulting from neutropenia and it can lessen the need for subsequent or continued hospitalization during the post-incident period when medical resources and personnel may be extremely limited. The current challenge is that the local supply of cytokines would be quickly depleted in the absence of adequate triage to ensure that those that may not require this treatment immediately are referred for later evaluation and possibly treatment in a more resource-rich environment.

All patients with confirmed neutropenia or medical history of radiation exposure and physical injuries strongly suggestive of combined injury (radiation *plus* trauma and/or burns) are potential candidates for myeloid cytokines if they are deemed to have survivable exposure/injuries. Studies in non-human primates suggest that *initiating myeloid cytokines within 24 hours of exposure* may improve outcomes [29]. Myeloid cytokines [granulocyte colony-stimulating factor (G-CSF; filgrastim), granulocyte–monocyte colony-stimulating factor (GM-CSF; sargramostim) or pegylated G-CSF (pegfilgrastim)] can reduce the duration of neutropenia, hospital length-of-stay, and overall costs.

Myeloid cytokines should be initiated as soon as there is evidence a casualty will develop severe neutropenia (i.e., less than 500 neutrophils per mm^3). Specific indications for initiating myeloid cytokines prior to the onset of neutropenia include a projected whole body dose of 2 Gy or more based on (1) physical dose reconstruction using geographic information, (2) clinical signs, and/or (3) lymphocyte depletion kinetics. Drugs should be continued until normalization of the granulocyte count. Supportive care measures are equally important. Anti-emetics for vomiting, hydration, and antibiotics to prevent bacterial infections during the neutropenic period have been shown to improve survival in animal models of ARS. These suggestions are not different from those that are in daily practice in the hematology-oncology or transplant wards.

The essential role of biodosimetry

Appropriate triage and care after radiation exposure depends on accurate and timely estimates of radiation dose. Dose information will be important for classifying victims into groups that: (1) will not require medical intervention, (2) could benefit from supportive care (e.g., colony-stimulating factors) to facilitate autologous marrow recovery, (3) require evaluation for HSCT to treat potentially irreversible marrow damage, and (4) cannot be salvaged. A variety of information can be used to estimate an individual's radiation exposure. Unlike the homogenous dosing associated with therapeutic total body irradiation, shielding from nearby structures (e.g., buildings) during accidents or terrorist attacks will result in heterogeneous exposures. Therefore, a careful history of the victim's location and subsequent symptoms will be essential. Initial clinical assessment will include the time from event to first emesis and peripheral blood counts, with subsequent lymphocyte depletion kinetics.

Approaches that use only clinical and routine laboratory findings to stratify victims into risk groups are valuable for a small-sized accident, but their utility during large events is not clear [30–32]. Biodosimetry, the use of biologic markers to assess dose, can enhance the predictive value of clinical findings after radiologic or nuclear events. The "gold standard" for biodosimetry is the quantification of dicentric chromosomes using metaphase cytogenetics in peripheral blood lymphocytes (PBLs). Unfortunately, dicentric quantification requires multiple days to perform and is currently available only in select centers. Plans have been formulated to develop major radiation laboratory networks to perform dicentric quantification on a mass scale [33]. Newer methods for biologic dosimetry, including rapid genomic analysis of PBLs, serum proteomics, and measurements of DNA damage, are also under development [34–43]. Treating hematologists will need to calculate radiation doses using the information they have available. Online algorithms for estimating dose based on clinical and biologic data are available from the Radiation Event Medical Management (REMM) website at http://www.remm.nlm.gov/ars_wbd.htm or from the Armed Forces Radiobiologic Research Institute at http://www.afrri.usuhs.mil/www/outreach/biodostools.htm.

Radiation Injury Treatment Network (RITN)

Beginning in 2001, the National Marrow Donor Program (NMDP) established a working group to address the issue of a nuclear disaster. In 2006, the American Society for Blood and Marrow Transplantation (ASBMT) formally joined this effort and established the Radiation Injury Treatment Network (RITN), a voluntary consortium of 61 (and growing) HCT centers, donor centers, and umbilical cord blood banks (Figure 38.2), made possible through partnerships with the Office of Naval Research and the Center for International Blood and Marrow Transplant Research. The goals of RITN (www.ritn.net) are:

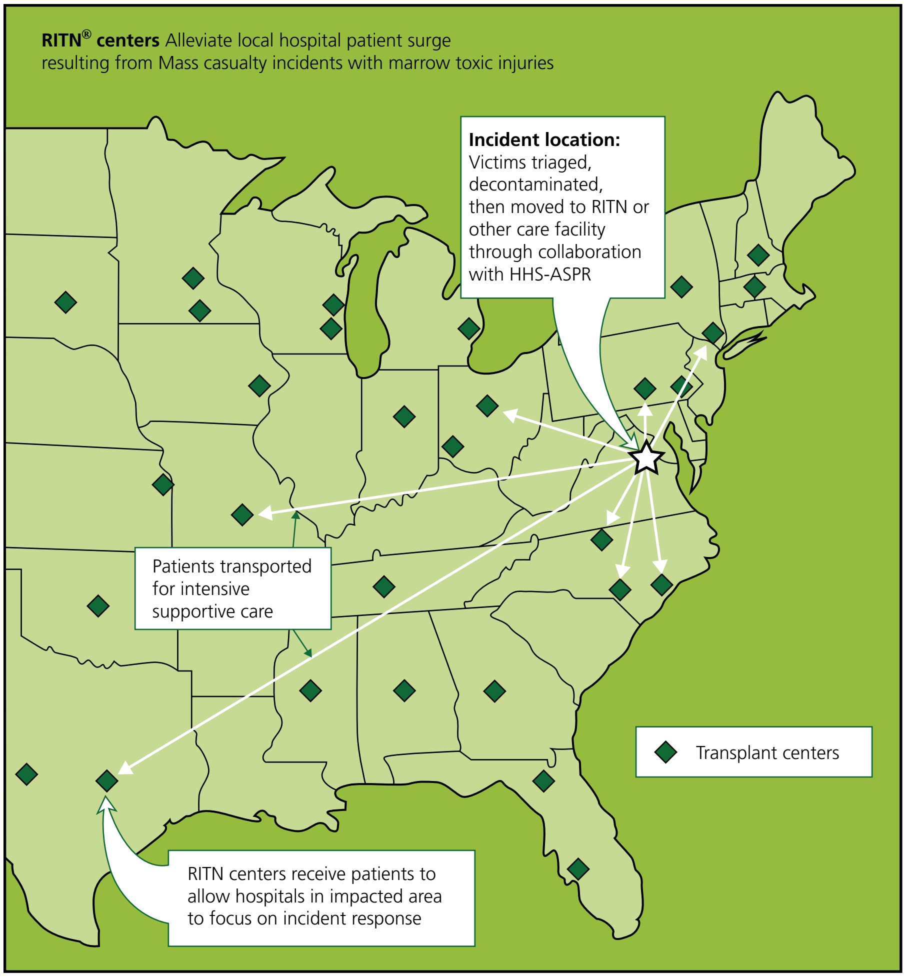
RITN® centers Alleviate local hospital patient surge resulting from Mass casualty incidents with marrow toxic injuries
Incident location: Victims triaged, decontaminated, then moved to RITN or other care facility through collaboration with HHS-ASPR
Patients transported for intensive supportive care
RITN centers receive patients to allow hospitals in impacted area to focus on incident response
Transplant centers

Figure 38.2 The Radiation Injury Treatment Network (RITN). Transplant centers, donor centers, and umbilical cord blood banks are divided into 10 regions designated by the US Federal Emergency Management Agency (FEMA). Primary transplant centers act as the lead institutions within their region during event response.

1. to develop treatment guidelines for managing hematologic toxicity among victims of radiation exposure,
2. to educate healthcare professionals about pertinent aspects of radiation exposure management,
3. to coordinate situation response after a radiation event,
4. to provide comprehensive evaluation and treatment for victims at participating HCT centers, and
5. to collect data on the outcomes of treatment.

The European Group for Blood and Marrow Transplantation (EBMT) is establishing a similar network to offer training courses and improve cooperation between institutions [29].

The number of patients who will require care after a large-scale event, such as an improvised nuclear device, exceeds the capacity of RITN centers by many orders of magnitude. For example, if a device similar to the bomb detonated over Hiroshima struck a metropolitan area, the number of casualties would depend on many factors, such as the size of the device, the time of day, type of buildings, weather conditions, and the precise location of the detonation. Models suggest that as many as 175,000 victims would require intensive medical care and nearly 30,000 would need intensive management for myelosuppression [44]. Such an event would clearly overwhelm any health system. The care of such victims would require altered standards of care, including the use of alternative treatment sites such as armories, auditoriums, or gymnasiums. Thus the logistical complexity for planning is clearly daunting, some would say too daunting. However, despite the obstacles, experience from radiation accidents indicates that many victims can be salvaged with appropriate care. The LD_{50} (lethal dose to 50% of persons exposed) for total body irradiation in humans is approximately 3.5–4 Gy without supportive care, but the use of antibiotics and transfusions may increase the LD_{50} to 4.5–7 Gy and survival at doses greater than 10 Gy may be possible with HCT [45]. If a significant fraction of victims with acute injuries could be saved with supportive care alone, appropriate planning and response after a large-scale event could save thousands of people. Thus, a large-scale event will offer extraordinary opportunities for qualified practitioners at large and small centers throughout the United States and potentially internationally to support such an effort. Who would be more qualified than hematologists and oncologists, especially HCT physicians, if properly trained?

Resources for assisting hematologists with clinical management

Acute radiation syndrome (ARS) can affect virtually any organ but primarily manifests as injury to the hematologic, dermatologic, gastrointestinal, and central nervous systems. The severity of ARS increases proportionally with the radiation dose from mild (<1–2 Gy) to invariably lethal (>10–20 Gy). The clinical course of ARS generally includes a prodromal phase, followed by a period of apparent clinical remission, manifest illness, and ultimately recovery or death. Importantly, the latency between exposure and severe manifestations of ARS affords the time to transport victims to RITN sites.

A large fraction of patients with radiation exposure significant enough to induce cytopenias will have multi-organ system damage. Persons exposed to radioactive material may present unique and exceedingly complex management challenges. Few physicians are familiar with the basic manifestations of acute radiation injury or have training in the prospective management of patients with significant radiation exposure. In addition, esoteric aspects of care such as decorporation therapy for internal radiation contamination are extremely important but very few healthcare practitioners have experience in this realm. A number of resources are available to assist with the evaluation and care of radiation exposure victims (Table 38.3). Among these, the Radiation Event Medical Management website (www.remm.nlm.gov) was developed in a collaboration among the National Library of Medicine, Office of the Assistant Secretary for Preparedness and Response, and medical experts from around the world. With assistance from RITN members, REMM includes admission and treatment order templates directed toward victims of radiologic or nuclear events.

To ensure optimal care and enhance preparedness for subsequent events, it is essential that, to whatever extent possible, data on exposure and clinical complications be collected prospectively from victims and compiled centrally. The NMDP has developed a data collection protocol (http://www.RITN.net) for use at RITN centers across the United States. After a large-scale event, centers that accept patients with radiation injury may be asked to contribute patient data to central repositories, either through RITN or governmental agencies.

A role for stem cell transplantation?

Some victims of a large-scale event may receive doses of radiation to cause irreversible myeloablation. As discussed earlier, these patients will commonly have multiorgan damage. What remains unclear is whether allogeneic HCT can be a life-sustaining measure in this setting. To date, 31 patients have undergone allogeneic HCT after accidental radiation exposure. Median survival after transplant for these patients is ∼1 month [4]. All four patients who survived >1 year reconstituted autologous hematopoiesis, raising the question whether the HCT provided any benefit. Particularly troubling was the contribution of GVHD to mortality in >20% of patients. More recently previously unavailable data on the use of stem cell administration to aid recovery of victims of the Chernobyl disaster became available [46]. There were nine patients heretofore unreported in the scientific literature who underwent intraosseous injections of allogeneic bone marrow cells in Kyiv. Transplantation was associated with significantly shortened time to recovery of

Table 38.3 Websites containing additional information on approaches to medical triage, assessment and management after radiation exposure (websites accessed January 2015)

Source	Website
Armed Forces Radiobiology Research Institute	www.afrri.usuhs.mil
Centers for Disease Control and Prevention	www.bt.cdc.gov/radiation/
Federal Emergency Management Agency	www.fema.gov/hazard/index.shtm
Health Physics Society	www.hps.org
National Institute of Allergy and Infectious Diseases Radiation Countermeasures Program	http://www3.niaid.nih.gov/research/topics/radnuc/
Radiation Emergency Assistance Center/Training Site	www.orau.gov/reacts
Radiation Emergency Medical Management	www.remm.nlm.gov
Radiation Injury Treatment Network	www.ritn.net
Uniformed Services University of the Health Sciences Center for Disaster and Humanitarian Assistance Medicine	www.usuhs.edu/afrri/outreach/pdf/AFRRI_Pocket_Guide.pdf
U.S. Department of Veteran Affairs Emergency Management Strategic Healthcare Group	www.va.gov/emshg
U.S. Food and Drug Administration Office of Crisis Management	www.fda.gov/aboutfda/centersoffices/officeofoperations/officeofcrisismanagement/default.htm
U.S. Nuclear Regulatory Commission	www.nrc.gov/about-nrc/emerg-preparedness/in-radiological-emerg.html

granulocyte and platelet counts in these patients. While current guidelines would certainly include the use of cytokines, these data provide an indication of the effectiveness of stem cell transplant to treat victims of radiation exposure.

In many regards, patients with myeloablation from radiation exposure are similar to those with aplastic anemia. A reduced intensity conditioning regimen for severe aplastic anemia (where immunosuppression but not myeloablation is required) is being tested in the Blood and Marrow Transplant Clinical Trials Network (BMT CTN Protocol 0301) [47]. Of note, NMDP has plans in place to conduct large numbers of urgent searches for victims following an event, recognizing that only a few searches would likely lead to transplants.

Current efforts to develop medical radiation countermeasures

Several promising medical countermeasures are under development. However, the complex nature of radiation injury is such that no single drug is likely to provide benefit in all circumstances and against all aspects of radiation injury. Antioxidants and radioprotectants are presumably most effective if present at the time of irradiation, while therapeutics such as a growth factor may target one or more but not all affected organ systems. For this reason, many experts believe that combination therapy will be required to produce substantial improvements in outcomes.

The US Government, particularly the Department of Defense, has a long-standing interest in the development of medical countermeasures against radiation. Beginning in 2005, the Radiation Countermeasures Program at the US National Institute of Allergy and Infectious Diseases (NIAID) has supported the development of medical countermeasures for civilian populations exposed to radiologic or nuclear hazards as a result of accidents or terrorist attacks [48].

Conclusion

Whether a radiologic or nuclear incident occurs domestically or abroad, work continues to ensure that the United States is prepared to assist in planning and response. Many governmental and nongovernmental agencies are involved in the planning. Although the logistical difficulties inherent to any large-scale event are enormous, the potential for life-saving measures is equally large. Future efforts will focus on streamlining these processes, providing training to medical practitioners around the country, and validating medical countermeasures to reduce the morbidity and mortality of radiation exposure. Practitioners and institutions across the country are encouraged to become involved and participate in the planning. The widespread availability of radioactive material has made future exposure events, accidental or intentional, nearly inevitable. Hematologists, oncologists, and HSCT physicians are uniquely suited to care for victims of radiation exposure, creating a collective responsibility to prepare for a variety of contingencies.

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CHAPTER 39

Cord Blood Hematopoietic Cell Transplantation

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Introduction

The field of hematology has had a long and distinguished history, dating back at least to studies of blood by Galen in the years 131–201CE [1]. As our knowledge of the hematopoietic stem (HSC) and progenitor (HPC) cells, and the cytokines and stromal cell populations that regulate the HSC and HPC for production of the blood-forming elements grew [2–5] we learned how to best use this information for therapeutic intervention. Cord blood (CB) hematopoietic cell transplantation (HCT) is an accepted alternative treatment for patients with malignant and non-malignant disorders, with certain advantages compared to bone marrow (BM) and peripheral blood stem cells (PBSC) for HCT. Advantages presented include: ease of collecting the cells which entails little or no risk to the mother or the child, relatively quick access to donor human leukocyte antigen (HLA)-typed CB collections stored as cryopreserved samples in public or family CB banks, and lower stringency HLA matching requirement in unrelated and related transplants compared to BM. Lower stringency in HLA matching is in part due to the relatively naïve state of CB immune cells, and the lesser immune cell-mediated activity that manifests in decreased graft-versus-host disease (GVHD). These advantages of CB have helped recipients to receive a HCT who might not otherwise have a suitable HLA-matched donor. Over 30,000 CB transplants had been performed by 2012 [report of the Bone Marrow Donors Worldwide (BMDW) program] starting with the first HLA-matched sibling CB transplant in October 1988 for Fanconi anemia [6]. The recipient of this first CB transplant is alive and well. However, disadvantages to using CB for transplantation include limiting numbers of total nucleated cells (TNC), and hematopoietic stem (HSC) and progenitor (HPC) cells in CB, which have in part been responsible for increased graft failures. CB HCT is associated with slower time to neutrophil, platelet, and immune cell recovery compared to that for BM or PBSC. Despite this, and counterbalanced by decreased incidence of GVHD, survival has been comparable to that after BM or PBSC transplants. Double CB HCT has extended the use of CB HCT for adults, but has not significantly addressed the issue of delayed cell recovery.

Brief historical perspective of the first CB HCTs

A number of original papers and reviews have been published that highlight the studies that led to the first [6] and subsequent HLA-matched sibling CB HCTs [7–13]. The clinical field of CB HCT was ushered in with the report that HLA-matched sibling CB could be used to save the life of a child with Fanconi anemia [6], a clinical study performed after laboratory studies described CB as a potential source of transplantable HSCs and HPCs [9]. While previous studies had reported the presence of HPCs in CB (reviewed in [9–13]), the critical paper that initiated the concept of using CB in a clinical setting for efficacy [9] demonstrated a number of important points. It was estimated that there would be sufficient cells in a single collection of CB for HCT based on quantities of HPCs in a large number of single CB collections, even though the amount of total nucleated cells per kg recipient body weight would be at least 10-fold less than that used for BM HCT. The high proliferative capacity of CB HPCs compared to those in BM [9] (later described in greater detail [14–16]), was also apparent. In addition, the CB could be maintained in viable form without separation of cells for a few days at room temperature with no obvious loss in HPC viability or numbers. The collected CB cells could be shipped by overnight express mail to a distant laboratory, where they could then be frozen and subsequently thawed months later with a high-efficiency retrieval of functional HPCs [9]. This led to the reality that banks could be set up to store cryopreserved CB cells. High-efficiency recovery after 20-plus years of CB storage has been reported [17]. The first proof-of-principle CB bank was set up in the laboratory of Dr. Broxmeyer, and collections from this bank were used for the first five, and a further two sibling CB HCTs [6,11–13].

Within a few years of these first transplants, sufficient CB HCTs had been done to warrant a report summarizing the effects of sibling CB HCTs [13]. By that time, CB HCT had been extended to treatment of leukemias and other disorders. The first HLA-matched sibling transplant for a child with leukemia using myeloablative conditioning was performed by Dr. John Wagner at Johns Hopkins School of Medicine in Baltimore, MD, USA [12]. The first partially HLA-matched related CB HCTs were reported by Vilmer et al. [18] and Kurtzberg et al. [19]. The patient for the study by Kurtzberg was a 6-year-old African American child with relapsed acute myeloid leukemia (AML) who received a haploidentical related HCT, on February 19, 1993. The patient had previously received an auto transplant (Kurtzberg, personal communication, December 11, 2012). The first bank to store and provide unrelated CB units for

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HCT was started by Dr. Pablo Rubinstein at the New York Blood Center, New York, NY, USA [20,21]. The first unrelated, and many subsequent unrelated CB HCTs, were performed by Dr. Joanne Kurtzberg at the Duke University Medical Center using CB units obtained from the New York Blood Center bank.

CB banking

It was recognized early on, and prior to the first CB HCT, that CB could be frozen in a cryopreserved state, with efficient recovery of viable and functional HPC after thawing months to years later (reviewed in [17]). The outstanding engraftment potential of HSC, assessed by isolating $CD34^+$ cells from unseparated populations cryopreserved more than 20 years previously and assaying their engrafting capability in mice with non-obese diabetic, severe combined immunodeficiency, and with a gene deletion in the interleukin (IL)-2 receptor gamma chain [17], was also acknowledged (Figure 39.1). Currently, both public and private (e.g., family-directed) CB banks exist, but the overwhelming majority of CB units used for HCT have come from the public CB banks. Regardless, there is a clear necessity for both types of CB banks to operate under the highest level of regulatory standards [22]. A number of recent

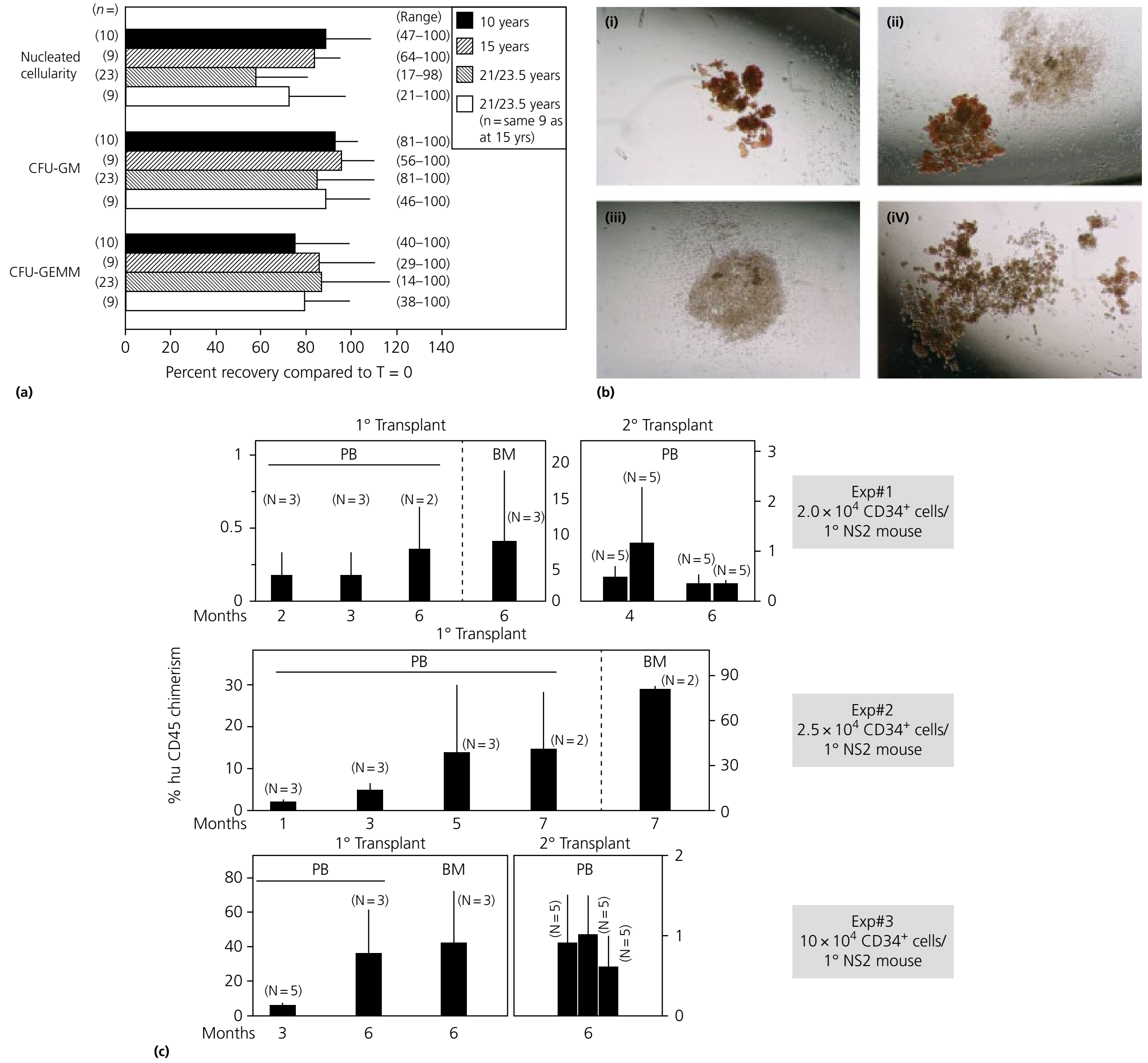

Figure 39.1 Recovery of cells after thawing of unseparated CB stored frozen for over 20 years. (a) Comparative percent recovery of nucleated cells, granulocyte macrophage (CFU-GM), and multipotential (CFU-GEMM) progenitor cells after 10, 15, and 21/23.5 years of cryopreserved storage. (b) Representative examples of colonies grown from CB thawed after 21–23.5 years in frozen state: (i) CFU-GEMM colony; (ii) CFU-GEMM (left) and CFU-GM (right) colonies; (iii) CFU-GM colony; (iv) CFU-GEMM colony. (c) Engraftment of $CD34^+$ CB cells (isolated from unseparated cells stored frozen for more than 20 years) in primary and secondary HCT in sublethally irradiated NOD/SCID IL-2 receptor gamma chain null mice. Three different experiments shown, each with a different frozen CB unit. Source: Broxmeyer et al. 2011 [23]. Reproduced with permission of the American Society of Hematology.

reviews have highlighted the development and application of CB banking processes, standards, regulations, and the accreditation of the banks [23–25], as well as the process for CB search and distribution [26], and the management of the units of CB to be used for HCT at the transplant center [27]. There are now CB banks in both developing and developed countries [28]. Most banks cooperate through international registries that list publicly banked CB units in searchable databases such as the BMDW program, the NetCord Foundation, the National Marrow Donor Program (NMDP), and other national registries. International accreditation bodies, and governmental regulatory requirements are in place to assure that publicly available CB units meet strict rules for quality [25,29,30]. Outcome data are collected and are available for analysis through international data registries that allow monitoring and analysis of CB HCT activity and outcomes.

Clinical results

Related or familiar CB for allogeneic HCT in children

Approximately 700 sibling HCT cases have been performed in Europe, most reported to the Eurocord registry (V. Rocha, personal information). Of these, around 150 children have received CB cells in association with BM cells from the same donor, due to low CB cell numbers at collection. Numbers of related CBTs have not increased year to year. Most recipients were children, and all but 29 were completely HLA matched. Table 39.1 lists the diagnosis of children transplanted with an HLA-identical sibling CB. Among 519 recipients of HLA-matched CB with available information on outcomes, 4-year overall survival was 91% for patients with non-malignant diseases and 56% for patients with malignant diseases. The cumulative incidence (CI) of neutrophil engraftment was 91% with a median time of 22 days (range 12–80 days). The number of CD34$^+$ cells infused was associated with engraftment, which was 95% if CD34$^+$ cell counts were greater than 1.4×10^5/kg and 90% if the counts were less ($p = 0.02$). The CI of acute and chronic GVHD at day 100 and at 4 years was 12% and 13%, respectively (V. Rocha for Eurocord, unpublished data).

To compare outcomes after CB HCT with BM HCT in the HLA-identical setting, Eurocord in collaboration with the International Bone Marrow Transplant Registry (IBMTR) evaluated 113 HLA-identical sibling CB HCTs in which the patients were transplanted from 1990–1997, and compared them to 2052 HLA-identical sibling BM HCTs carried out during the same period [31]. This comparative analysis demonstrated lower risks of acute and chronic GVHD, but delayed neutrophil recovery after CB compared to BM HCT. Survival was similar for both malignant and non-malignant disorders. Although the mechanism of such GVHD reduction is not fully understood, the immunologic immaturity of the newborn, the lower number of T cells in the graft, and the enrichment of CB regulatory T (Treg) cells as well as other immune cells within CB may account at least in part for this phenomenon. These lesser GVHD results were in part used as the basis for advocating HLA-mismatched CB HCTs and for further development of unrelated CB banks.

Related CB for malignant disorders

Risk factors and long-term outcomes have been reported for 147 HLA-identical sibling recipients with hematologic malignancies [32]. Median follow-up was 6.7 years. Median patient's age was 5 years. Acute leukemia was the most frequent diagnosis (74%). In brief, higher cell dose, not using MTX in GVHD prophylaxis, and disease remission, are the main factors influencing outcomes after related HLA-identical CB HCT.

Table 39.1 Diagnosis of children transplanted with HLA identical sibling CB

Diagnosis	**Number of patients (%)**
Related CBT *n* = 692	
Malignant diseases	
ALL	149 (22)
AML	46 (7)
MDS/MPN	27 (4)
CML	19 (3)
Lymphoma	10 (1)
Solid tumor	10 (1)
Non-malignant diseases	
BMF	
Aplastic anemia	25 (3)
Fanconi	39 (5)
Other	42 (6)
Hemoglobinopathies	
Thalassemia	185 (27)
Sickle cell disease	67 (10)
Immune deficiencies	
CGD	6 (1)
Wiskott–Aldrich	6 (1)
Other SCID, or not specified	35 (5)
Metabolic disorders	
Hurler syndrome	9 (1)
Other	8 (1)
Histiocytosis	4 (1)
Other diagnosis	5 (1)

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; MDS, myelodisplastic syndrome; MPN, myeloproliferative neoplasm; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; BMF, bone marrow failure syndrome; CGD, chronic granulomatous disease; SCID, severe combined immune deficiency.

Most related CB HCTs are performed in children with genetic diseases such as hemoglobinopathies [thalassemia major (TM), or sickle cell disease (SCD)] and genetic bone marrow failure syndromes (BMFS; with the majority being Fanconi anemia) (Table 39.1). Forty-four patients (median age 5 years, range 1–20) given a CB HCT for either TM ($n = 33$) or SCD ($n = 11$) were analyzed [32]. No patient died and 36 out of 44 children remained disease-free with a median follow-up of 24 months (range 4–76). Only 4 patients experienced grade II acute GVHD; only 2 out of the 36 patients at risk developed limited chronic GVHD. The 2-year probability of DFS was 79% and 90%, respectively, for patients with TM and SCD. Use of MTX for GVHD prophylaxis was associated with an increased risk of treatment failure. More recently, long-term outcomes were determined in 485 patients with TM or SCD who were treated by either HLA-identical sibling CB HCT ($n = 96$) or BM HCT ($n = 389$) (V. Rocha for Eurocord group, unpublished data). In comparison with patients receiving BM ($n = 259$ for TM; $n = 130$ for SCD), those given CB ($n = 66$ for TM; $n = 30$ for SCD) had slower neutrophil recovery, decreased incidence of acute GVHD and none had extensive chronic GVHD. With a median follow-up of 70 months, 6-year OS was 95% and 97%, respectively, after BM and CB. The 6-year DFS was 86% and 80% in patients with TM after BM and CB, respectively, while DFS in SCD patients was 92% and 90%, respectively. In a multivariate analysis, DFS was not statistically different between CB and BM HCT recipients. In summary, patients with TM or SCD had excellent outcomes after both CB and BM HCT from an HLA-identical sibling. However, in these cases CB may be preferable over BM because of the lower incidence of chronic GVHD and no harm to the donor.

Table 39.2 Advantages and limitations of unrelated donor BM, CB, and haploidentical cells from a relative, and main criteria to be considered for choosing an alternative donor for patients without an HLA-identical sibling

	Unrelated-HCT	CB-HCT	Haplo-HCT
Information on A+B+DRB1 (DNA) typing	16–56%	~80%	100%
Median search time	3–6 months	<1 month	<1 month
Donors identified but not available	20–30%	~1%	None
Rare haplotypes represented	2–10%	20–40%	Non-applicable
Main limiting factor to graft acquisition	HLA identity	Cell dose for single CB	Poor mobilization of HSCs
Ease of rearranging the date of cell infusion	difficult	easy	easy
Potential for immunotherapy	yes	no	yes
Potential for viral transmission to recipient	yes	no	yes
Potential for congenital disease transmission to recipient	no	yes	no
Risk for the donor	very low	no	very low
Main problems to be overcome	GVHD	Graft failure, delayed immune recovery	Delayed immune recovery, lack of T-cell-mediated GVL effect (for T-cell-depleted transplant only)

Table 39.3 Diagnosis and numbers of children transplanted with unrelated CB cells and reported to Eurocord registry

Diagnosis	Number of patients (%)
Unrelated CBT *n* = 4218	
Malignant diseases	
ALL	1380 (33)
AML	725 (17)
MDS/MPN	345 (8)
CML	85 (2)
Lymphoma	88 (2)
Solid tumor	21 (1)
Non-malignant diseases	
BMF	
Aplastic anemia	97 (2)
Fanconi	202 (5)
Other	97 (2)
Hemoglobinopathies	50 (1)
Immune deficiencies	
Wiskott–Aldrich syndrome	81 (2)
Other SCID	437 (10)
Metabolic disorder	
Adrenal leukodystrophy	31 (1)
Osteopetrosis	58 (1)
Hurler syndrome	164 (4)
Other or not specified	162 (4)
Histiocytosis	169 (4)
Other diagnosis	6 (1)

Abbreviations: See legend to Table 39.1.

Directed donor CB banking

A number of studies have reported on efforts to systematically identify, collect, and store CB units for family-directed use [22,32,33] or for research that investigates the use of autologous CB cells, also for using those cells for gene therapy. Care must be taken to ensure that such CB banks meet the necessary critical criteria needed to store these units [22]. Recent surveys on directed family cord blood banking have been published by Eurocord, NetCord, the NMDP, and others [22,23]. The successful use of family banked CB to treat patients was reported [34–36], although the probability of using an autologous CB is very low (4 per million). Whether or not to bank a child's CB for autologous or family use is a personal decision, based on informed knowledge.

Clinical use of unrelated CB cells for allogeneic HCT

The first public CB bank was established in 1991 [37], and the first unrelated CB HCT was performed in 1993 for a 4-year-old child with leukemia [38]. There are substantial logistic and clinical advantages to the use of CB in the unrelated setting particularly for underrepresented minority and mixed ethnicity patients who often cannot identify a suitably matched related or unrelated donor. Table 39.2 lists the advantages and disadvantages of CB compared with alternative sources of donor cells for unrelated or HLA-mismatched family members and patients lacking a HLA sibling HCT donor.

CB HCT has been marked by improved outcomes, especially in adults, as knowledge of TNC dose requirements led to improved collection, and to availability of CB units with higher cell doses. Improvements in supportive care have also contributed. The use of double CB HCT [39] opened up CB HCT for essentially all patients without suitable donors. However, double CB HCT does not result in faster neutrophil, platelet, or immune cell recovery.

Unrelated CB HCT in children

Unrelated donor CB HCT in children has been carried out for a multitude of disorders (Table 39.3), and results are comparable to other sources of HSCs [40–42]. For metabolic diseases and primary immunodeficiencies, CB cells is considered a preferable source of HSCs for transplantation. Results of the first prospective phase II multicenter trials on unrelated CB transplantation (COBLT) for children with hematologic malignancies [43] noted that the CI of neutrophil recovery, acute GVHD, chronic GVHD, and relapse were 80%, 20%, 21%, and 20%, respectively. Two-year overall survival was 49.5%. Risk factor analysis for outcome was associated with cell dose, HLA disparities, ABO compatibility, patients' CMV positivity, disease, and disease status at transplantation. Outcomes of CB HCT in children have been reported for acute lymphoblastic leukemia (ALL) [42], acute myeloid leukemia (AML) [43], myelodysplastic syndrome (MDS) [44], Hurler syndrome [45], Krabbe disease [46], Fanconi anemia [47], and primary immunodeficiencies [48]. Figure 39.2 shows the overall 2-year survival for 3546 children with malignant (*n* = 2163) and non-malignant (*n* = 1383) disorders transplanted with an unrelated CB graft and reported to Eurocord.

Malignant hematologic diseases

Children with ALL

About 20–30% of children with ALL will need to be treated with an allogeneic HCT, either because of very poor prognostic factors at diagnosis or following relapse. Children with ALL (*n* = 532) were retrospectively analyzed in first (1st) complete remission (CR) (*n* = 186), 2nd CR (*n* = 238), 3rd CR, or advanced disease patients (*n* = 108) who received CB cells as a first transplant. The most common immunophenotype was B-cell precursor ALL; 17 patients had biphenotypic ALL. Of 186 patients transplanted in 1st CR, poor risk cytogenetic [t-(4;11) and t-(9;22)] was present in 40%. Most of the CB grafts were single units, HLA 5/6 and 4/6, and median TNC dose infused was 4 × 10^7/kg. Cumulative Incidence (CI) of neutrophil recovery, acute GVHD, and transplantation-related mortality (TRM) was 82%, 27%, and 21%, respectively. In multivariate analysis, TNC infused (>4 × 10^7/kg), and remission status at time of CB HCT were associated with improved neutrophil recovery. The CI of

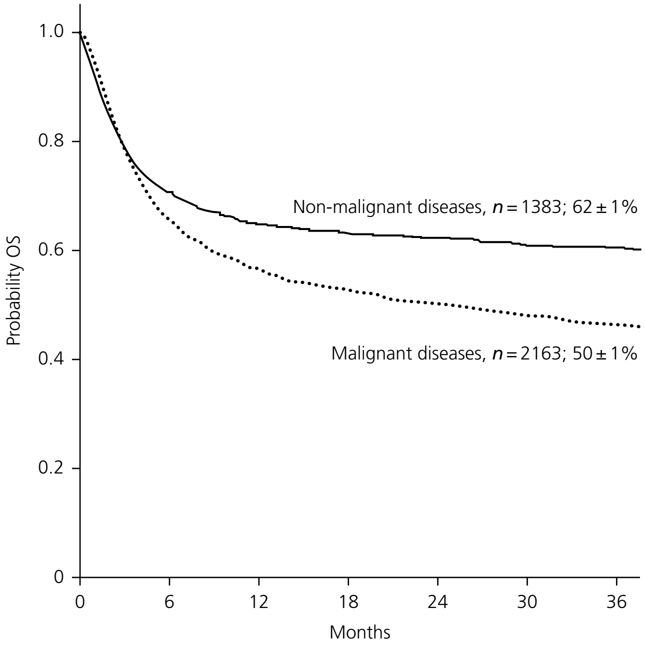

Figure 39.2 Two-year survival in children given an unrelated single CB HCT based on non-malignant and malignant disease state.

2-year relapse was 37% (31% for 1st CR, 34% for 2nd CR, and 50% for more advanced disease). In multivariate analysis, disease status at CB HCT and use of total body irradiation (TBI) were independently associated with lower CI of relapse. The 2-year probability of leukemia-free survival (LFS) was 38% (49% for 1st CR, 42% for 2nd CR, and 10% for more advanced disease). To address the prognostic value of minimal residual disease (MRD) before CB in children with ALL, 170 ALL children transplanted in CR after myeloablative conditioning regimen were analyzed [49]. At 4 years, CI of relapse was 30%, incidence being lower in patients with negative minimal residual disease (MRD) before CB HCT when compared to those with a positive MRD (24% vs. 39%; $p = 0.05$). There was also an impact of MRD on LFS. LFS was 54% when patients with negative MRD were transplanted compared to 29% for those patients with a positive MDR before CB HCT ($p = 0.003$). Thus, MRD assessment before CB HCT allows identification of patients at higher risk of relapse after transplantation.

Children with AML

Eurocord published results of CB HCT for childhood AML [43]. A cohort of 95 children was analyzed. LFS was 59% in children transplanted in 1st CR, 50% in 2nd CR, none among 5 in 3rd CR or beyond, and 21% for children transplanted in relapse. The results of CB were particularly encouraging for AML with a poor karyotype. With the aim to assess the impact of cytogenetic and molecular markers on outcomes after CB HCT for children with AML, 390 children transplanted from 1994 to 2010 were studied. At the time of transplantation, 37% were in 1st CR, 42% in 2nd CR, and 21% had more advanced disease. The majority of children had either one (44%) or two (37%) HLA disparities out of six with their graft. Median infused TNC and CD34 cells dose were respectively 4.95×10^7/kg and 1.9×10^5/kg. The majority of patients (86%) received myeloablative conditioning (MAC). The CI of neutrophil recovery was 85% and was associated with TNC and CD34$^+$ cell dose infused. At day 100, the CI of grade II–IV acute GVHD was 34%. At 1 year, the CI of non-relapse mortality was 24% and this was associated with the TNC dose ($>4.9 \times 10^7$/kg) and disease status. At 2 years, the probability of LFS was 63% in 1st CR, 43% in 2nd CR, and 22% in more advanced patients. LFS of 49 children transplanted in 1st CR with unfavourable disease was 70%, not statistically different from the overall 1st CR group. For CB HCT in 2nd CR, LFS was 71%, 33%, and 40% in the favorable, intermediate, and unfavorable subgroups, respectively. Multivariate analysis for 2nd CR cohort identified two significant prognostic factors: favorable disease subgroup ($p = 0.005$) and duration of previous CR longer than 7 months ($p = 0.03$) (V. Rocha for Eurocord, unpublished data).

CB HCT for children with MDS and juvenile myelomonocytic leukemia (JMML)

There are few reports investigating the outcome of children with MDS given an unrelated donor HCT. Most of these reports are limited to a small and heterogeneous series of studies [50,51] for JMML and/or secondary AML, including adult recipients in those reports [52,53]. In a collaborative study between the European Working Group on childhood MDS (EWOG-MDS), CIBMTR, and Eurocord–EBMT, factors were examined that influence the outcome of 70 children with MDS (refractory cytopenia ($n = 33$), refractory anemia with excess blasts ($n = 28$), and refractory anemia with excess blasts in transformation ($n = 9$) at diagnosis), who received a CB HCT after receiving a myeloablative regimen [44]. At day +60, the probability of neutrophil recovery was 76%; with a TNC $> 6 \times 10^7$/kg, transplantation of HLA-matched or 1-locus-mismatched CB cells and an irradiation-containing conditioning regimen favoring faster engraftment. The overall 3-year probability of DFS was 39%. It was 66% when HCT was performed within 6 months from diagnosis, as compared to 20% for patients grafted after a longer interval from diagnosis. TRM was lower in children receiving an allograft after the year 2001.

In a separate analysis, outcomes and risk factors were studied for 110 children with JMML, given a first single unmanipulated CB HCT from 1995 to 2010 (V. Rocha for Eurocord and CIBMTR, unpublished data). Monosomy of chromosome 7 was the most frequent cytogenetic abnormality (24%). Nineteen percent of CB units were HLA-identical or 6/6 (antigen level for HLA-A and -B, allelic for DRB1), while 43% and 38% had 5/6 or 3/6 compatibilities. At 60 days after CB HCT, the CI of neutrophil recovery was 80%, with a median time of 25 days. The CI of grade II–IV acute GVHD was 40% at 100 days. At 4 years, the CI of relapse was 37%; children who were older than 1 year at diagnosis were independently associated with increased relapse. At 4 years, DFS was 43%. In multivariate analysis, factors associated with improved LFS were: age younger than 1 year at diagnosis, graft with a 6/6 or 5/6 HLA CB unit, and absence of monosomy 7.

CB HCT outcomes compared with unrelated donor cells in children with acute leukemia

Comparison of results of CB and BM HCT from unrelated donors in children is of paramount relevance, because for many patients the search process will identify both CB units and BM donors. Briefly, recipients of CB were transplanted sooner compared to children given BM; neutrophil and platelet recovery were delayed, acute GVHD was decreased, and OS was not significantly different after CB compared to BM HCT [40]. Since 1998, the choice of an unrelated donor has been based on HLA high resolution typing of class I and II. For this reason, a study has compared the outcomes of 503 children with acute leukemia given CB versus 282 BM recipients transplanted with grafts that were matched for allele level for HLA-A, HLA-B, HLA-C, and HLA-DRB ($n = 116$), or mismatched ($n = 166$) [41]. Recipients of CB were transplanted with grafts that were HLA matched ($n = 35$) or HLA mismatched for one ($n = 201$) or two antigens ($n = 267$) out of six. In comparison with allele-matched BM transplants, 5-year LFS was similar to that after transplants of CB mismatched for either one or two antigens. TRM rates were higher after transplants of two-antigen HLA-mismatched CB and possibly after one-antigen HLA-mismatched low-cell-dose CB transplants. Relapse rates were lower after two-antigen HLA-mismatched CB transplants. These data strongly suggest that CB is an acceptable alternative to HLA-matched unrelated BM in children and support the concept of starting a simultaneous search for BM and CB unrelated donors. The final selection of unrelated donor BM versus CB should be based on the urgency of the transplant, and characteristics of the BM and CB unrelated donor such as cell dose availability and HLA compatibility.

Non-malignant hematologic diseases

Among the congenital bone marrow failure syndromes (BMFS), Fanconi anemia is the most frequent indication for allogeneic HCT. The outcomes of CB HCT in 72 patients with Fanconi anemia were reported [47]. Outcomes of CB HCT were analyzed in 44 patients with other congenital BMFS [54]. Diagnosis was: Diamond-Blackfan anemia, amegakaryocytic thrombocytopenia, dyskeratosis-congenita, Shwachman–Diamond syndrome, Kostmann disease, agranulocytosis, and unclassified BMFS. The majority of the grafts were HLA mismatched and three patients received double CB HCT. Median TNC infused was 6.1×10^7/kg. Day 60 CI of neutrophil recovery was 55%, 100-day CI of acute GVHD (grade II–IV) was 24%, 2-year CI chronic GVHD was 53%. Improved OS was associated with age <5 years and number of infused TNC $\geq 6.1 \times 10^7$/kg. In both analyses cited above a higher incidence of graft failure was observed [47,54]. Outcomes after CB HCT for patients with BMFS can be improved by selecting units more closely HLA matched and with higher cell dose. In addition, the use of more immunosuppressive drugs such as fludarabine in the conditioning regimen and the avoidance of MTX in the GVHD prophylaxis may also improve engraftment and survival.

Allogeneic HCT is considered an effective treatment to prevent disease progression for patients with metabolic disorders, with Hurler syndrome being the most common disease in this setting. The 3-year event-free survival (EFS) and 3-year OS rates were 70% and 77%, respectively, after CB HCT in 93 children [45]. More recently, outcomes were compared after HCT in 258 children with Hurler syndrome according to graft source and donor type (V. Rocha for Eurocord, EBMT, and CIBMTR, unpublished data). OS and EFS at 5 years, were 74% and 63%, respectively, after HCT. However, EFS after HLA-matched sibling donor and 6/6 HLA-matched unrelated CB HCT were similar at 81%. It was 66% after 10/10 HLA-matched unrelated donor and 68% after 5/6 matched CB HCT. EFS was lower after transplantation of 4/6 matched CB cells (57%; $p = 0.031$), than after HLA-mismatched unrelated donors 41% EFS ($p = 0.004$) and T-cell depleted unrelated donor (36%; $p = 0.005$). Full donor chimerism and normal enzyme levels were higher after CB HCT (92% and 98%, respectively) compared to the other grafts sources and donors (69% and 59%, respectively). Results of allogeneic transplantation for Hurler syndrome are encouraging with similar EFS rates after matched sibling donors, 6/6 matched unrelated CB, 5/6 unrelated CB, and 10/10 matched unrelated donors. The use of mismatched unrelated donors and 4/6 matched unrelated CB was associated with lower EFS.

CB HCT has been successfully used in children with various primary immunodeficiencies [48,55]. Since severe primary T-cell immune deficiencies are pediatric emergencies, immediate HSC donors are mismatched related donor (MMRD) or CB HCT, in the absence of an HLA-identical sibling donor. Recently, 175 MMRD HCTs were retrospectively compared with 74 CB HCTs performed in 32 transplant centers [55]. T-cell depletion was used in all MMRD HCT. T-cell engraftment was not statistically different between the two groups. CB recipients had a higher frequency of complete donor chimerism at day 100 and faster total lymphocyte recovery compared to MMRD recipients, and independence of immunoglobulin replacement therapy was achieved faster and more frequently after CB HCT. Estimated 5-year OS was 62% after MMRD and was 57% after CB HCT. In the CB group, OS rate was improved in the group of patients with severe combined immunodeficiency given a CB graft with an HLA-identical 6/6 (76%; $n = 21$) or 5/6 (62%; $n = 29$ match) compared to 4/6 HLA disparities (35%; $n = 24$).

Outcomes after HLA-matched unrelated donor HCT for TM have been reported with encouraging results, but larger series of patients with longer follow-up are needed to draw definitive conclusions [56]. The use of unrelated HCT for TM and SCD is still controversial, and its efficacy is under evaluation in clinical protocols. A recent phase II study in the United States, evaluating the use of CB HCT after reduced intensity conditioning (RIC) regimen in 8 children with SCD resulted in suspended recruitment due to high incidence of graft failure [57]. The Eurocord group in collaboration with CIBMTR retrospectively examined the efficacy of CB HCT in children with TM ($n = 35$) and SCD ($n = 16$) [58]. Donor–recipient pairs were matched at HLA-A and -B (antigen level) and DRB1 (allele level) in 7, or HLA mismatched at one ($n = 18$), two ($n = 25$), or three loci ($n = 1$). Transplant preparative regimen was MAC ($n = 39$) or RIC ($n = 12$). Neutrophil recovery with donor chimerism was documented in 24 patients; 11 patients developed grade II–IV acute GHVD, and 10 patients developed chronic GVHD. OS and DFS was 62% and 21% for TM and 94% and 50% for SCD, respectively. In multivariate analysis, engraftment rate and DFS were improved with a CB unit containing a TNC dose $>5 \times 10^7$/kg. The 2-year probability of DFS was 45% in patients who received grafts with cell dose $>5 \times 10^7$/kg and 13% with lower cell doses. Novel approaches aimed at improving engraftment will be needed before unrelated CB HCT can be widely adopted for transplanting patients with TM and severe SCD.

Unrelated CB HCT in adults

Numbers of adult CB HCTs have increased and since 2006 adults have surpassed the numbers of children being transplanted with CB. This increase is mainly due to: (i) similar outcomes of CB HCT to that of HLA-matched BM or MPB donors, (ii) double CB units to circumvent problems of cell dose, and (iii) reduced-intensity conditioning regimens that decrease TRM. Most CB HCTs are performed in adults with malignant disorders using double cord blood units.

Adults with ALL

Single center experience has shown interesting results in CB HCT in adult recipients with high-risk ALL, but reports analyzed a small series of patients. One [59] described 22 patients who received reduced-intensity conditioning regimen (RIC) followed by allogeneic transplantation with either matched related ($n = 4$) or CB ($n = 18$) donor grafts. OS, TRM, and relapse were 50%, 27%, and 36% at 3 years, respectively. Another published results on 27 patients receiving single CB HCT after myeloablative conditioning (MAC) [60].

With a median follow-up of 47 months, the probability of LFS at 5 years was 57%. A more recent study reported outcomes after CB HCT for 256 adults with ALL transplanted from 1997–2006 in Japan [61]. The CI of neutrophil engraftment at day 100 was 78%. Infused CD34$^+$ cell dose ($>1 \times 10^5$/kg) was associated with successful neutrophil engraftment. CI of grade II–IV acute GVHD at day 100 was 37%. Two-year LFS and OS rates were respectively 36% and 42%. Multivariate analysis showed that in patients older than 51 years, active disease at time of the CB HCT, presence of grade III–IV acute GVHD, and absence of chronic GVHD were negatively associated with OS.

The Eurocord group has performed a survey and risk factor analysis of outcomes after CB HCT for adults with ALL (V. Rocha for Eurocord, unpublished data). From 2000 to 2011, 421 adult patients received a CB HCT for ALL in European centers. Median age at time of CB HCT was 32 years (18 to 76 years) and 59% of patients (n = 247) were older than 35. At time of transplant 46% (n = 195) were in 1st CR, 32% (n = 136) in 2nd CR, and 22% (n = 90) of patients were not in remission. Of 314 patients with available karyotype at diagnosis, 229 had abnormalities. Double CB HCT was performed in 173 patients (41%). Most patients received CB units with one (24%; n = 103) or two (55%; n = 231) HLA disparities. MAC was given to 314 patients (75%), and 103 patients (25%) received RIC. Median follow-up was 27 months. The CI of 60-day neutrophil recovery was 78%. CI of acute and chronic GVHD was 33% and 26%, respectively. CI of TRM at 2-years was 42% and was lower in patients <35 years in CR at transplant and using RIC. Two-year RI was 28% and the factors associated with lower RI were use of MAC, and CR at the time of transplant. Estimated 2-year LFS was 39% for patients in 1st CR (n = 195), 31% for 2nd CR (n = 136), and 8% for advanced disease (n = 90). Three factors were associated with improved LFS: age <35 years, CR at transplant, and not using antithymocyte globulin as part of the conditioning regimen. In patients with Ph+ ALL (n = 129), 2-year CI of TRM and relapse were 35% and 28%, respectively, and 2-year LFS was 36%. As shown in children [49], presence of MRD before CB HCT was also associated with final outcomes in adult CB HCT recipients. Sixty-five patients with Ph+ ALL in remission were analyzed for whom a MRD was performed before CB HCT (34 were positive and 31 were negative). LFS at 2 years was 48% for those patients transplanted with a negative MDR and 38% for those with a positive MRD ($p = 0.05$) (V. Rocha for Eurocord, unpublished data). Thus, CB HCT for patients with ALL is an alternative option for those without an HLA-identical sibling.

Adults with AML

In spite of reports and retrospective comparisons with other HSC sources, reports on outcomes and risk factor analysis on larger series of AML patients given CB HCT with longer follow-up and more homogenous populations are still missing. Some single center experiences and registry data have reported encouraging results [62–65]. LFS varied from 40% to 46% in 1st CR, from 26% to 39% in 2nd CR, and from 11% to 20% in advanced phase of the disease.

Eurocord conducted a survey on outcomes of CB HCT for adults with AML. Adult patients (n = 604) with de novo AML in 1st CR (n = 229), 2nd CR or 3rd CR (n = 228), and advanced disease (n = 147) who received CB HCT as a first transplant were retrospectively analyzed (V. Rocha for Eurocord, unpublished data). CB HCT was performed from 2000–2010 in 131 European centers. Median age of recipients was 41 years, and 18% of the patients had previous autologous transplants. Based on available cytogenetic and molecular markers at diagnosis (n = 339), 56% were in intermediate risk and 31% in unfavorable risk groups. Grafts were single (n = 361) or double (n = 243) CB units; 39% of CB units were identical to recipient or had 5/6 HLA matched (antigen level for HLA-A and -B allelic level for DRB1), while 61% had 4 or 3/6 HLA compatibilities. At infusion median, TNC dose was 3.1×10^7/kg (2.4×10^7/kg with single CB and 3.7×10^7/kg with double CB) and median CD34$^+$ cell count was 1.2×10^5/kg (1×10^5/kg with single and 1.3×10^5/kg with double). MAC was given to 51% and RIC to 49% of recipients. The most common regimens used were busulfan in combination with fludarabine and thiotepa for MAC, and cyclophosphamide plus fludarabine and TBI 2Gy for RIC. GVHD prophylaxis consisted of cyclosporine A (CSA) associated or not with mycophenolate mofetil (MMF) treatment in 58% and CSA ± corticosteroids in 32% of patients. Median follow-up was 13 months. The CI of neutrophil recovery, acute GVHD (II–IV) and 1-year TRM was 80%, 26%, and 21%, respectively. CI relapse at 2 years was 38% (1st CR 27%, 2nd CR and 3rd CR 29%, and non-remission 56%); it was 31% for those patients transplanted with MAC (n = 291) and 30% with RIC (n = 282). For patients given MAC, 2-year LFS was 50% for 1st CR, 27% for 2nd CR and 3rd CR, and 17% for those patients transplanted in non-remission, whereas it was 35%, 44%, and 18% for RIC, respectively. In this large series of patients, but still short follow-up time, CB HCT appears to be an option for treatment of adults with high-risk AML after MAC or RIC without an HLA-identical donor.

Adults with MDS

Reports of CB HCT for patients with MDS have been scarce [66–68]. Through Eurocord and EBMT registries, outcomes and risk factors have been evaluated for adult patients who underwent single or double CB HCT for MDS or secondary AML [69]. Adults with MDS (n = 39) or secondary AML (n = 69) were analyzed. Median age was 43 (18–72) years. Seventy-seven patients (71%) received a single CB HCT. MAC was given to 57 (53%) patients. Median numbers of nucleated and CD34$^+$ cells at freezing were respectively, 3.6×10^7/kg and 1.1×10^5/kg. At 60 days, CI of neutrophil recovery was 78% and was independently associated with numbers of CD34$^+$ cells/kg ($>1.1 \times 10^5$/kg) and advanced disease status (blasts <5% at time of CB HCT). Two-year non-relapse mortality (NRM) was significantly higher after MAC (62% vs. 34%; $p = 0.009$). Two-year DFS and OS were 30% and 34%, respectively. In multivariate analysis, patients with high-risk disease (blasts >5% and international prognostic scoring system, intermediate-2 or higher) had significantly poorer DFS. Neutrophil recovery seemed inferior to that in other malignant disorders in adults, and a CB unit containing a high number of CD34$^+$ cells or a double CB HCT should be recommended. DFS of 30% appears to be comparable to that after HCT using other sources of donor cells in large multicentric studies in high-risk MDS patients. No retrospective comparisons with other sources of donor cells have been made in this disease-specific setting.

Adults with lymphoid malignancies

There have been some reports of CB HCT for refractory non-Hodgkin lymphoma (NHL) [70–72] and malignant lymphoma treated by RIC-CB HCT [73,74]. The Eurocord group evaluated 104 adult patients (median age, 41 years) who underwent unrelated donor CB HCT for lymphoid malignancies [75]. The CI of neutrophil engraftment was 84% by day 60, with greater engraftment in recipients of higher CD34$^+$/kg cell dose. CI of TRM was 28% at 1 year, with a lower risk in patients treated with low-dose TBI. CI of relapse or progression was 31% at 1 year, with a lower risk in recipients of double-unit CB HCT, and those with chemosensitive disease. The probability of DFS was 40% at 1 year, with improved survival in those with chemosensitive disease (49% vs. 34%), who received higher cell doses ($\geq 2 \times 10^7$/kg) (49% vs. 21%) and conditioning regimens containing low-dose TBI (59% vs. 23%). Thus, CB HCT is a viable treatment for adults with advanced lymphoid malignancies. Several important strategies to be considered for patients with malignant lymphoma and CLL are: greater use of less toxic RIC regimens, such as those containing low-dose TBI, better selection of CB units, and broader use of double CB HCT.

Results of unrelated CB

Adults with acute leukemia and other hematologic malignancies after myeloablative conditioning regimen

Currently the choice of unrelated donor is based on high-resolution typing or allelic typing for HLA-Class I and II. Therefore, Eurocord in collaboration with CIBMTR compared results of unrelated CB HCT in adults with acute leukemia, given a CB graft (with more than 2.5×10^7/kg TNC at freezing and not more than 2 out of 6 HLA disparities), with BM or PB grafts (allelic typing for HLA-A, -B, -C, -DRB1) after MAC. Data were available on 1525 patients aged >16 years with acute leukemia transplanted between 2002 and 2006 using CB ($n = 165$), PBSC ($n = 888$), and BM ($n = 472$) [76]. CB units were matched at HLA-A and -B at an antigen level and -DRB1 at an allele level ($n = 10$), or were mismatched for one ($n = 40$) or two antigens ($n = 115$). PBSC and BM grafts from unrelated adult donors were matched for allele-level HLA-A, -B, -C, -DRB1 ($n = 632$; $n = 332$) or mismatched at one locus ($n = 256$; $n = 140$). LFS after CB HCT was comparable to that observed after allele-matched (at HLA-A, -B, -C, -DRB1) and mismatched PBSC or BM transplantation. TRM was higher after CB HCT than after allele-matched MPB ($p = 0.003$) or BM ($p = 0.003$), since the engraftment rate was decreased in CB recipients. Grades II–IV acute and chronic GVHD were lower in CB HCT recipients compared to allele-matched MPB ($p < 0.001$ and $p < 0.001$, respectively). Chronic, but not acute GVHD, was lower after CB compared to allele-matched BM HCT ($p = 0.011$). Therefore a search for CB units is recommended in absence of an HLA-matched or single antigen-mismatched donor, or in urgent situations when time to search for a donor is limited.

Adults with lymphoid malignancies after RIC HCT compared to unrelated PBSC

The Eurocord group in collaboration with the Lymphoma Working Party of EBMT studied 645 adult patients with lymphoid malignancies who received a single or double CB HCT ($n = 104$) or a mismatched unrelated donor (MUD) HCT ($n = 541$) after a RIC regimen (V. Rocha for Eurocord, unpublished data). Median follow-up time was 30 months. The CI of engraftment at day 60 was 81% after CB and 97% after MUD HCT, whereas at day 100, the CI of grade II–IV acute GVHD was 29% and 32%, respectively. At 36 months, the CI of TRM was 29% after CB and 28% after MUD HCT, relapse or progression was 28% and 35%, and chronic GVHD was 52% and 26% ($p < 0.01$), respectively. Two-year progression-free survival (PFS) and OS were 43% and 58% after CB and 36% and 51% after MUD HCT, respectively. In a multivariate analysis, TRM, relapse or progression, PFS and OS were not statistically different between CB and MUD HCT, but MUD HCT was associated with higher risk of chronic GVHD and CB HCT with delayed engraftment. Thus, CB HCT is a valuable alternative for patients with advanced lymphoma and CLL who lack an HLA-matched related or unrelated donor, since CB HCT is associated with a lower risk of chronic GVHD.

Unrelated double CB HCT for adults with leukemia and hematologic malignancies

Results with double CB HCT support the safety of the procedure [76,77]. In double-unit CB HCT, only one of the two CB units wins out early on in the vast majority of reported cases. Reasons for this are still unknown Towards a better understanding of this phenomenon, an in vivo mouse model double-unit human CB engraftment was developed that correlated with clinical engraftment of the same CB units as used in the mouse model [78]. It was suggested that unit dominance was an in vivo effect perhaps associated with a graft-versus-graft immune interaction mediated by $CD34^-$ cells. Figure 39.3(a) and 39.3(b) shows survival curves of 354 and 282 adult patients with acute leukemia transplants with double CB HCT, respectively, in the RIC and MAC settings. In 2007, in this context, 110 patients with hematologic diseases were evaluated in the setting of a RIC regimen [79]. Neutrophil recovery was achieved in 92% of the cases. TRM was 26% at 3 years. Survival and DFS at 3 years were respectively 45% and 38%. More recently, the Société Française de Greffe de Moelle et de Thérapie Cellulaire in collaboration with Eurocord analyzed 155 consecutive CB HCTs performed using a RIC regimen (V. Rocha for Eurocord, unpublished data). The CI of neutrophil engraftment at day +60 was 80%; autologous recovery was seen in 14% of patients. In multivariate analysis, factors independently associated with better neutrophil recovery were $CD34^+$ cell dose ($>1.2 \times 10^5$/kg) ($p = 0.04$), HLA compatibility (0–1 vs. 2–3) ($p = 0.05$), and previous autograft ($p < 0.01$). The CI of TRM was 18% at 18 months. The estimated probability of OS and DFS at 18 months was 62% and 51%, respectively. A regimen of low-dose TBI, fludarabine, and cyclophosphamide used as RIC was associated with better outcomes than other RIC regimens. More recently, the Minnesota group in collaboration with CIBMTR has confirmed this observation [80].

Outcomes after double, compared with single, CB HCT in adults with acute leukemia

In a single center analysis of 177 patients with acute leukemia, relapse was significantly lower for early stage (1st and 2nd CR) patients who received double CB units compared to single-unit CB HCT, suggesting a possibly greater GVL effect. DFS was 40% and 51% for single and double CBT recipients, respectively [81]. The Eurocord group has published comparable results of single and double UCBT for patients with lymphoid malignancies [75]. In preliminary analysis, Eurocord with the Acute Leukemia Working Party of EBMT have compared outcomes after double with single CB HCT (in the single CB setting, patients receiving $>2.5 \times 10^7$/kg) in adult patients with AML and ALL in remission in two different analyses according to type of conditioning regimen. Double CB HCT was associated with decreased relapse incidence and improved LFS only in patients transplanted in 1st CR after RIC (V. Rocha for Eurocord, unpublished data), results needing confirmation.

Outcomes after double CB HCT compared with HLA-matched sibling and HLA-matched and -mismatched unrelated HCT after myeloablative conditioning

Analysis of 536 patients with malignant disease (AML, $n = 211$; ALL, $n = 236$; CML, $n = 70$; and MDS, $n = 19$) transplanted with an HLA 8/8 allele-matched related (MRD, $n = 204$) or unrelated donor

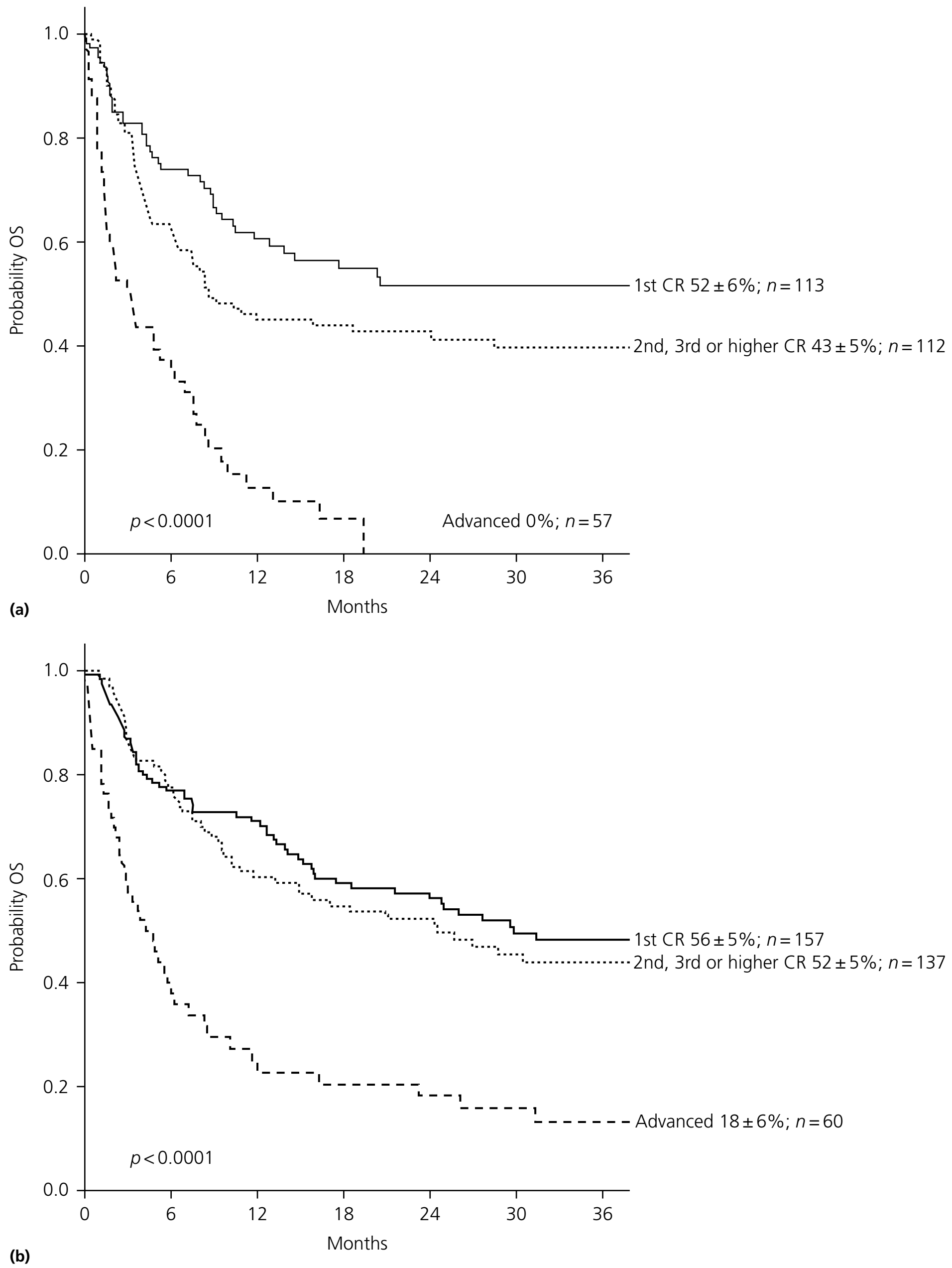

Figure 39.3 Two-year survival for adults with acute leukemia given (a) an unrelated double CB HCT using reduced-intensity conditioning based on disease status and remission; (b) unrelated double CB HCT using myeloablative conditioning based on disease status and remission.

(MUD, n = 152), 1 allele-mismatched unrelated donors (MMUD, n = 52), or double UCB (n = 128) has been published [82]. All patients received MAC with cyclophosphamide and TBI, with fludarabine administered prior to double CB HCT and recipients receiving GVHD immunosuppression with a calcineurin inhibitor and either methotrexate (MRD, MUD, and MMUD) or MMF (MRD and double CB). Median follow-up of survivors was 3.1 years. Double CB HCT was associated with slower hematopoietic recovery compared to other HSC sources. Despite greater HLA mismatch, the CI of grade II–IV acute GVHD and chronic GVHD were lowest after double CB HCT, but risk of relapse was remarkably low while TRM was elevated, resulting in similar DFS. Thus, in the absence of a MRD donor, using either MUD or double CB HCT yields encouraging DFS results.

Outcomes after double CB HCT compared with unrelated MPB HCT in adults with acute leukemia after RIC regimen

Efficacy of double CB HCT was compared with PBCS from 8 of 8 or 7 of 8 HLA-matched unrelated donors receiving RIC regimens [83]. Compared with matched 8/8 PBSC, TRM and overall mortality were similar after double CB (low-dose TBI, fludarabine, cyclophosphamide = TCF) (p = 0.72; p = 0.60), but higher after double CB-other RIC (p = 0.0001; p = 0.004). Compared with 7/8 PBSC, TRM (but not overall mortality) was lower after double CB-TCF-HCT (p = 0.04; p = 0.41). Probabilities of survival after double CB-TCF-HCT, double CB HCT-other RIC, and 8/8 MPB and 7/8 PBSC were 38%, 19%, 44%, and 37%, respectively. With similar survival after 8/8, 7/8 matched PBSC, and double CB-TCF, results support use of double CB-TCF-HCT in adults with acute leukemia who may benefit from RIC transplantation urgently, or who lack a 7/8 or 8/8 unrelated donor.

Recent reviews on CB HCT are available for: leukemia in children [84], treatment of non-malignant disorders [85], extending use in adults [86], double CB HCT for malignant disorders [87], engraftment after double unit CB HCT [88], RIC and double CB HCT [89], and why CB may be underutilized [90].

Selection of CB units

Table 39.4 lists the steps that can help search coordinators and clinicians choose a CB unit based on a patient's disease and donor-related factors.

The combined impact of prefreeze TNC dose and HLA match upon CB HCT outcome was analyzed in recipients of 6/6 matched to 3/6 HLA-A, -B antigen, and -DRB1 allele-mismatched CB units [91]. The study demonstrated that the best outcome for neutrophil and platelet engraftment, acute GVHD, TRM, treatment failure, and overall mortality was associated with the transplantation of 6/6 HLA-matched units, regardless of the TNC dose. The next best survival outcomes were observed in recipients of 5/6 HLA matches with a TNC dose of 2.5 × 10^7/kg or greater, or 4/6 HLA-matched units with a TNC dose of 5.0 × 10^7/kg or greater. However, other factors such as diagnosis (hemoglobinopathies or bone marrow failure syndromes) have an important role in rate of engraftment, due to the fact that most patients with hemoglobinopathies have a full marrow and have not received chemotherapy or immunosuppression before conditioning, or, in the cases of aplastic anemia, they have often received previous multiple transfusions or had a severe infection at the time of transplantation, thus increasing the risk of non-engraftment and mortality. Recently, given that requirements regarding cell dose and HLA matching may differ in malignant and non-malignant diseases, an algorithm was constructed to guide clinicians in choosing the "best" cord blood unit, taking into account the impact of diagnosis, cell dose, and HLA incompatibilities, in patients receiving a single CB HCT. If the TNC dose with a single unit is not available, a double CB HCT should be investigated. In the malignant disease group, Eurocord and others have found that cell dose was the most important factor influencing outcome, increased the risk of delayed engraftment, and led to a higher incidence of TRM and chronic GVHD; however, the number of HLA disparities decreased the risk of relapse, resulting overall in a

Table 39.4 Recommendations for unrelated cord blood unit selection and transplantation

To start, choose a CB unit, consider the diagnosis and also the *presence of a patient's HLA antibodies against the HLA of the CB unit*. HLA compatibility is more important for patients with non-malignant disorders than for malignant disorders.

If the below criteria on cell dose for a single CB HCT is not achieved, a double CB HCT should be proposed, or patients be included in other prospective trials.

Recommendations for HLA and cell dose

HLA (defined based on HLA antigens for -A and -B and allelic typing for HLA-DRB1)

- **CB unit with 6/6 or 5/6 HLA match.** Prefer HLA-A or HLA-B than DRB1 mismatches. Probably, HLA-DRB1 mismatch can lead to high GVL effect in patients transplanted in non-remission (based on Eurocord unpublished and preliminary data). If CB unit 4/6, avoid mismatches in HLA-DRB1 and avoid using this unit for transplantation in patients with non-malignant disorders; use those mainly for bone marrow failure states.
- It is not recommended to transplant CB units with 3/6 or 4/6 HLA disparities.
- **HLA-C** (antigenic typing, 2 digit level) should be performed when there are many CB units available: 6/6 or 5/6 or for those units 4/6 with HLA-DRB1 mismatches [126]. The association of HLA-C mismatches with HLA-DRB1 is associated with higher mortality.
- **HLA and double CB HCT.** There is insufficient data on the role of HLA in double CB HCT.

TNC and CD34$^+$cell dose

Malignant disorders:

Nucleated cell dose: **at freezing, minimum cell dose 2.5 to 3.0 × 10^7/kg, or after thawing, minimum dose of 2.0 to 2.5 × 10^7/kg***

(This recommendation is based on Eurocord, New York Placental Blood Center, and CIBMTR data.)

* If the nucleated cell dose infused is inferior to 1.0 × 10^7/kg, an immediate second transplant should be discussed since early mortality associated with this TNC dose is around 70%.

* If the TNC dose infused is between 1.0 to 2.0 × 10^7/kg, it is recommended to take into consideration the number of CD34$^+$ cells and CFU-GM in order to try to predict the probability of neutrophil recovery and to discuss the possibility of a second transplant. If both cell doses are lower than recommended, a BM aspirate and chimerism analysis should be performed between day +20 and day +28 to confirm the absence of engraftment that indicates a need for a second transplant.

For **double CB unit**: the minimum cell dose of one unit should be higher than 1.5 × 10^7/kg at freezing. Although there is no data to support this recommendation, it was based on the first studies of double CB HCT [79]. Eurocord data was unable to find any association between the sum of TNC dose of both units and the outcomes.

CD34$^+$ cell dose: **at freezing, 1.0 to 1.7 × 10^5/kg, or after thawing, around 1.0 to 1.2 × 10^5/kg**

This recommendation is based on published data [61,63,66]. Due to variation in techniques of counting CD34$^+$ cells, this recommendation should be received with caution. **For double CB HCT**, Eurocord and Société Française de Greffe de Moelle et de Thérapie Cellulaire data have shown an association between the sum of CD34$^+$ cell dose of both units and neutrophil recovery and transplantation-related mortality of more than 1.2 × 10^5/kg pre-freeze CD34$^+$ cells.

Colony-forming unit assay: when available, the CB bank should provide this information with that of the methodology used to set up the assay (semi-solid medium, cytokines, O_2 tension) and score the colonies. This assay is important to evaluate the functional capacity of HPCs after thawing an aliquot or after thawing the product; however, it is difficult to establish a generalized CFU-GM dose due to variations of colony setup and counting between centers.

Non-malignant disorders:

Nucleated cell dose: **at freezing, minimum cell dose 3.5 × 10^7/kg, or after thawing, minimum 3.0 × 10^7/kg**

For patients with BM failure syndromes (aplastic anemia or congenital bone marrow failure states) or hemoglobinopathies, the number of TNC at freezing should be greater than 5 × 107/kg [89,104].

CD34$^+$ cell dose: **at freezing or after thawing, >1.7 × 10^5/kg**

(There is not enough data for this recommendation. This cut-off is based on the greater cell dose for a malignant disorder.)

Colony-forming unit assay: See above section on malignant disorders.

Other considerations:

If there are many CB unit choices available following the above criteria, take into consideration the following:

1. **Allele HLA typing of HLA-A, -B, -C**: A still preliminary analysis by Eurocord and CIBMTR indicates that CB units with 3/8, 4/8 and 5/8 matches should be performed only using a CB unit with a TNC dose >5 × 10^7/kg.
2. **NIMA**: Two retrospective registry-based analyses have demonstrated the favorable impact of NIMA matching on outcomes after CB HCT [162]. The frequency of NIMA matching for CB units in those studies was below 10%. CB banks are currently capturing the HLA type of the mother of the donor CB unit with the aim of prospectively selecting a CB unit based on NIMA.
3. **KIR ligand**: There is conflicting data on the role of KIR on outcomes. If there are many choices of CB units, KIR ligand should be determined for patients with AML [154,155].
4. **ABO compatibility**: In spite of limited evidence, ABO should be taken into consideration when there is a choice between many CB units.
5. **Accredited cord blood bank**: For safety only accredited banks recognized by national and international organizations should be used.

lack of influence of HLA mismatching on OS and DFS, except for those given three or more disparities out of six. Type of HLA mismatch did not influence outcome, but matching for HLA-DRB1 appeared better for patients receiving a graft that had two HLA incompatibilities. Increasing cell dose abrogated effects of HLA mismatching, but not for grafts with three or four HLA incompatibilities. Thus, patients with non-malignant disease should receive a higher cell dose to obtain engraftment than patients with a malignant disease. In non-malignant disorders, HLA mismatching played a major role in engraftment, GVHD, TRM and survival, which was partially abrogated by increasing cell dose.

Other considerations when selecting a cord blood unit

Although availability of adequate cell dose and HLA-matched CB units is important, a number of other factors need to be considered when selecting one or two CB units for transplantation. The first consideration is related to the best CB cell content marker before freezing or after thawing. Most of the studies have analyzed the impact of TNC dose at freezing or after thawing. Among nucleated cell subpopulations, numbers of erythroblasts should be considered [92]. CD34$^+$ cells are probably a better cell marker than TNC since they may better reflect the HSC and HPC content; however, numbers of TNC or CD34$^+$ cells do not necessarily reflect functional HSC content. Due to logistic, technical, and economic conditions, use of HSC or HPC functional cell assessment is difficult to perform routinely in all collection centers as a surrogate marker for CB unit choice.

Studies in BM HCT have identified the importance of allelic disparity of HLA class I (-A, -B, and -C) and class II (mainly DRB1) in determining transplant outcomes. Although HLA matching at an antigen level (low or intermediate resolution) for HLA-A and -B, and allele level matching for HLA-DRB1, continues to be the current standard for CB unit selection, retrospective analyses have evaluated the impact of undetected allelic disparities in a subset of CB recipients. Data from 122 donor-recipient pairs showed important changes in HLA-A, -B, and -DRB1 matching status between the initial assignments (antigen level for HLA-A and -B) and allele level for HLA-A and -B [93]. Over half (9 out of 16) were previously matched at 6/6 at an allele level. However, when additional HLA loci such as HLA-C and HLA-DQB1 were determined, only 14% of the patients were matched for 9–10/10 alleles, 63% were matched for 6–8/10, and the remaining 23% were more extensively mismatched. It was not possible to associate allele typing and outcomes, since numbers of patients were small in the different categories of mismatches, and this was a heterogeneous group of patients. The COBLT study [42] reported 179 patient–donor pairs using high-resolution typing for HLA-A and HLA-B. Matching at high-resolution typing was associated with decreased incidence of acute GVHD, but not with time to neutrophil or platelet engraftment. To determine the real value of allele typing in CB HCT, hundreds of patient–donor pairs will be needed. CB banks and transplant centers are encouraged to perform this typing or to freeze DNA samples in order to retrospectively evaluate the impact of HLA allele typing on CB HCT outcomes.

Eurocord in collaboration with CIBMTR analyzed the impact of matching for HLA-C [94] in CB HCT, knowing that the current selection of CB units is based on antigen-level HLA typing at HLA-A and -B and allele level at DRB1. Eight hundred and three patients/CB donors were studied with HLA-C data at an antigen level. These data suggest that the present strategy for CB selection should be reassessed. Matching at HLA-C for units that are matched at HLA-A, -B, or -DRB1 or in the presence of a single locus mismatch at HLA-A, -B, or -DRB1 should be included to minimize mortality risks.

Donor KIR-ligand incompatibility in the GVHD direction is associated with decreased RI and improved LFS after haploidentical and HLA-mismatched unrelated HCT. Outcomes of 218 patients with AML ($n = 94$) or ALL ($n = 124$) in CR were assessed who had received a single unit unrelated CB HCT from a KIR-ligand compatible or incompatible donor [95]. KIR-ligand incompatible CB HCT showed decreased RI ($p = 0.05$), improved LFS ($p = 0.0016$), and overall survival ($p = 0.004$). Results were more evident for AML transplant recipients, in whom 2-year RI and LFS with or without KIR-ligand incompatibility were 5% versus 36% ($p = 0.005$), and 73% versus 38% ($p = 0.012$), respectively. The Minnesota group examined clinical impact of KIR-ligand mismatch in a heterogeneous cohort of 257 patients with all malignant disorders, transplanted in remission or advanced phases of the disease, given RIC ($n = 102$) or MAC ($n = 155$) and single ($n = 91$) or double ($n = 166$) unit CB grafts [96]. Following RIC, KIR-L mismatch between the engrafted unit and the recipient resulted in significantly higher rates of grade III–IV acute GVHD and TRM with inferior survival [95]. Therefore, more studies are needed to analyze the impact of KIR-ligand matching.

Donor-specific anti-HLA antibodies have an impact on neutrophil and platelet engraftment after CB HCT. Since most CB HCTs are HLA mismatched, presence of anti-HLA antibodies in the patient, donor-specific against the HLA of the CB should be tested. In a Japanese study [97], 648 recipients of a single CB unit and myeloablative CB HCT were analyzed retrospectively. The results suggested that patients' pre-transplantation anti-HLA antibodies should be examined and considered in the selection of CB units.

In the double CB HCT setting, two studies with differing results are available on the impact of anti-HLA antibodies and outcomes. One reported 73 patients receiving double CB HCT showing an increased risk of graft failure and lower survival for patients with positive anti-HLA antibodies [98]. Another showed no association between presence of anti-HLA antibodies and transplant outcomes in 126 double CB HCT recipients [99]. Donor-specific anti-HLA-antibodies (DSA) in recipients of unrelated CB HCT appear to be associated with graft failure and higher mortality, and screening patients for DSA before unrelated CB HCT is recommended before choosing an HLA-mismatched CB unit [100].

Non-inherited maternal antigens (NIMA) promote tolerance in solid organ transplant recipients implying that fetal exposure to NIMA may promote lasting tolerance in humans. NIMA-matched single CB HCT was associated with higher neutrophil recovery and lower mortality rates [101]. A Eurocord–CIBMTR study [102] showed a lower TRM and better survival after CB HCT when selecting a NIMA-matched CB unit; however, the frequency of NIMA matching was below 10%. Cord blood banks are currently capturing information on the HLA of the cord blood unit's mother with the aim to prospectively select a CB unit based on NIMA. Recently, indirect evidence has suggested that perhaps microchimerism from maternal donor cells may mediate a GVL effect in CB HCT [103]. Whether or not this observation is of value in selecting a CB unit has to be further investigated.

ABO major incompatibility has been associated with decreased survival and disease-free survival rates in CB HCT for children and adults with hematologic malignancies [42,104]. However, in many analyses, ABO compatibility is not as robust a factor as cell dose and

HLA. When many CB units are available, choice of the CB unit that is ABO compatible or with minor incompatibilities should be taken into consideration.

A relationship between race and ethnicity and survival of recipients after single CB HCT has been reported for both adults and children with leukemia and MDS [105]. Black recipients had inferior survival to Caucasians but the outcomes were improved when single CB units with a higher TNC dose were used.

In addition to Table 39.4, other reports on selection of an optimally engrafting CB unit have been published [106,107]. Moreover, efforts have gone into predicting the quality of transplantable CB collection through a multiparameter Apgar scored system [108,109].

Improving outcomes after CB HCT

Factors related to procedures of HCT, such as conditioning regimen and GVHD prophylaxis may be associated with time to engraftment. In a Eurocord study, the use of fludarabine in MAC was associated with improved neutrophil and platelet recovery in adult CB HCT (V. Rocha for Eurocord, unpublished data). In Fanconi anemia patients, use of fludarabine in the preparative regimen has been associated with improved engraftment independent of cell dose and HLA in CB HCT [47]. Conversely, use of MTX-containing regimens for GVHD prophylaxis has been associated with delayed engraftment and increased risk of graft failure in patients with hemoglobinopathies and malignant diseases transplanted with an HLA-identical sibling CB unit. In Europe and the United States, the most common regimen is calcineurin inhibitor-based GVHD prophylaxis alone or in combination with corticosteroids or MMF. New protocols for GVHD prophylaxis, using sirolimus-based reagents are reported in the RIC and double CB HCT setting [110]. Prospective studies are needed to establish the best GVHD prophylaxis in the setting of CB HCT.

Immune reconstitution after CB HCT

Immune cell recovery after HCT is a complex process [111]. Effects of delayed immune reconstitution after CB transplantation is most obviously manifest by increased incidence in opportunistic infections [112]. A high incidence of serious infections and infection-related mortality is apparent in the first 3–6 months following CB HCT [113–115], due, in large part, to phagocytic cell- and T-cell-mediated immune deficits before the onset of thymopoiesis which becomes appreciable 6 months or so after HCT [116].

The kinetics of lymphocyte subset recovery after CB HCT has been well described [117–121], and are summarized in Figure 39.4. Natural killer (NK) cells are detectable in peripheral blood at 1 month after transplantation in almost all patients, and reach age-appropriate normal levels at a median time of 2–3 months [118,120]. Early recovering NK cells are predominantly CD56$^{\text{bright}}$ and preferentially express the inhibitory receptor NKG2A, but have a high potential for cytokine production, including interferon (IFN)-γ [122–124]. NK cell recovery remains rapid after CB HCT because CB is a rich source of HPC with the capacity to develop into NK cells upon influence of instructive cytokines [125,126], including a novel myeloid-like NK cell progenitor [127,128].

B cells also rapidly recover reaching normal numbers at a median time of 6 months after transplantation, with IgG, IgM, and IgA levels remaining normal in the post-transplant period in the vast majority of patients [118]. This recovery is associated with a well-diversified B-cell repertoire by day +90 as assessed by B-cell receptor V_H complementarity determining region 3 (CDR3) [129]. A high proportion of the B cells recovering in the first 6 months after CB transplantation have the phenotype of regulatory B cells ($IgM^+IgD^+CD38^+CD5^+$ B cells), the number of which exceeds that found in normal donor peripheral blood [129].

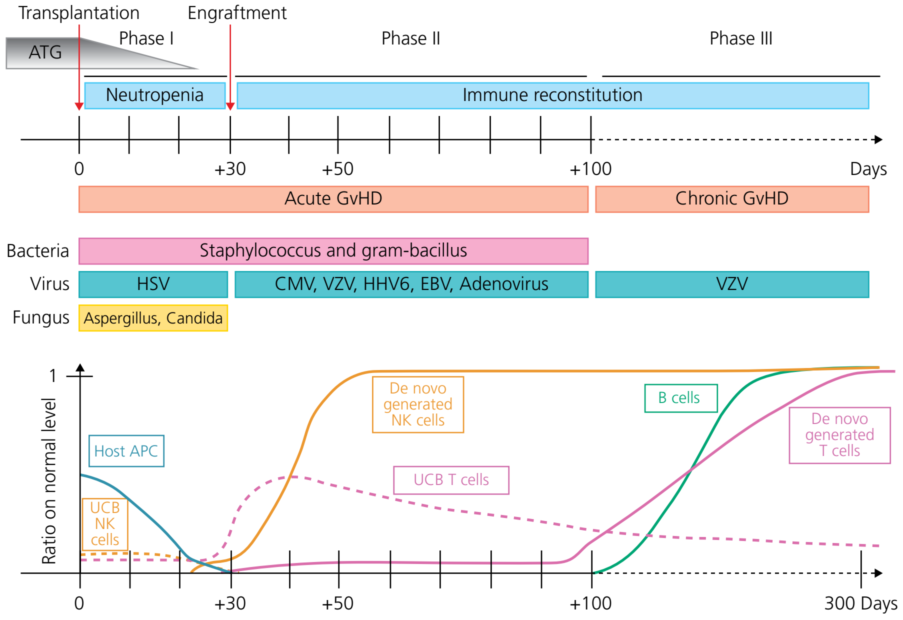

Figure 39.4 Kinetics of immune reconstitution and transplant-related complications following UC HCT. The pre-engraftment period (Phase I, Days 0–30) is characterized by general immunosuppression, including neutropenia, with high susceptibility to bacterial and fungal infections. Host residual antigen-presenting cells (APC) progressively disappear during this phase. In the post-engraftment period (Phase II, Days 31–100), NK cells reach normal levels by about 1 month after transplantation, and may represent 80% or more of the recipients peripheral blood lymphocyte count. UCB-derived T cells also persist during this phase and undergo antigen-driven thymic independent expansion. During this phase, there is susceptibility to bacterial as well as viral infections. In phase III (beyond Day 100), there is progressive B-cell reconstitution and de novo generation of T cells through thymopoiesis. Recovery of thymopoiesis is usually significantly more prolonged in adults. Source: Merindol et al. 2011 [121]. Reproduced with permission of Wiley & Sons.

T cells are slowest to recover after CB HCT. $CD4^+$ T cells recover to normal numbers after a median of 12 months, but $CD8^+$ T cells recover somewhat faster, reaching normal numbers after medians of 8–9 months [118,120]. Recovered T cells appear to show normal responses to common mitogens, including phytohemagglutinin, concanavalin A, and pokeweed mitogen, by 6–9 months after transplantation [118]. Unlike PBSC or BM grafts, T cells contained in CB are predominantly naïve ($CD45RA^+CD62L^+CD27^+CCR7^+$) recent thymic emigrants [130]. Therefore, the overwhelming majority of T cells that emerge during the first 3–6 months after CB HCT are peripherally expanding graft-derived T lymphocytes. While naïve T cells present in CB grafts give rise to antigen-specific T-cell-mediated immunity, protection against infection may not be entirely effective [131], possibly because this pathway of expansion leads only to a skewed T-cell repertoire with limited responses against newly encountered antigens. By 3–6 months after CB HCT, thymus-derived T-cell reconstitution becomes detectable, as assessed by TREC-containing T cells, reaching normal range at approximately 9–12 months post-transplantation in pediatric patients [132,133].

There is currently no evidence that kinetics of immune cell subset reconstitution are substantially different following double versus single CB HCT [58,134,135], with the possible exception of enhancing the development of TREC-containing cells following CB HCT in adult patients [136]. In the largest reported study to date, investigators recently compared lymphocyte subset reconstitution in children undergoing transplantation using CB ($n = 112$) with those undergoing unmanipulated unrelated BM HCT using a competing risk analysis [137].

A higher nucleated cell dose collected in the CB unit has been associated with faster total T cell and $CD8^+$ T-cell recovery [120]. Greater HLA mismatch and development of acute GVHD have been significantly associated with slower T-cell recovery, with the effect of acute GVHD impairing $CD4^+$ T cells [120]. While the intensity of the preparative regimen has not been shown to affect immune cell subset recovery or immunoglobulin reconstitution [138], TBI and anti-thymocyte globulin (ATG) administration have been reported to delay T-cell recovery [139].

Clinical immune outcomes following UCB transplantation

Few studies have attempted to correlate immune cell recovery with risk of opportunistic infections [118,140]. In the largest study including pediatric CB recipients from Duke University [140], a higher $CD3^+$ cell dose in the CB graft (together with a higher $CD34^+$ cell dose, negative recipient CMV serology, greater HLA match, and younger age) was found to predict a lower risk of death from opportunistic (mostly viral) infections at 6 months after transplantation, suggesting that in vivo expansion of infused post-thymic T cells contained in the CB graft could provide protection from infection. Additionally, CB recipients who developed opportunistic infections by day +100 have a significantly reduced probability of overall survival compared to those who did not have infection. Also, patients who developed opportunistic infections showed greater evidence of in vivo expansion of infused naïve T cells contained in the graft together with the emergence of T-cell subsets with a Th1/Tc1 cytotoxic phenotype as early as 2–3 weeks after HCT.

Immune cell recovery may impact risk of relapse and survival after CB HCT. Rapid lymphocyte recovery after CB HCT is associated with improved clinical outcome. In multivariable analysis, an absolute lymphocyte count (ALC) of $>0.2 \times 10^9$/L at day +30 ($n = 73$) after myeloablative conditioning was associated with superior 2-year overall survival (73% vs. 61%; $p = 0.02$), progression-free survival (68% vs. 54%; $p = 0.05$), and less transplant-related mortality (8% vs. 28%; $p < 0.01$) compared to patients whose ALC was $<0.2 \times 10^9$/L ($n = 43$). Similarly, for patients receiving reduced-intensity conditioning, an ALC $>0.2 \times 10^9$/L at day +42 ($n = 105$) was associated with superior 2-year overall survival (59% vs. 41%; $p < 0.01$) and progression-free survival (46% vs. 36%; $p = 0.05$). Notably, there was no significant relationship between ALC and relapse [141].

Although ALC and other immune cell numbers have not been predictive of relapse, antigen-specific immune reconstitution has been associated with reduced leukemia relapse and improved relapse-free survival following CB transplantation [142,143]. A robust T-cell response to at least one herpes virus was a strong predictor of relapse-free survival. Conversely, a high level of expansion of $CD8^+$ T cells co-expressing programmed death-1(PD-1), an inhibitory receptor of the CD28 family highly expressed on dysfunctional T cells during chronic viral infections [144], early following CB HCT has been associated with leukemic relapse [131], suggesting that excessive numbers of $PD\text{-}1^+CD8^+$ T cells may be a surrogate marker of defective immune reconstitution. The importance of reconstitution of thymopoiesis has been underscored by the observation that while $CD8^+$ T cells capable of secreting IFN-γ in a CMV-specific enzyme-linked immunosorbent spot (ELISpot) assay can be detected as early as 8 weeks after UCB transplantation, these cells are not capable of clearing CMV viremia [136]. Clearance of CMV viremia occurred later with recovery of $CD4^+CD45RA^+$ T cells, and was dependent on reconstitution of thymopoiesis and attainment of TREC levels of 2000 or more copies/μg DNA.

Future directions for enhancing CB HCT

Potential means to increase numbers of collected CB cells and/or ex vivo expand CB HSC and HPC

Means to enhance CB HCT have been reviewed [145]. A simple means to get more cells from a CB collection is to collect more cells at the birth of the baby. While perfusion techniques have been used for this purpose after the placenta is removed from mother [146], this ex vivo procedure is technically challenging, and is not likely to be available except at select collection sites. Another means is to collect and process cells under hypoxic conditions (e.g. 3% O_2) or in ambient air in the presence of cyclosporine A which prevents mitochondrial permeability transition pore opening, increased release of mitochondrial ROS, and enhances by 2-5 fold the numbers of transplantable HSC obtained [147,148]. Investigators have also focused efforts on ex vivo expanding of the HSC and HPC in CB already collected. Attempts at ex vivo expansion have met with limited success, but new studies may be promising. A potent combination of cytokines for ex vivo culture of human $CD34^+$ cells for expansion of HPC and repopulating cells in vivo in immune-deficient mice is stem cell factor (SCF), Flt3 ligand (FL), and thrombopoietin (TPO) with other cytokines, molecules, and/or other populations sometimes added to this cocktail of growth factors. Examples of other molecules tested with or without SCF, FL, and TPO include rapamycin [149], SR1 [150], other small molecules [151,152], Notch ligand Delta1 [153], the combination of Delta1 plus HoxB4 [154], as well as other factors, including selected members of the angiopoietin-like family of molecules [155] and chromatin remodeling agents [156,157]. Some have shown some efficacy for ex vivo expansion of phenotypic or functional CB HSC. Notch-mediated expansion of short-term repopulating HSC/HPC populations was demonstrated in a clinical setting [153], as was mesenchymal stromal cells to enhance cytokine-mediated expansion of CB cells [158]. Both clinical studies [153,158] were done in the context of a double CB HCT where ex vivo cultured cells were infused with an unmanipulated CB unit. Both approaches resulted in an apparent shorter time to neutrophil and platelet engraftment. However, it was the unmanipulated CB unit that engrafted long term, and it is not clear if any studies have resulted in ex vivo expansion of long-term repopulating HSC needed for use of such procedures in a single CB HCT setting.

Enhanced understanding of the cells within the microenvironment niche in BM that nurtures survival and other functions of HSC and HPC could also be of use for HSC expansion effort. This niche includes endothelial cells, osteoblasts, and mesenchymal stem/stromal cells (MSC). In mouse models it was demonstrated that parathyroid hormone or a related protein activated and stimulated osteoblasts, which in turn influenced numbers and function of mouse HSC [159]. This led to assessment of parathyroid hormone effects in a phase II trial of double CB HCT [160]. The results were not encouraging, with no enhancing effect on time to neutrophil or platelet engraftment. Mixtures of either endothelial cells, MSC, or other cells with CB cells have been assessed for enhancing the repopulation of CB HSCs in animal models [161,162], but it remains to be determined if such efforts are efficacious for enhancing human CB engraftment of humans. A better appreciation of the intracellular molecules that regulate HSC self-renewal and proliferation should help to increase HSC numbers or activity ex vivo. A large number of such intracellular mediators have been defined [5], but we need to appreciate how these signaling molecules interact with each other to modulate HSC function.

Efforts to enhance the homing and subsequent nurturing effect of the microenvironment on CB HSC

Clinical efforts have been made to bypass the circulation by direct intra-bone marrow injection of CB cells [163]. It is not clear whether this has overcome the problem of delayed time to neutrophil, platelet, or immune cell recovery. HSCs inefficiently home to the BM after intravenous (IV) infusion. Efforts to enhance the homing of HSC to their nurturing BM microenvironmental niche include pretreating donor cells prior to IV injection with either small molecule inhibitors of the enzyme dipeptidylpeptidase (DPP)4 [164–166], agents that enhance fucosylation [167], or prostaglandin (PG)E 1 or 2 [168–170]. In vivo treatment to inhibit DPP4 with small molecules has also enhanced engraftment [166]. Appropriate timing with combination of the above-mentioned targeting reagents may synergize to enhance engraftment. Efforts are ongoing to test the clinical feasibility of PGE, DPP4 inhibition, and fucosylation for enhancement of time to engraftment. An example of potentially improving upon first clinical efforts is that of a pilot clinical study of in vivo DPP4 inhibition to enhance engraftment of single-unit CB HCT in adults with high-risk hematologic malignancies [171], that utilized an FDA approved, orally active DPP4 inhibitor, sitagliptin, (used clinically to treat type II diabetes mellitus). Systemic DPP4 inhibition was well tolerated, and results were encouraging, but not definitive in significantly enhancing time to neutrophil engraftment. It is possible that more frequent administration of DPP4 and for longer periods of time than administered in the pilot study [171], might significantly enhance the time to engraftment with single CB HCT. In fact, using combinations of treatments such as ex vivo short-term culture of CB with either PGE or a DPP4 inhibitor and infusion of these treated cells in patients treated with a DPP4 inhibitor may be more efficacious than each one of these procedures alone [172].

Possibilities for regenerative medicine with cells present in or generated from CB

It is too early to tell if non-hematopoietic cells in CB will be able to be used successfully for therapeutic advantage, in the context of cell replacement or regenerative medicine. CB contains MSCs, which have the capacity, at least in vitro of differentiating down pathways that can produce bone, fat, and cartilage [173–175]. However, there has been variability reported in the literature with regards to the frequency of MSC in different collections of CB [176,177]. Endothelial colony-forming cells (ECFC; perhaps endothelial progenitor cells, EPC) have also been found in CB [178,179], and have been characterized by selected expression of antigenic markers which distinguishes them from blood progenitors [180]. ECFC are present in low frequency in CB, but can be recovered in viable form after thawing CB samples that had been stored in a cryopreserved state for up to 21 years [17]. However, ECFC were present after thawing at 10–20% of numbers found in freshly isolated CB samples. Also, the proliferative capacity of these cells, although still extensive, is not at the levels seen for freshly isolated CB ECFC, suggesting that the freezing procedure that works well for HPC, is not as effective for cryopreservation of ECFC. More efficient cryopreservation techniques may have to be developed for ECFC, and perhaps other non-hematopoietic cells in CB.

Induced pluripotent stem (iPS) cells can be generated from mature cell types, including $CD34^+$ CB cells, through enforced expression of transcription factor such as Oct4, Sox2, KLF4, and c-Myc [181,182] (Figure 39.5). Generated iPS cells have the capacity to produce in vitro, and by teratoma formation assays in vivo in mouse, cells of all three germ cell layers (endoderm, ectoderm, and mesoderm). IPS cells have been generated from $CD34^+$ cells isolated after thawing from unseparated CB cells that had been stored in a cryopreserved state for more than 20 years [17]. Only a low frequency of the iPS cell colonies generated from cryopreserved or fresh CB samples, and from other cells sources used to generate iPS cells, are fully reprogrammed to an ESC-like state [183]. A more fully reprogrammed iPS cell state could be generated in a larger proportion of the cells if in addition to enforced expression of Oct4, Sox2, KLF4, and c-Myc in $CD34^+$, as well as other, cell types, microRNA-302 cluster was also expressed from an exogenously added vector. Epigenetic regulation of Nanog (another important ESC-related transcription factor), by microRNA-302 as mediated through methyl-DNA binding domain protein 2, an epigenetic suppressor, allowed completed iPS cell reprogramming in a larger percentage (~16%) of the cells [183].

While iPS cell generation has the potential to be used to generate all cell types of the body's cells, including blood cells, and we have learned and will continue to learn about cellular processes with iPS cells, whether or not iPS cells, or in fact other non-hematopoietic cells in CB will be a safe and efficient means to be considered for human regenerative medicine remains to be determined [184]. Cells similar to CB endothelial colony forming cells with functional regenerative medicine potential have been differentiated from CB $CD34^+$ derived iPSCs [185]. If in the future, iPS cell technology is found to be of clinical cell replacement use, or other non-hematopoietic cells in CB can be used in a similar manner, this will likely extend the use of cryopreserved CB, which would also enhance the need for more CB banking efforts [184,186]. The future use of CB cells for other than blood cell replacement therapies remains to be proven, and great caution needs to be exercised in this context, including the possible need for enhanced governmental oversight and regulation [187].

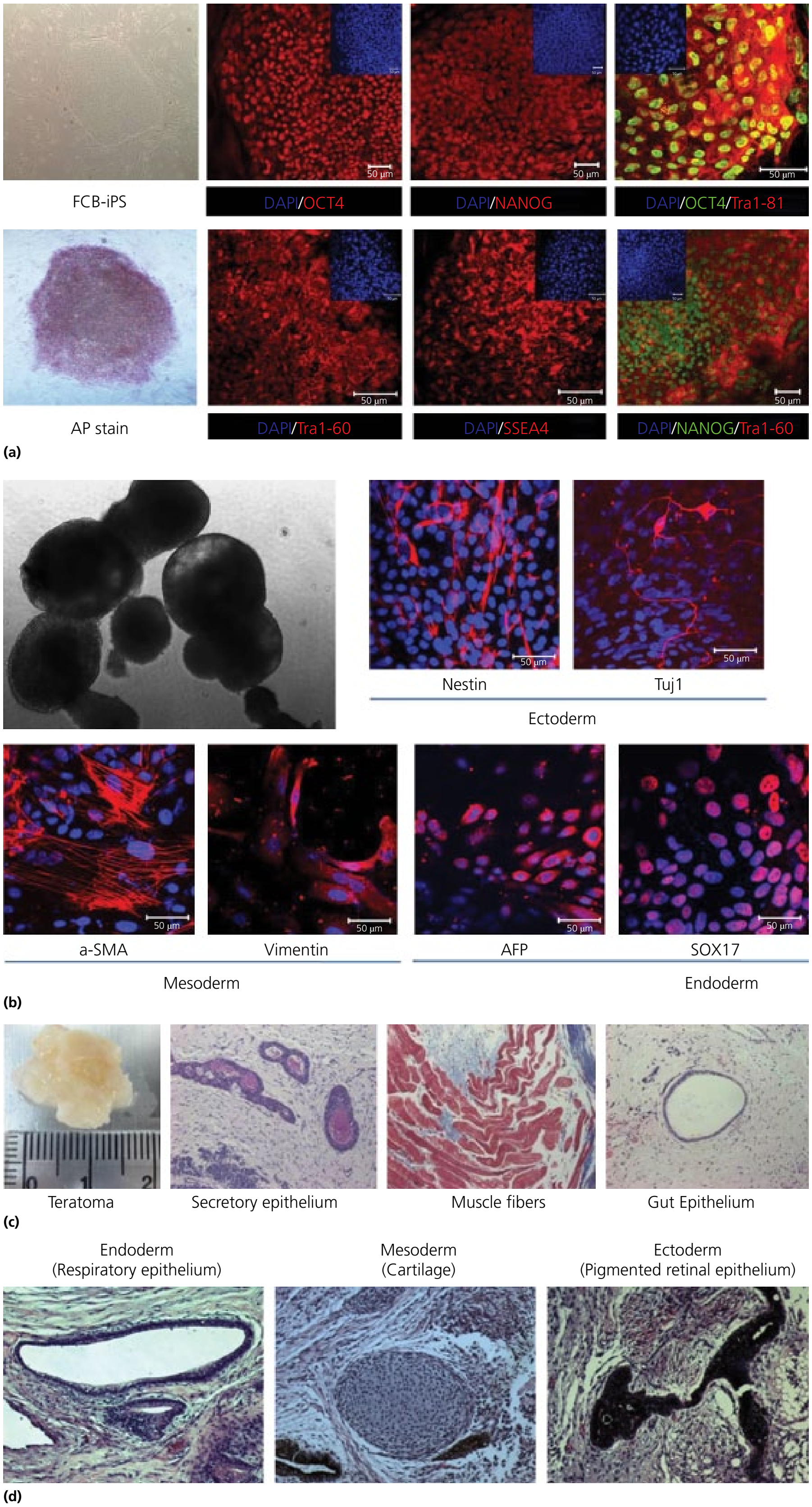
FCB-iPS
DAPI/OCT4
DAPI/NANOG
DAPI/OCT4/Tra1-81
AP stain
DAPI/Tra1-60
DAPI/SSEA4
DAPI/NANOG/Tra1-60
50 µm
(a)
Nestin
Tuj1
Ectoderm
a-SMA
Vimentin
AFP
SOX17
Mesoderm
Endoderm
(b)
Teratoma
Secretory epithelium
Muscle fibers
Gut Epithelium
(c)
Endoderm
(Respiratory epithelium)
Mesoderm
(Cartilage)
Ectoderm
(Pigmented retinal epithelium)
(d)

Figure 39.5 Reprogramming 21-year-old frozen human CB CD34$^+$ cells to iPS cells. (a) Immunohistochemistry for pluripotency markers Oct4, NANOG, TRA-1-60, SSEA4, and alkaline phosphatase (AP). (b) Morphology of 10-day embryoid body under phase contrast microscopy (top left), and immunostaining of iPS cell differentiated cells for ectoderm, mesoderm, and endoderm markers as noted. (c) Terotoma derived from immunodeficient mice injected with iPS cells (top left) with tissue sections representing all three embryonic germ layers. (d) Tissue sections from terotoma, as in C, but from different thawed and isolated CD34$^+$ cells. Source: Broxmeyer et al. 2011 [23]. Reproduced with permission of the American Society of Hematology. (For a color version of this figure, please see the color plate section.)

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CHAPTER 40

Mobilization of Peripheral Blood Hematopoietic Cells for Autologous HCT

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Introduction

High-dose chemotherapy has become an important therapeutic strategy for many malignancies. These regimens are often severely myelosuppressive or myeloablative, and autologous peripheral blood progenitor cells (PBPCs) have been used to provide rapid and sustained hematopoietic recovery to support such treatment. In the past, autologous bone marrow transplantation was used in this setting but, because of the less invasive collection methods and more rapid resolution of neutropenia and thrombocytopenia, PBPC transplantation has largely replaced bone marrow transplantation for the support of high-dose chemotherapy (Figure 40.1) [1,2].

Both hematopoietic stem cells and progenitor cells populate the resting bone marrow. Stem cells are single cells that are clonal precursors capable of giving rise to both identical stem cells (self-renewal) and a defined set of differentiated progenitors. Progenitor cells are multipotent precursor cells that lack the ability to self-renew but, like stem cells, maintain the capacity to differentiate and proliferate. Despite this important difference, these terms often are used interchangeably in the literature [3].

The ability of PBPC to reconstitute hematopoiesis was first described in 1980 and followed by additional reports in 1985 and 1986 [4–6]. Under steady-state conditions, hematopoietic progenitor cells circulate in the peripheral blood compartment at a frequency of approximately 10–100 cells/mL of whole blood. This low frequency of PBPC in the circulation made collecting adequate numbers of these cells a cumbersome process. As a result, methods to mobilize these cells from the marrow compartment and increase their numbers in the peripheral circulation have been developed to foster collection of sufficient cells for clinical use. The work by Abrams [4] and Richman [7] in animal models demonstrating an increase in PBPC following subablative chemotherapy administration in patients following cytokine administration have led to the widespread application of this approach for supporting high-dose therapy [8].

Identification and enumeration

Human hematopoietic stem cells express CD34 and Thy-1 at a high level and c-kit at a low level and do not express lineage specific markers ("Lin") and CD38 [3]. The ability of a mobilized apheresis product to engraft and permanently repopulate the bone marrow depends on its content of both stem cells and progenitor cells. In the transplant setting, this can be evaluated using several assays including colony-forming unit – granulocyte/macrophage (CFU-GM), colony-forming unit – granulocyte/erythrocyte/macrophage/megakaryocyte (CFU-GEMM), and burst-forming unit – erythroid (BFU-E), all of which have been correlated with time to engraftment [8,9]. Because these assays require significant laboratory manipulation, results often reflect technical variability and are difficult to compare.

Quantification of CD34 expressing cells has been used to assess the quality of the apheresis product and its adequacy for repopulating the bone marrow. $CD34^+$ cell content correlates closely with CFU-GM, BFU-E, and CFU-GEMM, as well as time to engraftment of both neutrophils and platelets [10]. $CD34^+$ cell quantification is also subject to intra-laboratory variation, but standardization of procedures can reduce this variation [11,12]. Efforts have also been undertaken to identify subsets of the CD34 cell population that are critical to bone marrow reconstitution, such as $CD34^+CD33^-$, $CD34^+CD33^+$, or $CD34^+CD38^-$ cells [13].

Mechanisms of hematopoietic cell mobilization

Hematopoietic stem cells (HSC) in the bone marrow (BM) exist in a highly organized three-dimensional microenvironment consisting of a diverse group of cell types including stromal cells and an associated extracellular matrix (ECM) rich in fibronectin, collagens, and various proteoglycans. Osteoblasts appear to play a pivotal role in HSC function via a physical interaction that exists between HSC and the BM osteoblasts, thus defining the osteoblastic niche [14]. Other subpopulations of HSCs exist in close proximity with sinusoidal endothelial cells, thus defining the "endothelial or vascular niche" [15]. It is assumed that each of these niches differentially regulate HSC trafficking (homing and mobilization) as well as basic functions such as survival, proliferation, self-renewal, and differentiation. To enter the circulation, HSCs must transmigrate through the vascular barrier (termed the BM–blood barrier) where both endothelial cells and subsequently macrophages are thought to play a role in stem cell migration and release from the marrow microenvironment [16,17]. Several excellent reviews on this topic address the various roles played by these and other factors in this complex process (Figure 40.2) [18–20].

A number of adhesion molecules have been implicated in HSC trafficking. These include stromal cell-derived factor-1

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(SDF-1/CXCL12), very late antigen-4 (VLA-4 or a4 integrin), c-kit, CD62 ligand (CD62L), P- and E-selectins, and CD44 [21–23]. In addition to these adhesion molecules, a number of cell-associated chemokines including Gro-beta, Interleukin-8, metalloproteinase-9 (MMP-9 or gelatinase B), and CD26 (dipeptidylpeptidase-4) have been implicated in mediating the release of HSCs from the BM into the PB via cleavage of the candidate adhesion molecule tethers mentioned earlier [24–27].

The kinetics of HSC mobilization can vary dramatically depending upon the mobilizing agents used. Granulocyte colony-stimulating factor (G-CSF) induces maximum HSC mobilization in 4–5 days while granulocyte–macrophage colony factor (GM-CSF) induces maximum stem cell mobilization in 5–6 days. When chemotherapy and G-CSF or GM-CSF are used together, mobilization is optimal after 10–14 days. In contrast, mobilization by chemokines (IL-8 or GRO-β) or chemokine receptor antagonists (plerixafor) result in mobilization of HSC in minutes to hours [18,19,24,28].

Relative to BM HSC, a higher percentage of mobilized PB HSCs mobilized with G-CSF or chemotherapy and G-CSF are in G_0 or G_1 phase of the cell cycle. HSCs are selectively mobilized after M phase of the cell cycle providing a potential explanation for the preponderance of HSC in the G_0 and G_1 phases of the cell cycle in the blood after G-CSF treatment [29]. In addition, consistent with the downregulation of various adhesion molecules contributing to mobilization, circulating HSCs display reduced expression of VLA-4, c-kit, and CXCR4 [29,30].

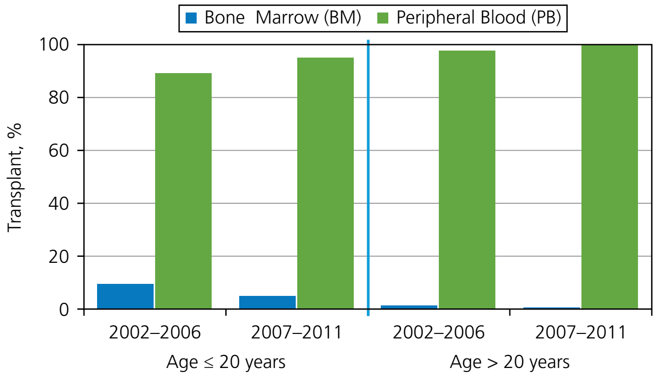

Figure 40.1 Use of peripheral blood versus bone marrow as the source for autologous hematopoietic stem cell transplantation according to data provided by Pasquini et al. from the Center for International Bone Marrow Transplant Research Summary Slides (2013). Source: Pasquini MC, Wang Z. Current use and outcome of hematopoietic stem cell transplantation: CIBMTR Summary Slides, 2013. Available at http://www.cibmtr.org. Reproduced with permission of Pasquini and Wang.

Evidence for the pivotal role of SDF-1/CXCR4 in HSC mobilization and trafficking

There is convincing evidence that the interaction of SDF-1/CXCL12 with its cognate receptor, CXCR4, generates signals that regulate HSC trafficking in the BM. SDF-1 is a CXC chemokine constitutively expressed in the BM by stromal cells and BM osteoblasts [31]. SDF-1 is a potent chemoattractant agent for HSCs and has been shown to regulate HSC survival, adhesion, and cell cycle status [21]. Analyses of CXCR4-deficient mice have established that both SDF-1 and CXCR4 are necessary for normal migration of HSCs from the fetal liver to the BM and for the efficient retention of these progenitors in the BM [27,28]. Elevation of the SDF-1 levels in the blood by administration of SDF-1 or by the injection of adenoviral vector expressing SDF-1 is associated with significant mobilization of HSCs into the blood. Likewise, injection of a competitive inhibitor of CXCR4 (AMD3100; plerixafor) induces rapid mobilization (9 hours in humans and 3 hours in mice) [28].

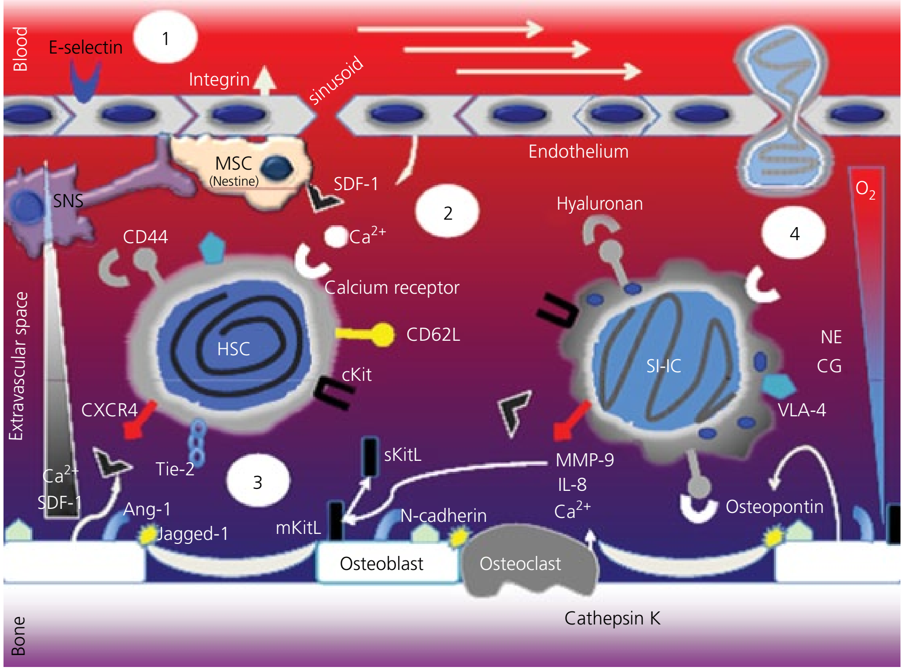

Figure 40.2 Cartoon depicting normal and leukemic cell interactions with the marrow microenvironment. Source: Schroeder and DiPersio 2012 [18]. Reproduced with permission of Society of Leukocyte Biology and John Wiley & Sons Ltd. (For a color version of this figure, please see the color plate section.)

Interruption of SDF-1/CXCR4 signaling/interaction represents a pivotal and key step in HSC mobilization by G-CSF. SDF-1 levels in the BM decline sharply during G-CSF mobilization [31]. There is evidence to suggest that CXCR4 (expressed on HSCs) and SDF-1 (expressed on BM stromal cells and osteoblasts) may be proteolytically cleaved and inactivated during G-CSF treatment [30] although the role of proteolytic cleavage of SDF-1 by these proteases as the mechanism of G-CSF-induced mobilization has been brought into question by elegant studies in which mice deficient in both NE, MMP-9, and cathepsin G (CG) mobilized HSCs normally in response to G-CSF treatment [32]. Recent data provide compelling evidence that G-CSF induces a dramatic transcriptional downregulation of SDF-1 mRNA in BM stromal cells. The downregulation of SDF-1 mRNA in response to G-CSF occurs in a temporally consistent way with G-CSF-induced mobilization of HSCs (maximum downregulation of SDF-1 and HSC mobilization occurring in 4–5 days) [33]. These data provide prima facia evidence that G-CSF regulates SDF-1 levels and thus HSC mobilization via transcriptional downregulation but they do not eliminate the possibility that G-CSF may also induce mobilization in part by activation of cell-associated proteases. Recent evidence suggests that G-CSF has no direct effect on BM osteoblasts or nestin$^+$ CCL12/SDF-1 abundant reticular cells (CAR) but mediates its suppressive effects on SDF-1 expression in these cells via intermediate cells phenotypically and functionally similar to monocytes. The mechanism by which G-CSF induces downregulation of SDF-1 via these intermediate monocytes remains unclear. Of interest, this same group found that IL-8 in contrast to G-CSF is completely dependent on neutrophils for mobilization [34].

Role of proteases and other cell adhesion molecules on HSC mobilization

Significant data suggest that a highly proteolytic environment is induced in the BM during HSC mobilization by G-CSF [35]. These proteases include NE, CG, and CD26 which appear to increase following G-CSF administration and cleave important adhesion molecules such as VCAM-1, SDF-1, and c-kit [21,22]. The metalloproteinase MMP-9 also appears important in the role of IL-8-induced HSC mobilization [24–26]. The interaction of these proteases in animal models has proven complex, however, leading to the conclusion that at this time, specific proteases or combinations of proteases have not clearly implicated any single or combination of proteases mechanistically in cytokine, chemokine, or chemotherapy-induced HSC mobilization [24,26,27,32,35]. Adhesion molecules that are thought to play a role in stem cell mobilization include VCAM-1, CD44, and P- and E-selectin, both of which are inhibited by fucoidan, the administration of which leads to stem cell mobilization in mice [21,22,35–37]. Recent reports have also helped elucidate the roles of microRNA126, sphingosine-1-phosphate, complement, and constitutional polymorphisms in SDF-1, VCAM-1, and CD44 on the response of individuals to G-CSF administration [38–41]. Several excellent reviews of these processes have recently been published [18,19].

Clinical applications

PBPCs have become the preferred source of autologous stem cells to support the use of high-dose, myeloablative chemotherapy in a variety of diseases [1,2,41–43] and are also being utilized widely in allogeneic transplants [44].

Collection techniques

Once PBPC mobilization is initiated, the timing and volume of apheresis is critical to maximizing yield. For steady-state mobilization with recombinant growth factors (in the absence of chemotherapy), the peak values of mobilized hematopoietic progenitor cells are observed after 4–6 days of treatment in most series [45,46]. Hematopoietic cells are usually collected with an apheresis machine using a technique that is similar to that used for collection of platelets. Small comparison studies have not clearly demonstrated the superiority of one machine over another, but at least one report suggested that use of the COBE Spectra unit resulted in a more rapid collection and higher yield of CD34$^+$ cells in patients undergoing apheresis prior to ASCT for multiple myeloma [47]. Chemotherapy-based mobilization results in an increase in the time required to initiate collection and less predictability as to when this will occur, as maximal mobilization occurs following resolution of the hematologic nadirs produced with chemotherapy-induced myelosuppression. While a number of studies indicate that the combination of chemotherapy and cytokines leads to increased yield of hematopoietic progenitor cells compared to cytokines alone, the variability inherent in blood count recovery following chemotherapy can present a challenge in trying to schedule apheresis procedures. Guidelines and general recommendations for target cell numbers in the peripheral blood have been developed to initiate apheresis and monitoring daily CD34$^+$ cell content in the peripheral blood has become the preferred means by which to predict PBPC yield [48–50]. In one study, a CD34$^+$ cell count of ≥50 cells/μL peripheral blood predicted a CD34$^+$ cell yield of $\geq 2.5 \times 10^6$ CD34$^+$ cells/kg body weight in a single apheresis [51].

In harvesting PBPCs, the goal is to minimize the number of apheresis procedures required to achieve the target hematopoietic progenitor cell dose. To date, the optimal apheresis volume that achieves this goal has not been defined. Large volume apheresis (>15L) has been investigated as a way to decrease the number of aphereses. Initially, there was concern that the large volumes would impair CD34$^+$ cell quantity harvested over collection time. However, in one randomized trial comparing 7-L and 10-L apheresis volumes, the use of a 10-L volume did not decrease the number of apheresis required to collect $\geq 2 \times 10^6$ CD34$^+$ cells/kg [52]. Most reports have demonstrated a correlation between total CD34$^+$ cell yield and the blood volume processed, resulting in varied approaches among institutions including individualized, patient-specific, collection techniques that are frequently based on practical issues such as scheduling and cost [48,52].

Identifying the optimal cell yield

Several groups have identified minimum thresholds ranging from 1×10^6 to 3×10^6 CD34$^+$ cells/kg body weight for sustained engraftment [10,53]. Other investigators have suggested an optimal CD34$^+$ cell dose of ≥ 5–8×10^6 CD34$^+$ cells/kg, with which neutrophil and platelet recovery is accelerated, the demand for other supportive measures (antibiotics, transfusion) is decreased and therapy schedule is more likely to be preserved [50,53]. One report highlighted the fact that although a higher dose of CD34$^+$ cells is unlikely to shorten the median duration of neutropenia or thrombocytopenia in a broad patient population, it will reduce the number of "outlier" patients with prolonged times to engraftment requiring prolonged transfusion support (Figure 40.3) [53]. While some investigators suggest that more primitive CD34$^+$ cell subsets such as CD34$^+$CD33$^-$ or CD34$^+$CD38$^-$ cells are better predictors of durable engraftment,

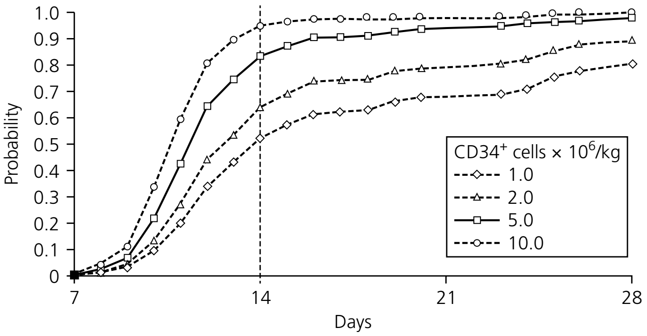

Figure 40.3 This figure demonstrates the achievement of platelet recovery to >50,000/uL post-transplant on the *Y* axis and days post-transplant on the *X* axis as a function of the number of CD34$^+$ cells infused. Note that the median days to platelet recovery does not change but that the frequency of patients with prolonged time to recovery decreases markedly as doses exceed 5 × 10^6 CD34 cells/kg infused. Source: Adapted with permission from Glaspy et al. 1997 [53] with permission of the American Society of Hematology.

Table 40.1 Comparison of mobilization with granulocyte colony-stimulating factor (G-CSF) or granulocyte–macrophage colony-stimulating factor (GM-CSF)

No. of patients	Comparison	Observation	Ref.
29	G-CSF at 6 µg/kg vs. GM-CSF at 8 or 16 µg/kg	G-CSF group: 5.4-fold higher CD34$^+$ cell yield	[45]
44	G-CSF at 5 µg/kg/d vs. GM-CSF at 250 µg/kg/d or 125 µg/kg BID	No difference in G-CSF vs. GM-CSF induced CD34$^+$ cell yield	[54]
156	G-CSF + chemotherapy vs. GM-CSF + chemotherapy	G-CSF group: 3.5-fold increase in CD34$^+$ cell yield	[55]
95	Unprimed vs. 5 or 10 µg/kg G-CSF	3–6-fold more CD34 cells with 5 or 10 µg/kg than for unprimed patients	[46]

BID, twice daily; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor.

Table 40.2 Comparisons of cytokine doses and combinations for mobilization

No. of patients	Comparison	Observation	Ref.
	Cytokine doses		
82	Mobilization with chemotherapy + filgrastim, molgramostim, or lenograstim	No statistically significant difference in CD34 cells collected in any one group over the others	[73]
52	Peg vs. G in autologous patients	6 mg of pegfilgrastim was comparable to 10 µg/kg G-CSF	[74]
45	6 vs. 12 mg pegfilgrastim vs. 8 µg/kg G-CSF + chemo	Comparable CD34 yields with allo doses and an insignificantly lower CD34 yield	[75]
144	No growth factor vs. GM-CSF: 125 µg/m^2/day vs. 250 µg/m^2/day	GM-CSF 125 µg/m^2/day and 250 µg/m^2/day groups: five- and 12-fold increase in PBPCs compared to no growth factor	[57]
75	G-CSF: 10 µg/kg vs. 24 µg/kg G-CSF in normal donors	1.7-fold increase with higher dose G-CSF	[56]
72	G-CSF: 10 µg/kg QD vs. 5 µg/kg BID vs. 8 µg/kg BID in healthy donors	Modest benefit from split dose	[59]
50	G-CSF: 10 µg/kg QD vs. 5 µg/kg BID to healthy volunteers in a crossover design with a washout period in-between	Split dose led to approximately a twofold increase in CD34 cells/donor.	[60]
	Cytokine combinations		
156	Chemo + G-CSF vs. chemo + GM-CSF followed by G-CSF	No difference in CD34$^+$ cell yield (but both regimens yielded 3.5-fold more CD34$^+$ cells than chemo + GM-CSF)	[55]
40	G-CSF or GM-CSF vs. GM-CSF followed by G-CSF	GM-CSF f/b G-CSF group: 2.3-fold increased yield of CD34$^+$ cells.	[68]
302, 298	G-CSF alone was compared to G-CSF + plerixafor in separate studies in patients with multiple myeloma or lymphoma who then underwent apheresis	In each case, CD34 yields were higher with the combination than with G-CSF alone resulting in fewer days of collection to achieve the targeted apheresis yields	[66,67]

BID, twice daily; chemo, chemotherapy; DFS, disease-free survival; f/b, followed by; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; OS, overall survival; PBPCs, peripheral blood progenitor cells; QD, every day.

most centers continue to rely on total CD34$^+$ cell numbers as the most reliable indicator of an adequate PBPC population [10,13,48,50].

Cytokine and cytokine plus chemokine-induced mobilization

G-CSF and GM-CSF have been used alone to increase circulating CD34 cells or CFU-GMs compared to that found in the unmobilized, steady state, and mobilize PBPCs from cancer patients at doses from 3 to 24 µg/kg/day by subcutaneous injection [8,9,45,46]. A number of studies have compared the use of G-CSF and GM-CSF for mobilization both alone and in conjunction with chemotherapy [45,54,55]. While most have found that G-CSF provides a higher yield of PBPCs, both growth factors reliably mobilize cells into the circulation with minimal toxicity (Table 40.1) [45,46,54,55]. A dose–response to G-CSF, with higher doses resulting in increased CD34$^+$ peripheral cells, has been demonstrated. One group compared G-CSF doses of 5 and 10 µg/kg/day [50] while other groups compared G-CSF doses of 10 or 24 µg/kg/day [56]. In these studies, the higher dose resulted in superior collections of CD34$^+$ cells (Table 40.2). Higher doses of GM-CSF have likewise been shown to improve yield of CD34$^+$ cells [57]. Higher doses of G-CSF have also been used successfully to mobilize and collect cells in patients who have failed to collect following lower cytokine doses [58]. Studies in normal donors providing hematopoietic progenitor cells for allogeneic transplant recipients or in autologous patients prior to transplants for lymphoma or myeloma have not found a consistent benefit for once versus twice daily administration [48,59–61] and patient/donor convenience and comfort should probably be the determining factor at this time.

The timing of apheresis initiation following cytokine administration has also has been examined. One study of normal donors receiving G-CSF at 10 µg/kg/day showed that CD34$^+$ cell yield was threefold higher when apheresis was started at day 5 versus day 6 [45]. Another study reported that the peak time for cell mobilization was approximately 10 hours following G-CSF injection [62].

Chemokine receptor 4 (CXCR4) and stromal cell-derived factor 1-alpha (SDF-1α) are a receptor/ligand couplet that is important in the normal adhesion and release of CD34 cells from the marrow compartment [30,31]. Both of these molecules are degraded by proteases released from neutrophil degradation, thus contributing to CD34 mobilization following the neutrophilia observed after administration of G-CSF [19,20]. It has been noted that inhibition of this binding occurs in the presence of plerixafor (previously known as AMD3100), a reversible bycyclam inhibitor of the binding between these molecules. Injection of plerixafor in doses between 80–240 µg/kg SC was associated with only mild abdominal bloating, injection site erythema, and perioral paresthesias. Optimal doses, when given alone to myeloma patients, NHL patients, and normal volunteers, was 240 μg/kg SC [28,63].

Plerixafor was shown to be a modest mobilizing agent when given alone to patients with myeloma and NHL [63]. When given either alone or after 4 days of G-CSF, optimal mobilization (two- to threefold above the level of CD34$^+$ cells/mL induced by G-CSF alone) occurred approximately 6–9 hours after the dose of plerixafor [63–65]. The combined use of plerixafor (240 μg/kg SC the night before each apheresis) plus G-CSF (10 μg/kg/day SC × 4 days) was significantly more effective than G-CSF or plerixafor alone for the mobilization of HSCs in patients with myeloma and NHL [65]. Data from the 25 patients in this phase II study demonstrated that none of the patients initially given plerixafor + G-CSF failed to reach the minimum target of >2 × 10^6 CD34/kg after four apheresis collections. In contrast, 9/25 patients failed to reach this target when initially given G-CSF alone. All nine of the patients that failed to mobilize with G-CSF alone were successfully remobilized with G-CSF and plerixafor. After mobilization with plerixafor plus G-CSF, 56% of the patients reached the minimum CD34 cell dose after one apheresis and all patients reached the minimum target after 2 days of apheresis. Mobilization with G-CSF alone resulted in only 36% of the patients reaching this minimum target after 2 days of apheresis. Thus, plerixafor appears to be an active mobilizing agent in humans especially when combined with G-CSF. These data have provided the foundation for two large randomized trials in which myeloma and NHL patients were mobilized with either G-CSF (10 μg/kg/day) or G-CSF plus plerixafor (240 μg/kg/day given the evening before each apheresis) in preparation for ASCT [66,67]. The primary endpoint of the myeloma study was acquisition of 6 × 10^6 CD34/kg (sufficient for tandem autologous hematopoietic stem cell transplants). A total of 106 of 148 (71.6%) patients in the plerixafor group and 53 of 154 (34.4%) patients in the placebo group met the primary endpoint ($p < 0.001$). A total of 54% of plerixafor-treated patients reached target after one apheresis, whereas 56% of the placebo-treated patients required four apheresis procedures to reach target [66]. The primary endpoint of the NHL trial was >5 × 10^6 CD34/kg in four or fewer apheresis collections. In this second study by DiPersio and colleagues, the addition of plerixafor to G-CSF led to significantly more patients collecting >5 × 10^6 CD34 cells in ≤ 4 days than G-CSF alone (59% vs. 20%; $p < 0.001$). One hundred and thirty-five patients (90%) in the plerixafor group and 82 patients (55%) in the placebo group underwent transplantation after initial mobilization [67]. Median time to engraftment was similar in both groups. In both studies, plerixafor and G-CSF were well tolerated with the most common adverse events related to plerixafor being gastrointestinal disorders and injection site reactions [66,67].

Combination cytokine, pegylated cytokine, and cytokine plus chemotherapy-induced mobilization

Despite a phase I/II report of the value of combination cytokine therapy, most notably the sequence of GM-CSF followed by G-CSF, the additional time and expense of using two cytokines in this fashion and a negative phase III study have prevented widespread adoption of this approach [55,68]. Other cytokines that are capable of PBPC mobilization as a single agent include SCF, IL-12, and IL-3 [69–72] but their efficacy has been marginal and toxicity has precluded their general use or FDA approval for this purpose.

Recently, pegylated G-CSF has been developed and approved for use to augment neutrophil recovery following chemotherapy. This compound, pegfilgrastim, has also been used to mobilize CD34 cells when administered following chemotherapy and in most phase II trials appears roughly equivalent to daily doses of G-CSF [73–75].

Because IL-2 stimulates cellular immunity and T-cell progenitors, it has the potential to promote antitumor immunity within an autograft. These characteristics make IL-2 an attractive candidate for co-stimulation of immunologic effector cells in concert with other cytokines, such as G-CSF, that are more effective for progenitor cell mobilization. While IL-2 may enhance the number and function of antitumor effector cells, it unfortunately also impairs the mobilization of the CD34$^+$ cells [76], thereby limiting its application in the context of hematopoietic progenitor cell mobilization.

Studies have shown that patients mobilized with cyclophosphamide (CY) plus G-CSF resulted in increased numbers of mobilized PBPCs compared to either chemotherapy or G-CSF alone (Table 40.3) [77,78]. In a randomized trial comparing mobilization with G-CSF alone with CY plus G-CSF in patients with refractory lymphoma, the addition of CY resulted in a threefold increase in CD34$^+$ cell yield, but this did not translate into a shorter time to engraftment [77]. A randomized, cross-over trial compared CY plus G-CSF with GM-CSF plus G-CSF within the same patients. The CY-containing regimen resulted in a 2.7-fold increase in CD34$^+$ PBPCs over that resulting from the cytokines alone [78]. A small randomized study compared 5 μg/kg daily GCSF plus CY, 2 gm/m^2 with 10 μg/kg G-CSF and found no difference in regard to CD34 yield or count recovery post-transplant, but increased toxicity with the addition of CY [79]. Another randomized, phase III trial compared regimens of G-CSF, GM-CSF and sequential GM-CSF then G-CSF following myelosuppressive chemotherapy. Compared to patients receiving GM-CSF alone, patients receiving G-CSF alone yielded more CD34$^+$ cells/kg, had fewer aphereses and had a faster time to neutrophil recovery. There were no significant differences between groups receiving G-CSF alone and sequential GM-CSF followed by G-CSF [54]. These studies illustrate the improved potency of regimens containing both myelosuppressive chemotherapy and hematopoietic growth factors. At the same time, these increased PBPC yields have been accompanied by greater toxicity as a result of the concurrent administration of chemotherapy and a potential increase in the risk of myelodysplasia compared to the use of cytokines alone in some [79,80], but not all reports [81–84]. In the study by Wood, an intermediate dose of etoposide (750 mg/m^2), split over 2 days, plus G-CSF resulted in collection of >5 × 10^6 CD34 cells/kg in 1 or 2 days in 99% of 152 myeloma subjects studied, including 109 subjects who had received prior IMID therapy [83].

Table 40.3 Comparison of cytokine alone versus cytokine + chemotherapy

No. of patients	Comparison	Observation	Ref.
47	G-CSF alone vs. chemo + G-CSF	Chemo + G-CSF group: 2.9-fold increase in CD34+ cell yield	[77]
96	GM-CSF followed by G-CSF vs. chemo + G-CSF	Chemo + G-CSF: 2.7-fold increase in CD34+ cell yield yield compared to cytokine alone group	[78]
79	Randomized trial of G-CSF 10 μg/kg/d vs. cyclophosphamide 2 gm/m^2 + G-CSF 5 μg/kg/d	Equivalent CD34 yields and post-transplant count recovery days of hospitalization and fever were fewer with G-CSF alone with fewer	[79]

chemo, chemotherapy; di-CEP, cyclophosphamide, etoposide and cisplatin; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor.

The optimal doses of G-CSF have been investigated in a number of studies. In a randomized controlled trial, patients received either 8 or 16 μg/kg/day G-CSF following standard mobilization chemotherapy. With the higher dose of G-CSF, the $CD34^+$ PBPC yield was significantly increased more than threefold and the time to neutrophil engraftment was significantly decreased [84]. A small dose escalation study by Lefrere compared G-CSF doses of 50, 75, 100, 125, and 10 $\mu g/m^2$ along with chemotherapy and found they achieved adequate CD34 collections in 84 of 96 of patients suggesting that lower doses of G-CSF may be sufficient for the great majority of patients [85]. Efforts have been made to determine an optimal CY mobilization dose as well. Intermediate dose CY, 4 g/m^2, has been used with G-CSF to collect PBPCs. Several studies have shown that a higher dose, 7 g/m^2, results in higher PBPC yield but also leads to increased toxicity without a significant reduction in time to engraftment and no overall improvement in engraftment or eventual disease outcome [86]. A retrospective study by Gertz indicated that while cyclophosphamide-induced mobilization led to a higher yield of CD34 cells/kg than did G-CSF alone, patients actually required a longer time to recover platelet counts of >50,000/μL, suggesting an impact of intermediate-dose CY (3 gm/m^2) on the marrow microenvironment [87]. In patients who failed mobilization with CY, high-dose cytarabine, or the combination of etoposide plus high-dose cyclophosphamide have been used with good results [88,89].

Optimal timing of G-CSF administration after mobilization chemotherapy is also uncertain. In one study, G-CSF doses ranging from 250 μg/day as a fixed dose up to 10 μg/kg/day were administered 4 or more days following completion of chemotherapy and led to adequate ($>2 \times 10^6$) or optimal ($>5 \times 10^6$) CD34 cells/kg in the majority of patients [90]. It also appears that among the various preparations of G-CSF that are available, there is no significant advantage for one preparation (filgrastim, lenograstim, or molgramostim) over another [73].

Because no single mobilization chemotherapy regimen is clearly superior to the others, another mobilization approach is to incorporate PBPC mobilization into a cycle of disease-specific chemotherapy. In patients with hematologic malignancies, regimens containing ifosfamide, carboplatin and etoposide (ICE), have been used successfully for concurrent tumor cytoreduction and mobilization chemotherapy [91]. Other standard regimens like DHAP [92], have also been used to mobilize and collect CD34 cells. It is also important to note that the addition of the anti-CD20 monoclonal antibody, rituximab, prior to CD34 mobilization and collection does not decrease CD34 yield [93,94] and may reduce contaminating tumor cells in the CD34 product.

Factors affecting yield of PBPC mobilization

It is important to note that most patients mobilize adequate numbers of $CD34^+$ cells using a regimen of G-CSF alone. Although the addition of chemotherapy improves $CD34^+$ yield, this comes at the expense of increased short-term toxicity and, possibly, the increased risk of secondary myelodysplastic syndrome [80]. Even with chemotherapy–growth factor combination regimens, it may be difficult to achieve an adequate $CD34^+$ cell yield in some patients. Most cancer patients undergoing mobilization therapy for PBPC harvest to support high-dose chemotherapy have been treated extensively with chemotherapeutic agents and may have malignancy involving their bone marrow. These factors impair the ability to mobilize PBPCs. Even in normal, previously untreated donors, mobilization with G-CSF results in a wide variability of $CD34^+$ cell doses with 5–15% of donors mobilizing a suboptimal yield of less than $2.5–5 \times 10^6$ $CD34^+$ cells/kg [95]. This variability makes predicting how well any one patient will mobilize quite difficult. However, several studies have identified predictors of poor PBPC yield. The amount of myelosuppressive therapy (both chemotherapy and radiation therapy) received prior to mobilization is the most important factor affecting $CD34^+$ yield [96,97]. In particular, stem cell toxic agents including nitrogen mustard, chlorambucil, procarbazine, melphalan, carmustine, and >7.5 g CY were strongly associated with poor mobilization as is the prolonged use of fludarabine, a purine analogue commonly used for treatment of follicular lymphoma and chronic lymphocytic leukemia [97,98]. The number of chemotherapeutic regimens and duration of exposure to chemotherapy (>12 months) [99] also predict poor mobilization, but shorter courses of alkylating agents do not necessarily preclude good CD34 cell mobilization [99,100]. The intensity of the most recent cytotoxic drug administration relative to mobilization and stem cell collection may also influence mobilization [101]. Finally, previous radiation, hypocellular marrow and refractory disease have been associated with poor mobilization [97]. Recent reports have also demonstrated that extensive prior lenalidomide (>4–6 cycles) can result in decreased mobilization capacity which may require the addition of chemotherapy or plerixafor as opposed to G-CSF alone to achieve a successful collection [19,49,81,83,84,102,103]. An especially important finding was recently reported by Ferraro and colleagues in which they described poor mobilization in a small cohort of patients who were diabetic and in mice made diabetic with streptozocin [104]. Given the prevalence of this disorder it will be critically important to assess the applicability of this finding to the large number of diabetic patients who would be candidates for high-dose therapy and autologous HCT.

Managing poorly mobilizing patients

Strategies to manage patients who do not mobilize well include the addition of harvested bone marrow, remobilization with a dose escalation of cytokines, or use of a more intensive or novel regimen (Table 40.4) [58,105–107]. In recent years, the inclusion of plerixafor either with G-CSF alone or in conjunction with G-CSF plus chemotherapy has significantly improved the yield of patients who had failed their initial mobilization [18–20,63–65,108–111]. One center has found that infusing a suboptimal PBPC product in combination with harvested bone marrow can achieve short- and long-term engraftment [106]. Others have used high-dose G-CSF at 16 and 32 μg/kg/day to successfully mobilize patients previously failing standard myelosuppressive chemotherapy plus G-CSF mobilization [107]. There will still be challenges for heavily treated patients and those for whom mobilization is unsuccessful even in

Table 40.4 Guidelines for the "hard to mobilize" patient [refs]

1. Add plerixafor at a dose of 0.24 mg/kg following G-CSF alone or G-CSF plus chemotherapy when circulating pre-apheresis CD34 count is low [63–67,108,109,111,115]
2. Increase chemotherapy dose: high-dose etoposide or 4–7 g/m^2 CY appears more effective than 2–4 g/m^2 but is more toxic [82,86,87]
3. Increase growth factor dose (up to 10–24 μg/kg G-CSF) and consider use of cytokine combinations such as G-CSF + GM-CSF [54,68]
4. Consider bone marrow harvest [106]
5. Prolong duration between last chemotherapy and planned collection
6. Use investigational agents such as defibrotide or BIO5192 [130,133]

BID, twice daily; CY, cyclophosphamide; G-CSF, granulocyte colony-stimulating factor; IL, interleukin.

the absence of identifiable risk factors, but the addition of plerixafor to initial therapy has significantly reduced the likelihood of poor mobilization [64–67,108].

While historical factors are often able to predict the likelihood of good or poor mobilization, an increasingly effective and widely used strategy is the monitoring of peripheral blood CD34 counts following either cytokine mobilization alone or following cytokines plus chemotherapy [47–49]. Whatever method is used to identify patients who are likely to provide a poor apheresis yield, there is no doubt that cost considerations are significant in regard to both the initial collection and subsequent efforts to re-mobilize patients [103,112]. In either case, it is increasingly common to include initiation of plerixafor if the circulating CD34 count is suboptimal on day 4 or 5 following initiation of G-CSF therapy (<10–20 CD34/μL of peripheral blood) or if the CD34 count is low following chemotherapy once the circulating blood counts reach a certain level [108,109,112–117]. Using an algorithm such as those proposed by Costa [113,114] or Attolica [115] allows the inclusion of plerixafor when it is needed ("just in time"), but not in those circumstances following either cytokine alone or cytokine plus chemotherapy-induced mobilization when the yield of CD34 cells is likely to be adequate without the addition of this new and effective, but costly agent.

Tumor contamination

Although PBPC products are less frequently contaminated with tumor cells than bone marrow, tumor cells have been detected in patients with lymphoma, multiple myeloma, and leukemia [118,119]. In a study that compared outcomes of patients with non-Hodgkin lymphoma receiving high-dose therapy followed by autologous transplantation, patients receiving tumor-free autografts had a significantly improved survival over those receiving minimally contaminated autografts, demonstrating the importance of decontaminating the PBPC product [118]. An important analysis of the IBMTR and EBMTR datasets was undertaken by Bierman [120] who reported that patients undergoing either purged or syngeneic donor transplants for non-Hodgkin lymphoma had a lower likelihood of relapse post-transplant than did patients undergoing unpurged transplants for this disease. While there was no improvement in overall survival in the purged group, this analysis nonetheless attests to the potential importance of tumor cell contamination in the infusion product as one cause for treatment failure following high-dose therapy for these patients. Several reports from the group in Milan using in vivo purging with rituximab for mantle cell lymphoma patients indicated that tumor cell polymerase chain reaction testing negative products could be obtained in the majority of patients and that this was associated with a durable remission of more than 50% at 5 years [121,122]. This is felt to be the principal reason why reports from several centers have suggested that long-term transplant results are improved for patients who receive rituximab prior to their autologous transplant for B-cell lymphomas [93,121,122].

In multiple myeloma patients, both positively selected CD34$^+$ Thy-1$^+$Lin$^-$ progenitor cells and B-cell lineage depleted PBPCs have been transplanted to support high-dose chemotherapy with elimination of detectable tumor contamination and good engraftment [119], but there has been no improvement in overall survival [123]. Although these techniques are promising, available results have not demonstrated a clear benefit as PBPCs are depleted in the selection process and overall survival has not been improved. These results suggest that while infused tumor cells may contribute to relapse following high-dose therapy and autologous HCT, the evidence for relapse following autologous, syngeneic, and allogeneic transplants all point to the need for more profound cytoreduction in patients as an equally if not more critical component of the development of curative strategies [120,122–124].

Future directions

Chemokines such as IL-8 and GRO-β induce both a rapid release of proteases (NE, CG, and MMP-9) and HSCs from the BM into the blood. This effect by IL-8, GRO-β and N-terminal truncated of GRO-β, SB-251353, currently in early clinical testing, occurs in 30 minutes to 9 hours in mouse and humans [24,25,125]. Other second-generation CXCR4 inhibitors are either in development or are in early stage clinical trials as both normal and leukemic stem cell mobilization-inducing agents [18–20].

While the majority of patients receiving conventional mobilization regimens do produce adequate PBPC products, a significant, and not always predictable, portion of the population does not. Patients at risk for poor mobilization yields represent a challenging clinical scenario requiring improved mobilization strategies. Higher cytokine and marrow suppressive chemotherapy doses can produce improved CD34$^+$ cell yields [55,77]; however, these higher yields do not always translate into a shorter time to engraftment. Cytokine combinations have also been used in heavily pretreated patients resulting in modestly increased CD34$^+$ PBPC yield [68]. Clearly, innovative approaches are needed to address these patients, excellent summaries of which are provided in the review of stem cell mobilization with agents other than G-CSF [18,19].

Novel methods of mobilizing PBPCs continue to be studied with a number of preclinical compounds under investigation (Table 40.5) [18,19,24,25,125–127]. Manipulation of the adhesive interactions between hematopoietic progenitor cells and the bone marrow stroma has much promise. Anti-integrin monoclonal antibodies have already been shown to increase circulating hematopoietic progenitor cells in non-human primates [127] and in mice [128]. Manipulation of the chemokine environment represents an exciting and potentially most readily available opportunity for improving mobilization. A truncated GRO-β chemokine agonist has been shown to induce a rapid mobilization of hematopoietic stem cells in mice and primates [24,25,125], as has IL-8 [18,19,25,129]. Preclinical and clinical investigations with compounds such as Flt3

Table 40.5 Preclinical mobilization studies

Preclinical compound	System	Observation	Ref.
GRO-β (SB-251353)	Mice/primates	Fivefold increase in CFU and CD34$^+$ cells relative to G-CSF alone and more primitive lineage negative cells than G-CSF alone	[24,125]
Flt3 ligand	Mice/primates	Marked increase in CFU or CD34$^+$ cells and synergy with G-CSF	[126,127]
Antibodies to LFA-1/Mac integrins	Mice	2.7-fold increase in cobblestone and CFU/mL	[128]
Small molecule inhibitor of VLA-4	Mice	30-fold increase which was additive with G-CSF and plerixafor	[133]
IL-8	Mice/primates	Increase in CFU/mL	[25,129]
IL-17	Mice	Increase in CFU/mL	[132]

CFU, colony-forming unit; G-CSF, granulocyte colony-stimulating factor; IL, interleukin.

Table 40.6 Recommendations for "standard" mobilization regimens

Regimen	Advantages	Disadvantages	Refs
G-CSF: 10–24μg/kg/day by QD or BID schedule	Adequate yield in most patients Low toxicity Reliable scheduling Low risk of MDS Appropriate for use in allogeneic patients	Lower yield than G-CSF + chemo	[45,47,53,54,56]
GM-CSF followed by G-CSF	Higher CD34+ cell yield than G-CSF alone Appropriate to consider for use in allogeneic patients	More expensive than G-CSF alone No proven benefit over G-CSF alone in allogeneic or autologous patients	[54,68]
Chemotherapy + G-CSF: CY 2–4g/m2 High-dose CY or VP16	Higher CD34+ cell yield than cytokine alone Enhanced cytoreduction pretransplant High CD34 yields	Greater toxicity Increased risk of MDS/AML post-transplant Uncertain scheduling; risk of MDS, mucositis, infection	[82,84,86,87]
Mid-dose VP-16	High CD34 yields and modest toxicity	10% risk of hospitalization	[83]
Standard chemotherapy regimen	Continued successful cytoreduction	Inappropriate for allogeneic patients	[91,92]
Add plerixafor following G-CSF or G-CSF + chemotherapy if CD34 count low on day of planned apheresis	Increased yield of CD34 cells and reduction in the frequency of aphereses required. More reliable scheduling than chemo + cytokine	Added expense if adequate yields obtained without the addition of plerixafor	[67–83,102,103,108, 110,111,113–117]

AML, acute myeloid leukemia; BID, twice daily; chemo, chemotherapy; CY, cyclophosphamide; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; MDS, myelodysplastic syndrome; VP16, etoposide.

ligand [126,127], defibrotide [130], human growth hormone [131], and IL-17 [132] and a small molecule inhibitor of VLA-4 [133], also hold promise but require additional study prior to clinical use. How best to integrate these newer cytokines and chemokines designed to manipulate the progenitor cell–bone marrow stroma interactions and whether these can provide a synergistic approach to PBPC mobilization remains to be determined. Finally, consensus groups such as the SIDEM and GITMO should be applauded in their efforts to provide useful guidelines for the practice of stem cell mobilization and apheresis as well as highlighting needed areas for research in the field [47].

Summary

The use of high-dose therapy and autologous PBPC-supported transplantation has become an essential component of contemporary therapy for hematologic malignancies. The majority of patients will undergo successful mobilization and collection of sufficient PBPC with standard approaches to permit safe and effective autologous transplant procedures (Table 40.6). Despite these advances in PBPC acquisition, a significant minority of patients will have inadequate yields of PBPC and will continue to be the focus of research efforts designed to understand better the mechanisms of PBPC mobilization and to identify improved methods for their collection and administration.

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CHAPTER 41

Peripheral Blood Hematopoietic Cells for Allogeneic Transplantation

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Introduction

The existence of hematopoietic cells (HCs) in the peripheral blood (PB) was postulated in 1909 by Alexander Maximow [1]. In 1962, Goodman and Hodgson were first to prove that circulating HCs were capable of reversing irradiation-induced marrow aplasia in mice [2]. The first clinical transplantation was published in 1980 by Abrams et al., who transfused large numbers of syngeneic blood leukocytes to a patient with Ewing's sarcoma [3]. In 1985 and 1986, four different teams reported successful hematopoietic reconstitution by autologous blood-derived HCs in cancer patients [4–7]. Although these latter case reports provided the long-sought proof of principle, the low concentration of HCs in unmanipulated PB remained a major obstacle to the wider use of clinical transplants from PB, especially in the allogeneic setting where the exposure of healthy individuals to mobilizing cytotoxic drugs was not possible.

Only with the advent of granulocyte colony-stimulating factor (G-CSF) and the discovery that this and other cytokines were able to substantially increase the concentration of CD34$^+$ cells in PB [8,9] autologous and, 5–6 years later, allogeneic PBHC transplantation (PBHCT) began to enter the clinic. With regard to PBHCT, in 1989 Kessinger et al. showed that T-cell-depleted blood HCs were able to restore hematopoiesis after myeloablative therapy [10]. Because of fears that the high numbers of T lymphocytes contained in PBHC harvests would induce severe graft-versus-host disease (GVHD) in most recipients, allogeneic PBHCT replete of T cells was first used in emergency situations such as the failure of a previous marrow graft [11] or the inability of the donor to undergo general anesthesia for marrow collection [12]. After pilot studies using unmanipulated PB from human leukocyte antigen (HLA)-identical sibling donors were published in 1995 [13–15], a surge of allogeneic PBHCTs ensued. Currently, more than 70% of all allogeneic transplants in Europe and worldwide are performed with PB instead of marrow [16].

The donors

When allogeneic PBHCT entered the clinical arena, the experience with healthy individuals donating HCs mobilized into the blood was limited. The major issues that had to be addressed were the following:

1. How could HCs be harvested most effectively?
2. Did harvest products from the PB contain stem cells in quantities and quality that would allow for timely, complete, and durable engraftment of allogeneic lymphohematopoiesis?
3. What were the acute side-effects and long-term sequelae of the mobilization and collection procedure?

For further information regarding donors for HCT see also Chapter 39.

Mobilization and collection of HCs from PB

The first attempts to "mobilize" HCs into the PB of normal donors followed the experience gained with granulocyte transfusions, and with the mobilization of autologous HCs in patients with lymphoma and other diseases where the stem cell pool was not compromised by marrow infiltration or extensive cytotoxic therapy. G-CSF at doses between 5 and 16 μg/kg/day was administered to the potential donor for 5–7 days. Such treatment allowed the collection of CD34$^+$ cell numbers ranging from around 2 to more than 20×10^6/kg recipient body weight in most instances. Infusion of the harvest products into recipients with leukemia or lymphoma after myeloablative therapy resulted in reliable and surprisingly fast engraftment.

Tens of thousands of healthy individuals have now undergone mobilization and collection of PBHCs using a variety of harvest protocols. A joint analysis from the International Bone Marrow Transplant Registry (IBMTR) and the European Group for Blood and Marrow Transplantation (EBMT) [17] summarized the experience with 1488 donations of PBHCs from HLA-identical siblings (n = 1322), other relatives (n = 149), or unrelated donors (n = 15). Nearly all donors had received G-CSF (filgrastim, lenograstim) for mobilization. Approximately 40% of the donors had undergone a single apheresis, 45% underwent two, 11% underwent three, and 5% had to undergo four or more leukaphereses to collect the number of HCs deemed necessary for engraftment by the investigator. A single-center study reported on 400 related and unrelated donors who had received 10 μg/kg/day subcutaneously of glycosylated or non-glycosylated G-CSF once daily for 4 consecutive days, with apheresis commenced on day 5 [18]. The target cell number of 4×10^6 CD34$^+$ cells/kg was reached after one (63%) or two aphereses in 81% of donors. Most other donors collected between 2 and 4×10^6 CD34$^+$ cells/kg, while only 11 donors (<3%) collected less than 2×10^6 CD34$^+$ cells/kg with a maximum of two aphereses. Hoelig et al. reported on 3928 unrelated donors who had undergone stem cell harvesting in Dresden, Germany [19]. Of the donors, 97.3 % had received 7.5 μg/kg of lenograstim for 5 to 6 consecutive days. With

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this schedule 99.5 % of donors collected enough CD34$^+$ cells (median 5.88 × 10^8 CD34$^+$ cells) in 1 or 2 leukaphereses. In female donors, a second leukapheresis had to be performed in 37.4 % of cases as opposed to 15.9 % of all collections in men.

Based on these and similar data, G-CSF at a dose of 7.5 to10 μg/kg given for 4 or 5 consecutive days is considered the standard regimen for mobilization of allogeneic HCs, although other G-CSF doses as low as 2 μg/kg or as high as 40 μg/kg given once or twice daily have been used for mobilization, with a clear trend towards higher CD34$^+$ cell numbers being collected after administration of higher doses of growth factor. Commencing leukapheresis on day 4 or 5 with any commercially available continuous flow blood cell separator will allow the collection of adequate numbers of HCs in nearly all healthy individuals with one or two daily collections if two to four times the donor's blood volume is processed. CD34$^+$ concentrations in PB decrease when G-CSF is continued for 7 days and beyond. Therefore, adequate timing of apheresis and effective collection procedures are key to achieve optimum harvest products. Other variables such as age, sex, body weight or baseline CD34$^+$ cell concentration in the blood of the donor have also been described to correlate with the stem cell yield, but these findings remain controversial, and none of these parameters will allow precise prediction of the stem cell yield.

More recently, pegfilgrastim, a covalently bound conjugate of filgrastim and monomethoxypolyethylene glycol with a significantly longer elimination half-life than unconjugated G-CSF, at a single dose of 12 mg, has been used to mobilize allogeneic PBHCs in healthy family and unrelated donors [20]. Adequate numbers of HCs for transplantation could be harvested from healthy donors mostly by a single apheresis. However, bone pain and headaches appeared to be more severe after injection of pegfilgrastim. Additional concerns were caused by the hyperleukocytosis and the spleen enlargement seen after administration of pegfilgrastim. Further investigations would therefore be needed before pegfilgrastim can be recommended for mobilization of HCs.

Growth factors other than G-CSF or its variants, mostly granulocyte–macrophage colony-stimulating factor in conjunction with G-CSF, have occasionally been used for mobilization in healthy individuals, with no obvious advantages over the administration of G-CSF alone. Interleukin-3 (IL-3) [21], stem cell factor [22], and other experimental cytokines like fms-related tyrosine kinase-3 (Flt3) ligand [23] have been shown to increase the CD34$^+$ cell concentration in the PB of rodents, primates or humans, but have never been used clinically because of their side-effects, which would put healthy volunteers at unnecessary and potentially severe risk.

Plerixafor is an inhibitor of chemokine (CXC) receptor-4/stromal cell-derived factor-1α (CXCR4/SDF-1α) interaction that mobilizes CD34$^+$ cells into PB. In normal volunteers, a single injection of plerixafor after 4–5 days of G-CSF resulted in a 3–3.5-fold increase of circulating CD34$^+$ cells. This agent has been successfully used in the autologous setting [24] but awaits further study in healthy donors.

The increasing use of non-myeloablative preparatory regimens, and of unrelated, mismatched and haploidentical donors, will call for high CD34$^+$ cell numbers to safeguard against the increased risk of graft failure associated with any of these procedures. It is for these reasons that the search for the optimum mobilization and collection procedure is ongoing.

Characteristics and dose of HCs from PB or marrow

As the human hematopoietic stem cell itself is still elusive, the precise answer to the question of how many hematopoietic stem cells are contained in a typical PB collection product awaits further study. It is, however, possible to describe the quality and quantity of committed and more immature HCs as they appear in G-CSF mobilized PB, and to compare the findings with marrow-derived HCs. This comparison would indicate that primitive and committed HCs are present in mobilized PB at least as frequently as in a typical bone marrow (BM) harvest. Using the CD34$^+$ antigen, which can easily be detected by flow cytometry, as a surrogate marker for the human stem cell, pilot studies found that G-CSF mobilized PB would contain two to five times more HCs than BM. This has been confirmed by the results of the large randomized trials [25–28].

What is the minimum CD34$^+$ cell number to guarantee quick and durable engraftment? And should a certain number of CD34$^+$ cells not be exceeded? A definite answer to both questions is not possible. However, it seems reasonable to recommend the collection of at least 2–4 × 10^6 CD34$^+$ cells/kg recipient weight, because clinical experience indicates that the risk of graft failure is increased if less than this threshold number is transplanted. It is also difficult to tell if there should be an upper limit for CD34$^+$ cell numbers and if higher numbers would be harmful if transferred to the recipient. Animal experiments [29] and clinical data indicate that the desirable number of HCs needed to ensure engraftment will depend on a number of interdependent parameters, the most important of which are the immunosuppressive potential of the conditioning regimen, the HLA disparity between donor and recipient, the number of T cells infused, and the regimen used for GVHD prophylaxis.

For a "standard" transplant from an HLA-identical sibling donor after myeloablative conditioning, doses between 4 and 10 × 10^6 CD34$^+$ cells/kg may be optimal, while other situations, for example haploidentical transplantation, will need much higher numbers of CD34$^+$ cells to secure engraftment. Higher CD34$^+$ cell numbers have generally been recommended because a significant correlation with improved survival has been demonstrated [30,31]. It probably is less important if high CD34$^+$ cell numbers come from BM or PB, although one study reported that rich marrow (containing >2.7 × 10^8/kg nucleated cells) may result in superior outcome compared with mobilized PB [32]. On the other hand, because PBHC collection products containing high CD34$^+$ cell numbers also contain more T cells, it has been postulated not to use transplants from PB with very high CD34$^+$ cell numbers. Przepiorka et al. evaluated risk factors for acute GVHD after allogeneic PBHCT and found a sharp increase of GVHD if more than 6.3–10.0 × 10^6 CD34$^+$ cells/kg were transplanted [33]. It will be difficult, however, to confirm this finding with prospective data because such high numbers of marrow or PBHCs are harvested from a small fraction of donors only.

A number of investigators have looked at immunophenotypically, genotypically, or functionally characterized subtypes of HCs in more detail. Körbling et al. reported that G-CSF at a dose of 12 μg/kg/day increased the concentrations of PB CD34$^+$ cells and more primitive subsets such as CD34$^+$Thy1^{dim} and CD34$^+$Thy1^{dim}CD38$^-$ cells by 16.3-, 24.2-, and 23.2-fold, respectively. The mean apheresis yield of CD34$^+$Thy1^{dim} and CD34$^+$Thy1^{dim}CD38$^-$ cells per kilogram of recipient body weight and per liter of donor blood processed was 48.9 × 10^4 and 27.2 × 10^4, respectively, indicating an additional "peripheralization" effect of G-CSF on primitive CD34$^+$ cell subsets [34,35].

Other reports confirmed these findings but also showed that the immunophenotypic profiles of HCs from blood or marrow differed in several respects (Table 41.1) (reviewed in [36]). The low expression of CD71 and the decreased retention of rhodamine-123 in CD34$^+$ cells from mobilized PB suggest that these cells are not actively proliferating or metabolically active [37]. Kinetic data corroborate the phenotypic findings, further demonstrating that

Table 41.1 Characteristics of $CD34^+$ hematopoietic cells (HCs) from bone marrow (BM) or mobilized peripheral blood (PB)

Characteristics	BM	Mobilized PB
Phenotype		
$CD13^+$, $CD33^+$	Standard	Higher
$CD38^-$	Standard	Higher
$HLA\text{-}DR^-$	Standard	Higher
$CD10^+$ $CD19^+$	Standard	Less
$CD7^+$	Standard	Less
c-kit	Standard	Less
VLA-4	Standard	Less
Metabolic activity		
$CD71^+$	High	Low
Rhodamine	High	Low retention
Clonogenicity		
Colony formation	Normal	Increased
LTC-IC	Normal	Increased
Cycling status	Active	Non-active
Genotype cell cycle progression	Standard	Less
DNA synthesis	Standard	Less
GATA 2		Increased
N-*myc*		Increased
Apoptotic activity		Increased

LTC-IC, long-term culture-initiating cells.

relatively more $CD34^+$ cells from PB exhibit characteristics of quiescent cells [38].

Steidl et al. [39] reported on the molecular phenotype of BM-derived and circulating $CD34^+$ cells from mobilized PB. Gene expression profiling confirmed that $CD34^+$ cells from BM cycle more rapidly, whereas $CD34^+$ cells from PB include more quiescent stem and progenitor cells.

Prosper et al. [40] investigated the number of long-term culture-initiating cells (LTC-ICs) in the PB of normal donors treated with G-CSF (7.5–10 μg/kg/day) and found that $CD34^+CD38^+$ cells and $CD34^+HLA\text{-}DR^+$ cells sorted from mobilized PB contained 0.5–5% of cells capable of sustaining hematopoiesis in long-term culture for up to 5 weeks. This is at least five times more than the number of LTC-ICs found in steady-state BM or non-mobilized PBHCs. However, 90–95% of the LTC-ICs present in $CD34^+CD38^+$ or $CD34^+HLA\text{-}DR^+$ fractions of mobilized PB were not able to sustain hematopoiesis for 8 weeks, as is the case with close to one-third of $CD34^+CD38^+$ and $CD34^+HLA\text{-}DR^+$ cells from non-mobilized PB. Plerixafor may mobilize more primitive precursors than G-CSF in normal donors; further differences in the cell content of collection products mobilized by G-CSF, plerixafor, or both, have been described [24]. It is not clear if these differences may influence clinical outcomes of patients transplanted with such cells.

Taken together, phenotypic, genotypic, and functional analyses of HCs from BM or G-CSF mobilized PB demonstrate that stem and progenitor cells are contained in both compartments. HCs from PB may be enriched in LTC-ICs and other clonogenic cells, they are metabolically less active, cycle less frequently, and show increased apoptotic activity. Stem cell biology including the differences of $CD34^+$ cells from mobilized PB or BM remains an area of active research because a better understanding of basic phenotypes and function is paramount to optimize the peripheralization, harvesting, and clinical use of HCs.

T lymphocytes and other immune cells in mobilized blood

There are many differences in the composition of PBHC harvests and marrow grafts if cells other than HCs are considered. Most importantly, PB harvests contain up to 10 times more T cells than BM; the ratio of $CD4^+$: $CD8^+$ lymphocytes, however, is identical. This had already been noticed with autologous PBHC harvests, and was the most important reason why investigators initially accepted allogeneic transplants with PB as an alternative to BM only hesitantly. To some extent, the numbers of T cells contained in PBHC harvests relative to the number of $CD34^+$ cells seem to depend on the mobilization protocol and the day of apheresis. Leukapheresis on day 5 after administration of G-CSF should give the highest stem cell yield with relatively low numbers of T cells contained in the final product. Plerixafor alone or in combination with G-CSF may be used to collect HCs from normal donors in the future but will require new schedules for stem cell harvesting to be developed in order to fully exploit the benefits such alternative collection methods may offer to donors and patients [24].

The observation that the high number of T cells in PB harvests did not lead to a dramatic increase in the incidence and severity of GVHD in patients allografted with mobilized PB prompted researchers to investigate the effects of G-CSF administration on the number and function of immune cells in more detail. With regard to T cells, Pan et al. [41] were first to show that pretreatment of healthy individuals with G-CSF polarizes T cells towards the production of type 2 cytokines (IL-4 and IL-10), while type 1 cytokine production (interferon-gamma and IL-2) is reduced. Other effects potentially explaining the unexpectedly low incidence of severe GVHD after transplantation of allogeneic PBHCs are the downregulation of allogeneic immune responses by post-transcriptional inhibition of tumor necrosis factor-alpha production [42], and the comobilization of $CD4^+$ and CD 8^+ T cells. Kusnierz-Glaz et al. [43] demonstrated that $CD4^+CD8^+$ αβ T cells are markedly enriched in the leukapheresis products of G-CSF-treated normal donors, and that these cells, further enriched in low-density fractions of mobilized PB, significantly suppress the mixed lymphocyte reaction. Regulatory T cells are thought to combat GVHD; studies with G-CSF mobilized PB showed upregulation of genes related to T helper type 2 (Th2) cells but also to T reg (Treg) cells [44].

Mielcarek et al. [45] calculated that the number of $CD14^+$ cells in mobilized PB was 50-fold greater than in marrow. These large numbers of monocytes suppress T-cell proliferative responses to alloantigen by increased IL-10 production and decreased secretion of IL-12 and tumor necrosis factor-alpha. Arpinati et al. demonstrated that the number of Th2 cell-inducing dendritic cells (pre-DC2s) was increased in mobilized PB. As pre-DC2s lead to polarization of naïve $CD4^+$ T cells toward a Th2 phenotype, this finding also may explain why transplantation of allogeneic PBHC does not lead to more severe GVHD in a larger fraction of patients [46].

Franzke et al. [47] showed that T cells do express G-CSF receptors after G-CSF treatment, and G-CSF can thus directly modulate T-cell immune responses. Among other possible mechanisms, G-CSF seems to diminish T-cell receptor-mediated activation and proliferation, downregulate leukocyte function associated antigen-1 alpha (LFA-1α) thus affecting the homing process of the graft and abrogating donor T-cell activation to host antigens, and upregulate GATA-binding protein-3 (GATA-3), the master regulatory factor for induction of a T helper immune response.

Natural killer cells are five- to 10-fold more frequent in mobilized PB than in BM. Joshi and colleagues analyzed peripheral blood mononuclear cells from G-CSF-mobilized blood cell harvests for their immunologic function and compared them with peripheral blood mononuclear cells from steady-state non-mobilized donors [48]. They found a significant decrease in NK- and lymphokine-activated killer cell-mediated cytotoxicity for G-CSF-mobilized effector cells. They also reported a significant decrease in both

T-cell and B-cell mitogen responses with G-CSF-mobilized versus non-mobilized cells. Neutrophils not only increase in number after administration of G-CSF, but their function also is enhanced immediately after G-CSF administration, as shown by upregulation of Fcγ RIII receptors, cell surface CD11b and CD66 molecules, and elevated levels of plasma elastase [49]. It is not finally clear which of the mechanisms described contributes to which extent to the striking observation that the infusion of 1 to 2 log more T cells into the recipient does not result in the development of severe GVHD in more patients grafted with PB.

Side-effects and long-term sequelae of mobilization and collection of HCs

The overall short- and long-term effects of G-CSF have repeatedly been reviewed. The acute toxicities of G-CSF administration have been well characterized, and comprise bone pain, headache, and fatigue as the most frequent side-effects. However, myalgias, chest pain, anxiety, insomnia, night sweats, fever, anorexia, weight gain, nausea and/or vomiting, and local reactions at the injection site have also been described [18,50]. Side-effects have been mild to moderate in the vast majority of donors and usually resolve within 48 hours of discontinuation of G-CSF. They can be treated successfully by the administration of minor analgesics (acetaminophen or ibuprofen) in most instances, and these side-effects have not been reported to require cessation of G-CSF administration or cancellation of the PBHC harvest. There is some indication that the frequency and severity of side-effects of G-CSF is dose dependent [51]. Therefore, doses as low as possible should be used.

A number of side-effects of G-CSF administration and the harvest procedure on blood chemistry, the coagulation system, and baseline hematologic parameters already described in patients with hematologic malignancies also occur in normal individuals. Laboratory abnormalities caused by G-CSF include transient increases of lactate dehydrogenase and alkaline phosphatase, uric acid, alanine aminotransferase, and/or glutamyl transpeptidase, and a decrease in serum potassium and/or magnesium [52].

Several authors have shown that G-CSF can induce a mild hypercoagulation state, as indicated by in vitro testing. Increases in fibrinogen and factor VIII levels, a reduction in protein C and S levels [53], and increases in prothrombin fragments, thrombin–antithrombin complexes, D-dimers, and platelet aggregation have all been reported [54–56]. Mild-to-moderate decrements in platelet, lymphocyte, and granulocyte levels have been described following the collection of PBHCs [57]. Thrombocytopenia may partly be due to the administration of G-CSF itself or the leukapheresis procedure. Cytopenias are usually mild and self-limited, although more profound thrombocytopenia following repeated large-volume leukaphereses has been reported.

A number of studies have been presented that compare the experience of donors who participated in one of the randomized trials comparing BM transplantation (BMT) with PBHCT [58–63]. A Cochrane review [64] summarized the findings of six randomized trials comparing the tolerability and safety of hematopoietic stem cell donation from blood or marrow. It was reassuring that none of these reports described life-threatening complications or long-lasting discomfort in any of the donors. The study reports generally confirm that the side-effects of PBHC harvesting are related mainly to G-CSF administration, while the side-effects of BM harvesting are caused by the harvest procedure itself. The incidence of adverse events occurring in 5% or more of donors of PB or BM who participated in the large European trial are presented in Table 42.2.

Table 41.2 Subject incidence of adverse events occurring in >5% of donors. Date from the EBMT study comparing bone marrow transplantation (BMT) with peripheral blood progenitor cell transplantation (PBPCT) [28]

	BM donors (n = 166)	PBHC donors (n = 164)
Any adverse event	95 (57%)	107 (65%)
Harvesting procedure-related adverse events		
Any harvest-related	91 (55%)	61 (37%)
Access pain	39 (23%)	2 (1%)
Anemia	17 (10%)	0 (0%)
Back pain	16 (10%)	4 (2%)
Nausea	10 (6%)	1 (1%)
Arthralgia	8 (5%)	1 (1%)
Vomiting	8 (5%)	0 (0%)
Skeletal pain	3 (2%)	10 (6%)
Filgrastim-related adverse events		
Any filgrastim-related		96 (59%)
Musculoskeletal		71 (43%)
Headache		19 (12%)
LDH increased		14 (9%)
Alkaline phosphatase increased		8 (5%)

BM, bone marrow; LDH, lactate dehydrogenase; PBHC, peripheral blood hematopoietic cell.

Some of these studies also measured the emotional status and pain of donors prior to the harvest procedure and at defined time points after the collection of BM or PB. In general, the symptom burden, the levels of maximal and average pain, fatigue, and anxiety were similar, although symptom peaks occurred at different time points during G-CSF administration (in PB donors) or shortly after BM harvesting. A quicker resolution of symptoms in PBHC donors was also described [59,60]. Only the Scandinavian trial reported a significant difference in the total burden of complaints in the two groups of donors, favoring the PB donors. This study also found that donors would prefer to donate PB rather than BM if asked to donate again [61]. This observation is in line with reports from unrelated donors.

Serious or lethal complications of G-CSF administration and leukapheresis are rare. At least five cases of splenic rupture associated with the harvest procedure have been reported [65–69]. Therefore, special attention should be paid to donors presenting with an enlarged spleen or a history of splenic disorders. Routine abdominal ultrasound has been recommended to reduce splenic complications. Also, cases of severe cardiovascular events [70], acute lung injury [71], acute iritis [72], pyogenic infections [73], gouty arthritis [74], and anaphylactoid reactions [75] have been reported.

Such case reports, as well as concerns that G-CSF may perturb normal hematopoiesis and increase the risk for developing malignant disorders of the blood, including myelodysplasia and leukemia, caused investigators to propose an international donor registry many years ago. A number of reports on acute and long-term events in large cohorts of donors have been published [19,69,76,77]. Overall, acute and severe complications of PB stem cell donation are rare (<1%) and differences in long-term side effects after donation of BM and PB were not detected.

The report by Halter et al. [69] summarizes all deaths and severe complications of PB or marrow donations for 51,024 recipients receiving a first HCT. Data were generated retrospectively from questionnaires sent out to the principal investigators of 338 transplant centers who participated in the annual EBMT activity surveys. Any serious event occurring within 30 days after donation and the occurrence of any hematologic malignancy in a donor had to be registered. Overall, five donor deaths occurred (one after BM and four after PB donation; incidence 0.98 per 10,000 donations) and 37 severe complications (12 in BM and 25 in PB donors; $p < 0.02$) were reported. Nineteen hematologic malignancies (8 after BM and 11 after PB donation; $p = 0.4$) were reported. Three deaths (pulmonary embolism, subarachnoidal hematoma, and cardiac arrest) were clearly related to the donation, whereas two cardiac arrests on days 15 and 17 after PB donation may or may not have been related to the harvest procedure. The severe, potentially life-threatening complications mostly were cardiovascular events, hemorrhages, and splenic rupture. The hematologic malignancies reported were acute myeloid leukemia (three), acute lymphoblastic leukemia (three), lymphoma (seven), nasopharyngeal plasmacytoma, chronic lymphoblastic leukemia, and myeloproliferative syndrome (one case each), and other non-specified disorders.

In Chapter 37, Confer et al. summarize data available on deaths that occurred worldwide after donation of BM or PB. It is unclear how much overlap might exist between their cases and those reported by Halter et al. Also, it remains uncertain what number of donations formed the basis to Confer's report. In any case, these data, together with the reports from other registries, suggest that life-threatening and fatal accidents are rare but do occur in donors of PB or marrow stem cells. No significant differences between PB and marrow donors regarding the long-term consequences of both harvest methods have been described.

The leukapheresis itself is generally well tolerated, but side-effects caused by the anticoagulant and placement of central venous access have been reported. Although an effort should be made to avoid a central venous access, 2–7% of donors ultimately will need it because peripheral veins cannot be used or the necessary blood flow cannot be established [13–15]. Rarely, donors have experienced tetany or severe symptoms of hypocalcemia caused by the anticoagulant used throughout the leukapheresis procedure.

The recipients

In 1995, pilot studies from the United States and Europe [13–15] indicated that transplantation of allogeneic PBHCs resulted in fast and stable engraftment; GVHD did not seem to occur more frequently than after marrow grafting. Larger series from other transplant centers or cooperative groups, as well as retrospective comparisons with BMT confirmed that transplantation of allogeneic mobilized PB seemed a viable alternative to marrow transplantation.

The largest retrospective analysis presented by IBMTR and EBMT compared results of 288 PBHCTs with 536 BMT procedures from HLA-identical sibling donors [78]. The paper confirmed that recipients of PBHCs attained more rapid recovery of neutrophils and platelets; significant differences in relapse rates and the incidence of grade II–IV acute GVHD between marrow and PBHCT were not observed. The incidence of chronic GVHD at 1 year, however, was significantly higher after PBHCT (65%) than after BMT (53%). Treatment-related mortality was lower, and leukemia-free survival rates were higher with PBHCT in patients with advanced leukemias, but were comparable in standard-risk leukemias.

Randomized trials in leukemia patients with HLA-identical family donors

Between 1998 and 2002, the results of eight prospective randomized trials comparing the transplantation of allogeneic PBHCs with that of BM were published [25–28,79–82]. These studies were almost exclusively done in patients with leukemia, patients with other hematologic malignancies were included only rarely, and non-malignant disorders were not represented at all. The donors were HLA-identical siblings, and BM or PBHCs were infused without any manipulation. The trials differed substantially with respect to the number of patients included, the status of the diseases, the mostly myeloablative conditioning regimes used, the doses of HCs and T cells infused, the GVHD prophylaxis administered, and a number of other variables that may have influenced outcome. Still, the results of these trials allow a number of important conclusions, which are summarized later.

Engraftment

Transplantation of allogeneic PBHCs results in faster engraftment of HCs of all lineages compared with BMT. Neutrophils exceed a threshold of 0.5×10^9cells/L between 2 and 6 days earlier with PBHC than after BMT. In the United States trial, the neutrophils surpassed 0.5×10^9/L on day 16 (range 11–29 days) with PB, and on day 21 (range 13–36 days) with BM ($p < 0.001$) [25]. The Canadian study reported neutrophil recovery on day 19 (range 12–35 days) with PB, and on day 23 (range 13–68 days) with BM [27]. Patients in the EBMT study had the shortest time interval from transplantation to an absolute neutrophil count of greater than 0.5×10^9/L: 12 days for PB and 15 days for BM [28]. However, this trial was the only one of the large randomized studies that used G-CSF (5 μg/kg/day) also after transplantation, and day 11 methotrexate was not included. Other randomized trials proved that the administration of G-CSF at a dose of 10 μg/kg/day after PBHCT can accelerate neutrophil recovery by 3–4 days.

Platelet recovery after allogeneic PBHCT is also faster by approximately 6 days: a platelet count of 20×10^9/L was reached on day 13 (range 5–41 days) with PB, and on day 19 (range 7–74 days) with BM in the US study ($p < 0.001$). The Canadian study reported platelet recovery after 16 (range 0–100) days for patients grafted with PB, and 22 (range 0–100) days for patients grafted with BM ($p < 0.0001$). The EBMT study had a median time to platelet recovery greater than 20×10^9/L of 15 days for patients transplanted with PB, and 20 days for patients transplanted with BM ($p < 0.0001$). This difference translated into a median of 8 (range 1–68) days of platelet transfusions in the PBHCT group, and a median of 10 (range 2–71) days in the BMT group ($p = 0.0029$).

It is well recognized that factors other than the source of HCs influence the pace of hematologic recovery. While G-CSF accelerates neutrophil recovery, methotrexate if given for prophylaxis of GVHD retards hematopoietic reconstitution [83]. The occurrence of GVHD, infection, the grade of genetic disparity between recipient and donor, and splenomegaly have all been reported to affect the speed of hematopoietic recovery, and may explain the slight variations in times to neutrophil and platelet recovery seen in the randomized trials. Graft failures after PBHCT are less frequent than after BMT particularly in situations where the risk of graft failure is increased. Comparative studies of hematopoietic chimerism after PBHCT and BMT support the notion that transplantation of PB results in fast, complete, and durable complete chimerism.

There is evidence that mobilized PBHCs in comparison to BM require less vigorous conditioning of the host to ensure full lymphohematopoietic chimerism. This essential feature of PBHCs was the basis for the introduction of reduced-intensity conditioning PBHCT making HCT the only potentially curative procedure for many hematologic malignancies occurring in older and comorbid patients.

Immune recovery

Recovery of immunity after unmanipulated allogeneic PBHCT is at least as quick as after BMT, but may be faster with respect to some subgroups of cells with immune functions. Circulating naïve

($CD4^+CD45RA^+$) and memory ($CD4^+CD45RO^+$) helper T cells, B cells, and monocytes are found in higher numbers after PBHCT compared with BMT between 1 and 11 months post-transplant [84]. Natural killer cell numbers did not significantly differ between patients grafted with PB or marrow over the same time period. Proliferative responses to T- and B-cell mitogens (phytohemagglutinin, pokeweed mitogen, tetanus toxoid, and *Candida*) were significantly increased in recipients of PB from HLA-identical donors [84].

Storek et al. [85] studied 115 patients who had been randomly assigned to receive allogeneic marrow or mobilized PB from HLA-identical donors. Between 1 month and 1 year after transplantation, the counts of $CD4^+CD45RA^{hi}$ and $CD4^+CD45RA^{lo/-}$ T cells were significantly higher after PBHCT. T cells appeared equally functional when challenged by phytohemagglutinin or herpes virus antigen. Median serum immunoglobulin G levels were similar in both groups of patients. Of note, the authors also reported that particularly severe infections were significantly reduced after PBHCT. The reduced risk was most prominent for fungal infections, intermediate for bacterial infections, but low for viral infections. This latter phenomenon may be a consequence of the marked loss of memory T cells that occurs after allogeneic PBHCT [86].

Another interesting observation published by Lapierre et al. [87] was that, 30 days after PBHCT, anti-A and/or anti-B blood group titers were significantly higher after PBHCT compared with BMT, suggesting that immune hematologic reconstitution after PBHCT is quicker than after BMT. This observation could explain the cases of acute hemolysis described after ABO-incompatible PBHCT. On the other hand, transfusion requirements are no different in ABO-, rhesus- or Kell-different transplantation; however, ABO-mismatched patients were reported to show poorer survival than matched patients after PBHCT.

Acute GVHD

The incidences of acute GVHD grades II–IV were similar for both groups of patients in all larger studies except for the largest one [28], which noted a 13% increase of grade II–IV acute GVHD ($p = 0.013$) and a 12% increase of grade III–IV acute GVHD with PB ($p = 0.0088$) (Table 41.3). The EBMT study also was the only study where the maximum grade of acute GVHD was the primary endpoint, and randomization was stratified for some of the known risk factors of GVHD. Other differences in the design of the EBMT study and the other trials were the inclusion of good-risk patients only, the omission of methotrexate on day 11, and the use of G-CSF post-transplant. Omission of day 11 methotrexate has been reported to predispose recipients to develop GVHD [83], although this finding was not supported by data from the international meta-analysis [88]. It also remains in question if the high numbers of T cells transferred with a typical PBHC graft are responsible for the higher incidence of acute GVHD. Although such a correlation between GVHD and T-cell numbers was detected in the EBMT study, another study failed to demonstrate such a correlation [89].

Chronic GVHD

The large randomized studies all showed at least a trend to more chronic GVHD with PBHCs, and two studies reported statistically significantly more chronic GVHD after PBHCT [26,28] (Table 41.4). There was also more extensive chronic GVHD in patients having received PBHCs compared with BM cells. This difference was significant in the EBMT trial and in the studies chaired by Blaise et al. [26] and Vigorito et al. [82].

Follow-up reports from the United States [90], the French [91], and the EBMT [92] trials were first published with a median follow-up of 41, 45, and 36 months, respectively. Flowers et al. still did not observe more chronic GVHD after PBHCT, and the clinical characteristics of chronic GVHD were similar after PBHCT or BMT [90]. However, the study showed that chronic GVHD was more difficult to control when it occurred after PBHCT compared with BMT: the number of successive treatments needed to control chronic GVHD was higher after PBHCT, and the duration of glucocorticoid treatment was also longer ($p = 0.03$ for both endpoints). In contrast, Mohty et al. found a highly significant difference in the 3-year cumulative incidence of chronic GVHD in the PBHC group compared with the BM group (65% vs. 36%; $p = 0.004$) [91]. They also observed significantly more extensive chronic GVHD. Chronic GVHD needed further immunosuppression and led to longer periods of hospitalization. The 3-year follow-up of the prospective EBMT trial (Table 41.4) and the 5-year follow-up of the retrospective IBMTR/EBMT study also showed more chronic GVHD in patients after PBHCT [93]. Recently, the US study [94] and the EBMT study [95] have been updated again with median follow-ups of 12.2 and 10.8 years, respectively. Whereas the investigators from the United States still did not find a significant different in the cumulative incidence of chronic GVHD or the need for systemic

Table 41.3 Acute graft-versus-host disease (GVHD) grades II–IV

Reference	PB (%)	BM (%)	*p*-value
Bensinger *et al.* [25]	64	57	$p = 0.35$
Blaise *et al.* [26]	44	42	Not given
Couban *et al.* [27]	44	44	$p = 0.9$
Heldal *et al.* [79]	21	10	Not given
Powles *et al.* [81]	50	47	Not given
Schmitz *et al.* [28]	52	39	$p = 0.013$
Vigorito *et al.* [82]	27	19	$p = 0.53$

All percentages represent cumulative incidences at day +100 post-transplant except for the studies by Blaise *et al.* [26], Heldal *et al.* [79], and Powles *et al.* [81] where the actual percentage of patients with acute GVHD at day +100 is given.

Table 41.4 Chronic graft-versus host disease (GVHD)

First report				Follow-up report at 3 years			
Reference	**PB**	**BM**	***p*-value**	**Reference**	**PB**	**BM**	***p*-value**
Bensinger *et al.* [25]	(46%)	(35%)	0.54	Flowers *et al.* [90]	(63%)	(52%)	0.33
Blaise *et al.* [26]	(50%)	(28%)	<0.03	Mohty *et al.* [91]	65%	36%	0.004
Couban *et al.* [27]	85% (40%)	69% (30%)	0.62 (0.37)				
Schmitz *et al.* [28]	(67%)	(54%)	0.0066	Schmitz *et al.* [92]	73% (36%)	55% (19%)	0.003

% overall chronic GVHD; (% in brackets), extensive chronic GVHD.
BM, bone marrow; PB, peripheral blood.

immunosuppression, the EBMT study again reported more chronic GVHD necessitating prolonged immunosuppression in patients transplanted with PBHCs. In both studies, however, overall survival of patients grafted with BM or PM did not significantly differ.

Relapse rates

Knowing that the graft-versus-leukemia effect is exerted mainly by donor T cells and that around 10 times more T cells are transferred with a typical PB collection product, it was anticipated that transplantation of PB cells would result in a reduction of relapse rates after PBHCT. Animal studies [96] and results of non-randomized studies also pointed in that direction. The randomized studies have not consistently confirmed these expectations so far, although the updated US study [94] and the small British study [81] reported significantly fewer patients with relapse after PBHCT than after BMT.

Transplant-related mortality

Retrospective analyses had suggested a trend in favor of lower transplant-related mortality (TRM) after PBHCT. Specifically, the EBMT/IBMTR study showed a significant advantage in TRM for patients transplanted with PB for advanced leukemias [78]. The randomized US study reported a cumulative incidence of transplantation-related deaths of 21% in the PB group and 30% in the BM group; this difference, however, was not significant [25]. The Canadian study also found a reduction in non-relapse deaths after PBHCT as opposed to BMT. At day 100 after transplantation, the actuarial probabilities of death were 7.4% for the PB group and 16.1% for the BM group, respectively (p = 0.07) [27]. Until now, significant differences have not been reported from any of the randomized studies, and it remains to be determined if subgroups of patients with specific diseases or disease status benefit from the transplantation of PB because TRM is reduced.

Survival

Survival is the most relevant endpoint of studies comparing BMT with PBHCT. This is particularly true because different sources of HCs may have different effects on secondary endpoints such as TRM, relapse rate or GVHD, and the interplay of these may give different results for overall survival if different clinical situations are considered.

At first presentation, the three largest randomized studies came to somewhat different conclusions with respect to survival. The EBMT study had overlapping survival curves for patients grafted with PB or BM [28]. The US study reported a difference in overall survival of 12% at 2 years between the two groups of patients, favoring PBHCT; this difference was borderline significant (p = 0.06) [25]. The subgroup of patients with more advanced disease had a statistically significantly better survival (57%) with PB than with BM (33%) (p = 0.04). The Canadian study reported better overall survival for the whole group of patients transplanted with PB (68%) compared with the BMT group (60%) at 30 months (p = 0.04). With longer follow-up neither the US study nor the EBMT showed any significant difference in overall survival [94,95]. However, the US study still found superior disease-free survival after PBHCT.

Marrow or mobilized blood: the meta-analysis

An individual-patient data meta-analysis using data from nine randomized trials enrolling 1111 adult patients has been published [97]. The study confirmed that PBHCT results in faster neutrophil and platelet engraftment than BMT. PBHCT was shown to cause more grades III–IV acute GVHD, and more overall and extensive chronic GVHD (68% vs. 52% at 3 years; $p < 0.000001$) compared with BMT (Figure 41.1). PBHCT decreased relapse rates in both late-stage (33% vs. 51% at 3 years; $p = 0.02$) and early-stage (16% vs. 20% at 3 years; $p = 0.04$) disease. Non-relapse mortality was no different between PBHCT and BMT. Overall survival and disease-free survival were only significantly improved in patients with late-stage disease (overall survival 46% vs. 31% at 3 years; $p = 0.01$). The major results of the meta-analysis are depicted in Figure 41.2. The study also made clear that the vast majority of data (75%) comparing PBHCT with BMT were generated in patients with early disease (CML in chronic phase, AML and acute lymphoblastic leukemia in first complete remission, and early MDS) and that CML – a diagnosis hardly treated by allogeneic transplantation in first chronic phase

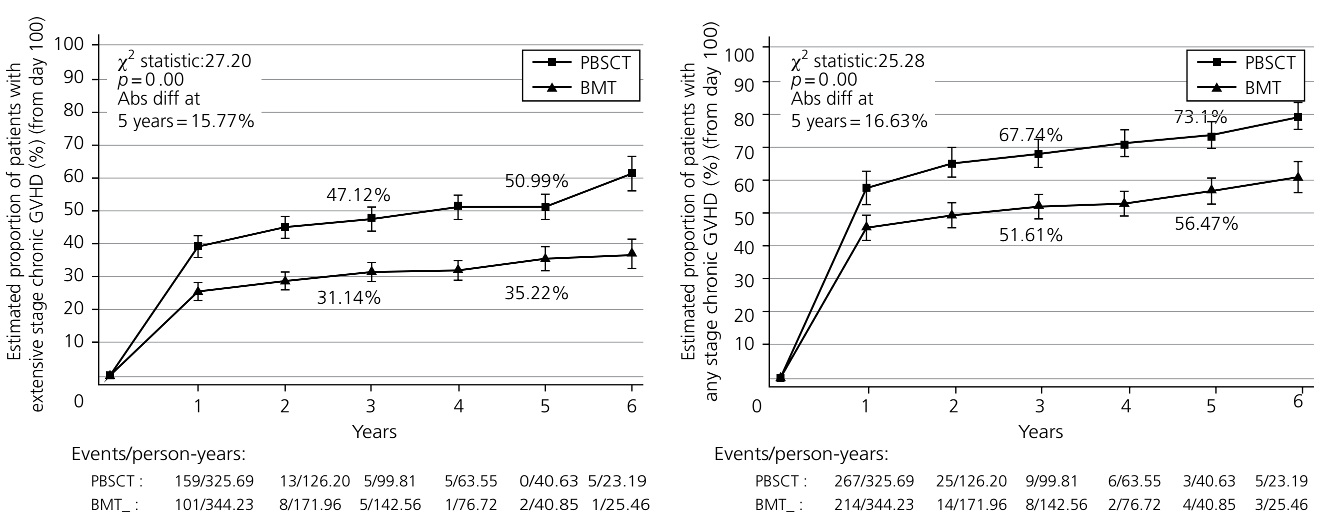

Figure 41.1 (a) Time-to-event plots showing the absolute risk for development of extensive stage of chronic graft-versus-host disease (GVHD) in patients with hematologic malignancies. (b) Time-to-event plots showing the absolute risk for development of any stage of chronic GVHD in patients with hematologic malignancies. Abs diff, absolute difference; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation. Source: Stem Cell Trialists' Collaborative Group 2005 [97].

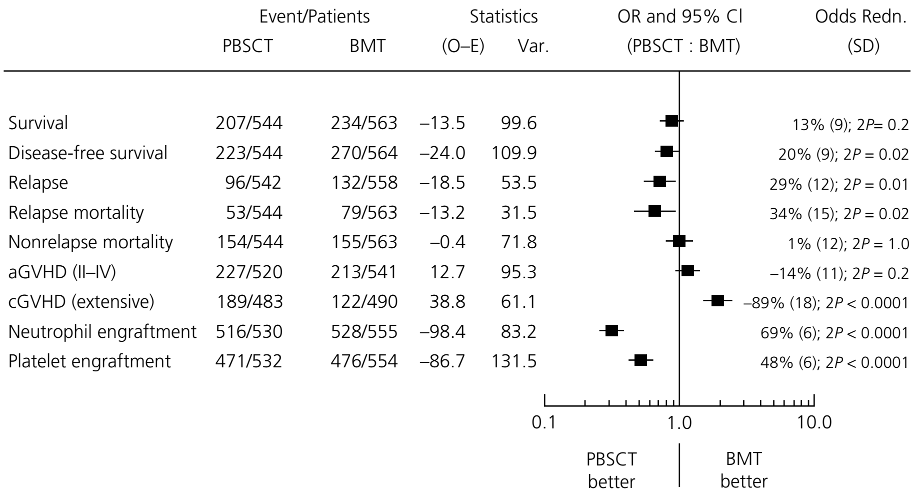

Figure 41.2 Summary forest plot showing the effects of allogeneic peripheral blood stem (hematopoietic) cell transplantation (PBSCT) versus bone marrow transplantation (BMT). If the square is to the left of the solid line, the odds ratio (OR) is better in the group receiving peripheral blood hematopoietic cells, but if the confidence interval (CI) crosses this line, this result is not statistically significant ($p < 0.05$). aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; O – E, observed minus expected; Redn., reduction; SD, standard deviation; Var., variance. Source: Stem Cell Trialists' Collaborative Group 2005 [97].

nowadays – was the most frequent diagnosis (approximately 40% of all patients treated).

Mobilized BM for allogeneic transplantation

Several investigators have used G-CSF-stimulated BM for hematopoietic reconstitution after myeloablative therapy, and compared engraftment and the development of GVHD with patients who were rescued with G-CSF-mobilized PB [98]. A small prospective study (57 patients being randomized) comparing G-CSF-primed BM with G-CSF-mobilized PBHCs concluded that engraftment after transplantation of either source of HCs was comparable; however, more steroid-refractory acute GVHD, chronic GVHD, and prolonged requirement for immunosuppressive therapy was found after PBHCT [99]. Survival did not significantly differ. Larger randomized studies would be needed to clearly define the role of G-CSF-stimulated BM compared with G-CSF-mobilized blood.

Allogeneic PBHCT in diseases other than leukemia

The prospective studies comparing BMT with PBHCT, as well as most retrospective analyses, included patients with leukemia and a few with lymphoma or myeloma. It is therefore largely unknown how patients with diseases other than leukemia would fare after either procedure. Some years ago, EBMT published their experience with BMT or PBHCT in patients with MDS [100]. The results of this retrospective study were similar to the large retrospective analysis of patients with acute leukemias and CML published by the IBMTR and EBMT: earlier hematopoietic recovery, and similar incidences of acute, but more chronic GVHD with PB. The 2-year TRM was significantly reduced with PB except for patients who had refractory anemia or high-risk cytogenetics. Treatment failure was significantly decreased with PB. Estimates of the 2-year event-free survival were 50% with PBHC versus 39% with BM. Gahrton et al. compared PBHCT with BMT in patients with multiple myeloma [101]. They did not find significant differences between BMT and PBHCT for TRM, acute and chronic GVHD, progression-free survival or relapse rates; overall survival tended to be better in the BMT group (53% vs. 44% at 3 years; $p = 0.05$).

Another EBMT study compared outcomes of 692 patients with severe aplastic anemia grafted with either mobilized blood or marrow from HLA-matched sibling donors. Hematopoietic recovery and grade II–IV acute GVHD were similar after PBHCT and BMT. In patients aged over 20 years, chronic GVHD and overall mortality rates were similar after PBHCT and BMT. In patients aged 20 or less, however, rates of chronic GVHD and overall mortality were higher after transplantation of PBPC than after BMT. In younger patients, the 5-year probabilities of overall survival were 73% and 85% after PBHCT and BMT, respectively. Corresponding probabilities for older patients were 52% and 64% [102]. These and other data for unrelated [103] or related donor transplants [104] indicate that BM grafts should be preferred to PBHCT in patients with severe aplastic anemia, and that results of PBHCT versus BMT may vary according to the underlying disease. No comparisons of BMT with PBHCT are available so far for patients with lymphoma.

Allogeneic PBHCT in children and adolescents

A study from the IBMTR cautioned against the use of PBHCT in children and adolescents [105]. One hundred and forty-three PBHCT and 630 BMT procedures from HLA-identical sibling donors in patients aged 8–20 years with acute leukemia were compared. Risks of grade II–IV acute GVHD were similar; however, the incidence of chronic GVHD was higher after PBHCT. The cumulative incidences of chronic GVHD at 3 years were 33% and 19% after PBHCT and BMT ($p = 0.001$). In contrast to reports in adults, rates of TRM, treatment failure, and mortality were also higher after PBHCT. The 3-year probability of overall survival, adjusted for other significant factors, was 48% and 58% after PBHCT and BMT, respectively ($p = 0.01$). These data serve as a cautionary note before a major change in the source of HCs used for allogeneic transplantation of children comparable to the situation in adults will occur. Cord blood is an attractive alternative, particularly for children (see Chapter 39) and prospective studies comparing cord blood transplantation, BMT, and PBHCT would be helpful to better define the role of the various sources of HCs.

T-cell-depleted mobilized blood

Because of concerns that the large numbers of T cells contained in mobilized PB would cause more frequent and severe GVHD, the interest in T-cell-depletion strategies was high from the beginning. Soon after the first transplants with mobilized PB had been reported, a number of studies using T-cell-depleted mobilized PB were initiated. Early results, however, were disappointing because – among other causes – depletion methods were far from optimal. With T cell numbers in excess of 1×10^5 T cells/kg recipient body weight remaining in the graft, it was not too surprising that acute and severe GVHD still occurred with incidences comparable to those observed after transplantation of unmanipulated PBHCs.

With further experience, technical problems were solved, and it became possible to routinely purge PB collection products to a residual T-cell content of below 1×10^4 T cells/kg. Such minimal T-cell load is not sufficient to elicit clinical GVHD even in the haploidentical setting. One of the major problems of T-cell depletion at the time when BM was exclusively used as the source of HCs was the increase in graft failures occurring after transplantation. Because CD34$^+$ cell numbers are much higher in mobilized PB, and the eventual loss of HCs caused by the depletion process can be compensated for by further leukaphereses, graft failure is less of a problem after the transplantation of T-depleted mobilized PB. Urbano-Ispizua et al. reported that the graft failure rate was still 18% after T-cell-depleted PBHCT if fewer than 2×10^5 CD3$^+$ T cells remained in the graft, while the incidence was very low (1%) if more than 2×10^5 T cells were infused [106]. It should be noted, however, that in this study relatively low numbers of CD34$^+$ cells (3.4×10^6/kg) had been infused into patients who subsequently suffered graft failure. Other studies suggest that the transplantation of very high numbers of CD34$^+$ cells can overcome the graft failure risk in the HLA-identical and the mismatched setting regardless of the residual number of T-cells in the graft.

Transplantation of CD34$^+$-selected cells from mobilized PB to patients with early myeloid malignancies was reported to result in less GVHD, less TRM, and better disease-free survival if compared with unmanipulated PBHCT. The randomized study published by Cornelissen et al. [107] made clear that the results of CD34 selection of PB or marrow are largely dependent on the efficacy of the T-cell-depletion strategy. With current methodology, high CD34$^+$ cell numbers can be transplanted after effective T-cell depletion ($<1 \times 10^5$ T-cells /kg recipients weight). Using such grafts can result in high overall and disease-free survival of patients grafted for early AML [108,109].

In summary, the availability of HCs from PB is important in the context of T-cell depletion strategies, because GVHD nowadays can virtually be eliminated by vigorous depletion strategies without a dramatic increase of graft failure rates when high to very high numbers of HCs are transferred. T cells, however, are mediators of the graft-versus-leukemia effect and contribute to quick immune reconstitution after transplantation. For these reasons, transplantation of T-depleted mobilized blood has often been restricted to cases of haploidentical transplantation where high numbers of CD34$^+$ cells are needed.

Unrelated donor transplants

Retrospective reports comparing PBHCT with BMT reported faster engraftment with PB; severe acute GVHD, 1-year TRM, and overall survival did not significantly differ after PBHCT or BMT but patients transplanted with PB experienced more cGVHD [110,111]. Recently, the first prospective randomized study comparing allogeneic PBHCT with BMT has been published [112]. The study enrolled 551 patients below the age of 66 years. Most patients suffered from acute myeloid or lymphoblastic leukemia or myelodysplastic syndrome. At 2 years, the overall survival (51% after PBHCT, 46% after BMT) did not significant differ. The overall incidence of graft failure was significantly higher after BMT (19% vs. 3% after PBHCT). The incidence of chronic GVHD in the PBHCT group was 53% as compared to 41% in the BMT group ($p = 0.01$). There were no significant differences in the incidence of acute GVHD or relapse. The authors conclude that PBHCT may be preferable over BMT if graft failure is the major clinical threat and BMT may be the better source of HCT in other settings. Also after publication of this important study, the clinician is left with more questions than answers: the study was largely restricted to patients with leukemia and MDS which were unsatisfactorily classified into good- or poor-risk disease by clinical parameters only (no cytogenetic or molecular characterization of the disorders are reported), GVHD prophylaxis probably was suboptimal in a significant proportion of cases who in 2013 would be considered mismatched transplants, and follow-up was still too short to allow for final conclusions.

Conclusion and future developments

A clear-cut picture of the advantages and disadvantages of G-CSF administration and collection of HCs from PB has evolved over recent years. Long-term side effects of PBHC and marrow harvesting do occur and make close follow-up of donors necessary. This is particularly an issue because the demand for HCs will steadily increase, and a rising number of older recipients and donors with comorbidities will be involved. Many potential donors and medical community members currently prefer to work with HCs from PB. This is a consequence of the results of the clinical trials available so far, but is also dictated by recent developments in other areas of blood and marrow transplantation, including non-myeloablative conditioning, exhaustive T-cell depletion, and transplantation across major HLA barriers, all of which require high to extremely high numbers of HCs that usually cannot be harvested from BM.

In the near future, patients will likely receive cocktails of HCs plus an array of other cells with distinct immune functions tailored to each patient's needs. Probably, all of these cells will be harvested or grown from the PB. One of the most exciting questions today is whether, with our increased understanding of basic immunology, it will be possible to dissect GVHD from the graft-versus-leukemia effect, both exerted by donor T cells. Future work will also show if blood can act as a source of truly pluripotent stem cells that can be used in the treatment of non-hematopoietic diseases, including cardiac failure, Parkinson's disease, and other degenerative disorders.

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CHAPTER 42

Cryopreservation of Hematopoietic Cells

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Introduction

Storage of hematopoietic cells (HCs) is required for transplantation, whether for the brief period required to deliver a freshly harvested bone marrow (BM) or peripheral blood HC (PBHC) product to an allogeneic recipient, or for the long-term (potentially decades) banking of cord blood HCs (CBHCs) intended for unrelated-donor transplantation. Room temperature or refrigerated (4 °C) storage may be appropriate for products stored for a few hours to a few days. Cryopreservation allows longer term storage and is the storage technique for virtually all products intended for autologous HC transplantation (HCT). Although cryopreservation of HCs is not a requirement for treatment with myeloablative, dose-intensive conditioning regimens, and patients have recovered marrow function after reinfusion of HCs stored for several days at 4 °C, a progressive loss of HCs occurs during non-frozen storage. The definition of ideal conditions (e.g., temperature of storage, initial processing of the cells, cell concentration, and additives) for the non-frozen storage of cells may slow this loss. In contrast, although some unavoidable loss of HCs occurs with processing and cryopreservation, progressive loss over months to years of proper storage is not obvious and may not occur if optimal storage conditions are maintained.

It is evident that HCs can be successfully cryopreserved from the successes of autologous and allogeneic HCT in regenerating marrow function after myeloablative conditioning regimens. Although some investigators have correlated poor HC cryopreservation with delayed engraftment after transplantation [1,2], this deleterious effect is most likely to be observed with products containing borderline quantities of HCs. Most cryopreservation laboratories use a variation of the technique outlined in Table 42.1, including differences in cryoprotectant concentration, product volume, and storage temperature. Although easily performed, HC cryopreservation and reinfusion are not without risk of toxicity to both the HC inoculum and the recipient of these cells. There are multiple aspects to successful cryopreservation of HCs. Each of these variables affects the survival of the cells and, as with any manufacturing process, must be rigidly controlled for reproducible results.

Non-frozen storage of HC products

Cryopreservation is not required for short-term storage of HCs. Non-frozen storage is a less costly alternative, especially for the immediate transportation of products and for the transplantation of patients conditioned with brief courses of chemotherapeutic agents with short in vivo half-lives [3]. The major advantages of cryopreservation are the lack of progressive loss of HCs over time and the greater flexibility in the timing of HCT relative to the collection of HCs, including the ability to modify or postpone a transplant conditioning regimen already started.

A progressive loss of HCs occurs during non-frozen storage. One author reported a 61% loss of myeloid colony-forming progenitor cells [colony-forming units – granulocyte macrophage (CFU-GMs)] from marrow after 72 hours of storage at 4 °C [4]. Yet another found only a 3% loss of CFU-GMs from BM stored for 96 hours, but a 95% loss if the source of the cells was peripheral blood [5]. As part of a clinical trial described later, Preti et al. compared the survival of mature hematopoietic progenitor cells isolated from marrow during frozen or non-frozen storage, and reported an immediate loss of myeloid (CFU-GM) progenitors of 33% during cryopreservation and thawing [6]. In contrast, cells stored at 4 °C showed a progressive, linear loss of total nucleated cells, cell viability, and HCs cloned in vitro. The quantity of erythroid colony-forming progenitor cells [burst-forming units – erythroid (BFU-Es)] in these non-frozen samples became significantly less than that of cryopreserved samples only after 5 days of storage. The difference for myeloid progenitor cells (CFU-GMs) was not yet significant even after 9 days of storage.

These differing reports demonstrate that storage conditions such as the concentration of cells, chemicals added, product volume, storage bag, and temperature of storage will affect the survival of cells kept in non-frozen storage. Most published reports of non-cryopreserved storage describe storage at 4 °C. This condition provides a stable temperature compared with storage at ambient temperatures, which can be quite variable (and which must be considered during the shipping of products). The optimum temperature is not known and will probably depend upon such concerns as the pre-storage processing, the quantity and concentration of mature blood cells, the acid buffering capacity of the solution, and the gas-diffusion capacity of the storage container. Beaujean et al., for example, reported a much lower pH for PBHC products stored overnight at room temperature compared with storage at 4 °C [3]. Although they did not find a difference in the recovery of progenitor cells in their experiments, this effect may be damaging to HCs in other circumstances. Antonenas et al. also reported a better survival of PBHCs stored at 2–8 °C but no effect of storage temperature on BM cells [7]. Yet another report describes better survival of HCs stored at 37 °C and at 25 °C compared with cells stored at 4 °C [8]. The conflicting reports about optimal storage temperatures are difficult to interpret. Storage conditions for non-frozen storage have not been tested in an engraftment model, so proper storage conditions are not adequately defined. For laboratories intending to store HCs for prolonged periods without freezing, rigorous validation of the storage conditions must be pursued and strictly controlled.

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Table 42.1 Hematopoietic cell cryopreservation: basic technical considerations

Item	Principle	Example
Prefreeze processing	Reduction of mature blood cells that are poorly cryopreserved	Separation of buffy coat cells or light-density cells
Cryoprotectant	Protection of the cells from ice formation and dehydration. May be "colligative" or "polymeric" or a combination of both	Colligative: dimethylsulfoxide, glycerol Polymeric: hydroxyethyl starch
Plasma protein	Reduces cryopreservation injury	Autologous plasma or plasma protein concentrates
Solvent	Provides a suspension of cells and diluent for cryoprotectants	Saline solutions
Cooling	Cooling rates are dependent on the cell being frozen and the cryoprotectant used	Hematopoietic cells in dimethylsulfoxide are cooled at 1–3 °C/min
Storage	Storage temperature must protect from ice recrystallization and progressive cell damage	Nitrogen liquid or vapor-phase. Addition of polymeric cryoprotectant may allow storage at warmer temperatures

There is limited experience with the autologous transplantation of non-cryopreserved HCs [4,6,9–11]. HCs appear to maintain viability for several days after collection if carefully stored. Preti et al. compared, in a non-randomized retrospective analysis, the engraftment kinetics of 54 patients who received cryopreserved marrow cells with 45 patients who received refrigerated cells [6]. The refrigerated cells were stored for a median of 4 days (range 3–9 days). The cryopreserved cells were stored a median of 69 days (range 5–981 days) at −80 °C using a cryoprotectant mixture of dimethylsulfoxide (DMSO) and hydroxyethyl starch (HES). Almost all patients were conditioned with a regimen consisting of carmustine (BCNU), etoposide, and cyclophosphamide. This group found no significant difference in engraftment kinetics for these two groups, although a small difference of a few days would not have been detected with the limited numbers of patients in this study.

Cryopreservation theory

The chemistry and biology of mammalian cell cryopreservation

The recovery of viable mammalian cells after cryopreservation and thawing became possible with the discovery that glycerol could be used for the freezing of bovine sperm [12]. Subsequent experiments demonstrated that marrow cells frozen with glycerol could be used to reconstitute the marrow function of irradiated mice [13]. Considerable exploration of the cryobiology of various mammalian and non-mammalian cells ensued. These studies defined the mechanism of cell damage incurred during cryopreservation and techniques to moderate that damage. The current understanding of cryobiology contends that ice crystal formation during cooling is the primary cause of cell damage [14]. This damage can be classified into two categories. At rapid rates of cooling, intracellular ice crystals may form, resulting in *mechanical* disruption of the cell and immediate cell death. At slower rates of cooling, ice crystal formation preferentially occurs in the extracellular space, resulting in increasing osmolality as free water is incorporated into the growing ice crystals. This loss of free water results in the concentration of extracellular solutes such as sodium that do not freely penetrate the cell membrane, extreme hyperosmolality, and *dehydration* injury. For example, the molality of sodium chloride (NaCl) in a saline solution at −10 °C is about 5.3 *m*, and that at −20 °C about 10.5 *m*.

Intracellular ice formation may be limited by cooling cells slowly. At slow rates, it is probable that ice nucleation will occur first in the larger volume outside the cell. Progressive dehydration of the cell will result if the rate of cooling is slow enough to allow water to shift to the extracellular space and be incorporated into the growing ice crystals. The optimal cooling rate differs for different cell types and appears to be defined by the permeability of water through the cell membrane [15]. Progressive dehydration with concentration of intracellular solutes prevents the supercooling of intracellular water and protects the cells from the formation of intracellular ice crystals. Ice that happens by chance to nucleate first within a cell will result in the death of that cell, but will also serve to promote the freezing of extracellular water as the ice crystal penetrates the cell membrane. The corollary is not true in that extracellular ice does not penetrate into the cell and promote intracellular ice formation, presumably because of some feature of the cell membrane.

Glycerol and DMSO are colligative cryoprotectants that prevent dehydration injury by moderating the increased concentration of non-penetrating extracellular solutes during ice formation, and by decreasing the amount of water absorbed by (in equilibrium with) the ice crystals at a defined temperature. "Colligative" refers to properties dependent upon the number of particles (solute) and not the composition of the particles. Freezing is the crystallization of water, and the freezing point is the temperature at which ice crystals can be sustained in equilibrium with water. The freezing point of water is depressed by the addition of solute. For any particular mixture of solute(s) and water, there will be a defined temperature at which ice crystals can initially form. Unlike pure water, ice crystal formation and growth in aqueous solutions occurs over a temperature range. Growing ice crystals absorb free water and exclude solute particles. The incorporation of water into ice results in concentration of the solute and further depression of the freezing temperature of the remaining water, thereby preventing additional ice formation unless further cooling occurs. Thus, temperature (and pressure) defines the equilibrium between ice and the non-frozen solution. With further cooling, a temperature is eventually reached at which the solute itself crystallizes (eutectic point). The molality of a solution in equilibrium with ice is therefore determined by the temperature of the solution, not the initial concentration of the solute, as depicted by the equation

$$m \approx \frac{273 - T}{1.9}$$

in which m represents the osmolal concentration of the solute and T is the temperature in degrees Kelvin [16]. Similarly, the fraction of water not frozen (q) is defined by the equation

$$q \approx \frac{1.9Mo}{273 - T}$$

where Mo is the osmolal concentration of the solution before freezing.

In our example above, a saline solution at −20 °C has an NaCl molality of about 10.5 *m*. For a three-component system such as DMSO and NaCl in water, both solutes will contribute to the molality of the unfrozen solution in the same relative proportion found in the initial solution. The molality (before freezing) of 10% DMSO [volume in volume (v/v)] in normal saline (140 mM) solution is about 1.6 *m*, with DMSO contributing about 10 times the molality contributed by NaCl to the medium. At −20 °C, this molal ratio between DMSO and NaCl will be maintained. The molality contributed by NaCl will be about 1.05 *m*, or about seven times the molality of NaCl in a solution without ice. DMSO freely penetrates the HC cell membrane so that the intracellular concentration of DMSO will equal the extracellular concentration throughout the temperature range, assuming adequate movement of water from the cell as the extracellular ice crystals form and grow. The addition of a penetrating cryoprotectant to an aqueous saline solution therefore reduces the osmotic stress across the cell membrane at −20 °C from about 75 times to about seven times that of an ice-free solution. According to this theory, colligative cryoprotectants must be capable of penetrating the cell to avoid merely contributing to the molality of the extracellular medium, and must be non-toxic to the cells at the concentration required for this effect.

The tolerance of cells to freezing at slow cooling rates depends on the ability of the cells to withstand osmotic stress, and initial studies of the osmotic behavior of HCs have been reported [17]. The osmotic tolerance of granulocytes is much less than that for lymphocytes or CFU-GMs, probably accounting at least in part for the difference in survival after cryopreservation [18]. With DMSO concentrations of less than 10%, the degree of dehydration caused by concentration of the non-penetrating solutes during freezing will be greater because non-penetrating solutes will contribute proportionately more to the molality of the non-frozen solution in the extracellular medium. With higher concentrations of DMSO, the osmotic stress will be less. The optimal concentration for a colligative cryoprotectant depends upon the osmotic tolerance of the cell to be frozen, the toxicity to the cell of high concentrations of the cryoprotectant [19,20], and the presence of other cryoprotectants. The ability of a cryoprotectant to penetrate the cell and its subcellular organelles explains, at least in part, the relative efficacy of different colligative cryoprotectants for different cell species.

Colligative properties do not explain the cryoprotection achieved by freezing cells in solutions of macromolecules such as HES. Solutions of high molecular weight, polymeric cryoprotectants, contain relatively few particles and, moreover, do not freely penetrate the cell. These cryoprotectants may protect the cell by forming a viscous, non-crystalline, glassy shell that retards the movement of water, thereby preventing progressive dehydration as water is incorporated into the extracellular ice crystals [21]. Solutions of some compounds, when present in sufficiently high concentrations, will solidify to an amorphous glass without first forming ice crystals, a process termed vitrification [22]. The "glass-transition temperature" (T_g) depends upon both the structure and concentration of the solute. At very high concentrations of cryoprotectants (6.3 M for DMSO [22]), the T_g is higher (warmer) than the temperature at which ice crystals can form, thereby preventing crystallization during cooling and its resulting mechanical and osmotic stresses.

The practical difficulty with achieving vitrification arises from the necessity to use these very high concentrations of cryoprotectants. Vitrification has been used for the cryopreservation of murine embryos, for example, with a solution of 20% DMSO, 15.5% acetamide, 10% propylene glycol, and 6% polyethylene glycol [23]. Prolonged exposure to this solution was toxic, however, with complete loss of viability after 30 min at 4 °C. Kurata et al. plunged CBHCs protected by high concentrations of ethylene glycol directly into liquid nitrogen [24]. The recovery of HCs in samples preserved with the highest concentrations of ethylene glycol was similar to that for samples more conventionally cryopreserved with 10% DMSO (Table 42.2). The toxicity of the cryoprotectant solution was not addressed by these authors. Also, the rapid cooling and warming rates required are easier to achieve with the small cell aliquots (0.5 mL) tested than with the large volumes characteristic of HC products.

Table 42.2 Recoveries of nucleated cells and hematopoietic cells after cryopreservation with ethylene glycol or dimethylsulfoxide (DMSO)

Cryoprotectant solution	Cell recovery	CFU-GM recovery
8 M ethylene glycol	89.5 ± 8.5	66.6 ± 20.8
4 M ethylene glycol	83.6 ± 9.4	36.4 ± 23.6
2 M ethylene glycol	75.0 ± 17.4	14.7 ± 24.6
10% (v/v) DMSO	88.5 ± 12.4	69.1 ± 9.7

CFU-GM, colony-forming unit – granulocyte macrophage.
Source: Adapted with permission from Kurata et al. 1994 [24]. Reproduced with permission of Nature Publishing Group

With lower concentrations of cryoprotectants, including extracellular macromolecular compounds and many sugars, crystallization occurs at warmer temperatures than the vitrification temperature and ice crystals will form during cooling. However, glass transition may occur even in the presence of ice crystallization. With the formation of ice crystals and resulting loss of free water, the concentration of the cryoprotectant in the non-frozen water will increase. With decreasing temperature causing increasing cryoprotectant concentration, a point will be reached at which the solution forms a glass. At this "limiting glass-transition temperature" (T'_g), the solution suddenly becomes viscous, retarding if not stopping the flow of water through the extracellular matrix. This has been proposed as the mechanism for cryoprotection afforded by the extracellular cryoprotectants [21]. In one study of peripheral blood monocyte cryopreservation using extracellular cryoprotectants, a limiting glass-transition temperature of −20 °C was optimal, which was achieved using a 20% solution of HES [21]. Substances forming glasses at higher or lower temperatures were less effective. Pure water forms a glass at about −139 °C [25]. The addition of cryoprotectants such as DMSO, glycerol or HES raises the T'_g [26]. This model of cryopreservation requires adequate dehydration to occur to concentrate intracellular solutes and decrease the probability of intracellular ice formation, but glass formation at an appropriate temperature (please recall that temperature defines the osmolality of the unfrozen aqueous solution) to prevent excessive dehydration of the cell.

Cells are not frozen in simple two- or three-component solutions but rather in complex solutions containing salts, sugars, penetrating cryoprotectants with or without extracellular cryoprotectants, and plasma proteins. Phase-transition temperatures (such as T'_g) for these solutions have not been published. The improved survival observed with increasing protein concentration [27], and, possibly, by combined penetrating and extracellular cryoprotectants [28], may be explained at least in part by these theories on ice and glass formation and their effects on the cell. Moreover, the existence of glass formation may explain the relationship between storage temperature and survival of HCs over time. Below the T'_g temperature, enlargement of previously formed ice crystals by recrystallization of unfrozen water cannot occur, and the cells are protected from progressive mechanical disruption. (The growth of an existing ice crystal through the process of recrystallization is thermodynamically more favorable than the nucleation of a new ice crystal.) The optimal storage temperature is below the T'_g for the cryoprotectant solution used. Warmer temperatures may be used, but at risk of cell damage, a risk that is dependent upon the temperature of storage and the stability (viscosity) of the solution at that temperature.

This simplified review of the effects of freezing and the cryoprotectant properties of penetrating and polymeric cryoprotectants does not completely explain the processes involved during freezing, and more detailed reviews of the freezing of aqueous solutions and mammalian cells have been published [29]. In addition to mechanical and dehydration injuries, cooling itself may be damaging to the cell [30]. Colligative effects alone are not sufficient to explain the cryoprotectant properties of DMSO or glycerol. Other freely penetrating chemicals such as urea and dimethylsulfone ($DMSO_2$) do not function as cryoprotectants for mammalian cells [31,32], and some chemicals such as ethanol and guanidine may actually function as cryosensitizers [33]. Obviously, the chemical structure of the cryoprotectant is important in the survival of mammalian cells, and a molecular interaction between the cryoprotectant and protein or lipid molecules appears necessary for optimal cryopreservation [34–37]. Examples of selective damage to cell membrane proteins or structure from freezing are illustrated by the loss of response to giant cell tumor-conditioned medium of myeloid progenitor cells after cryopreservation [38], the decrease in L-selectin expression by $CD34^+$ cells after cryopreservation and thawing [39], and the lineage-specific effects on granulocyte engraftment for patients receiving cells frozen with HES and DMSO in combination [40].

Induction of apoptosis during cell cooling

The recently demonstrated relationship of apoptosis with hypothermia may help explain HC losses during cooling and cryopreservation. Separate from cell necrosis resulting from the mechanical and dehydrating effects of cryopreservation, the induction of apoptotic pathways during cooling or after thawing may lead to further losses of HCs. Stroh et al. demonstrated caspase activation during the freeze–thaw process and reported improved survival of cells with the addition of a caspase inhibitor to the cryopreservation solution [41]. Benefit could be achieved also with the treatment of cells immediately after thawing, and the benefit was particularly significant for cells stored at warmer temperatures (e.g., –70 °C).

The physics of cooling and warming of cell products

The rationale for slow rates of cooling and rapid rates of warming is explained by the mechanical and dehydration injuries resulting from the formation and growth of ice crystals. Heat transfer is a physical process, not a biologic or chemical process. Cooling is a physical process dependent on properties of the object being cooled, such as the difference in temperature between the object and its environment, and the size and thermal conductivity of the object. The rate of heat transfer is described by the equation

$$q(r,t) = -k\nabla T(r,t)$$

in which $q(r,t)$ represents the heat-flux vector at a particular point on the object and a particular time, $-k$ represents the thermal conductivity of the material, and $\nabla T(r,t)$ represents the temperature gradient vector at that particular time and place. Objects with a greater thermal conductivity because of composition or smaller size will gain or lose heat more rapidly, as will objects placed in an environment with a much larger difference in temperature between the object and its environment.

Most centers use electronic rate-controllers to achieve an optimal cooling rate, but satisfactory rates of cooling can be achieved by simply immersing an appropriate volume of HCs into a –80 °C mechanical freezer [42–45]. Rowley cooled 50 mL aliquots in storage bags and found reproducible cooling rates of about 3 °C/min when placed in a –80 °C freezer, but rates between 10 °C/min and 16 °C/min when placed in a –135 °C freezer [42]. Clark et al. studied the effects of marrow volume on the cooling rates before and after the transition phase, and the duration of the transition phase for cells placed either in freezing bags or vials [45]. As would be predicted by the equation describing heat transfer, bags containing larger volumes cooled much more slowly and required longer duration of the transition phase (Table 42.3).

Table 42.3 Effect of product volume on cooling rate after immersion into a –70 °C freezer: rates of cooling before and after the transition phase, and the duration of the transition phase for samples of the stated volumes immersed into a 70 °C refrigerator

	Cooling velocity (°C/min)		Duration of transition phase (min)
Volume (mL)	Pre- transition	Post-transition	
30	6.1	2.0	0.5
60	3.1	1.6	3.0
90	1.6	1.0	8.6
120	1.8	1.0	12.8

Source: Adapted with permission from Clark et al. 1991 [45]. Reproduced with permission of Nature Publishing Group.

Cryopreservation using an immersion technique is not “uncontrolled,” unless the physical parameters used are also not controlled, resulting in widely varying rates of cooling. Before immersion techniques are adopted by individual laboratories, the rate of cooling and reproducibility for the type of bag and volume of cells must be determined and documented. The advantages of immersion cooling techniques are the avoidance of a rate-controlled apparatus and the decrease in personnel time required. The disadvantages are the lack of a recording documenting the rate of cooling, the limited adaptability to different volumes and containers, and possible detrimental effects related to the duration of transition phase, which may be critical if borderline quantities of cells are harvested.

Cryoprotectant solutions

DMSO

DMSO, glycerol, and a variety of other chemicals may serve as colligative cryoprotectants that protect the cell from excessive dehydration as extracellular water is drawn into growing ice crystals. The cryoprotectant properties of glycerol were described in 1949 [12], and those of DMSO 10 years later [46]. The rapid diffusion of DMSO through the cell membrane, and the difficulty in removing glycerol before reinfusion (infusion of DMSO-containing products being generally tolerated), have made DMSO the favored agent for HC cryopreservation.

DMSO, a byproduct of paper manufacturing, is a hygroscopic polar compound developed originally as a solvent for chemicals such as insecticides, fungicides, and herbicides. Pure DMSO is a colorless, virtually odorless liquid (specific gravity 1.108, molecular weight 78.13 g/mol), although industrial grades may have a strong sulfur odor [47]. The serum half-life of DMSO is about 20 hours, although that of $DMSO_2$, a renally excreted metabolite, is 72 hours [47]. A small proportion of DMSO is reduced to dimethylsulfide, which is expired through the lungs for about 24 hours after administration, and which accounts for the characteristic odor resulting from DMSO infusion.

The optimal concentration of either DMSO or glycerol for the cryoprotection of HCs appears to be about 10%, although concentrations as low as 5% have been used successfully for HCT [48]. In their original report, Lovelock and Bishop demonstrated a dose–response with improving red cell survival as the concentration of DMSO was increased to 15% [46]. The effect of yet higher concentrations was not reported. Subsequently, Ragab et al. studied the survival of HCs from human donors after freezing with various concentrations of DMSO [27]. They found a significant increase in progenitor cell recovery when the concentration of DMSO was increased from 7.5% to 10%. No further improvement was found with an increase to 12.5%. This improvement resulted from an increase in nucleated cell recovery from 17.2% to 35.6% and 32.1%, respectively, after washing. Cell recovery before the post-thaw wash did not differ, nor did the numbers of colonies per 10^5 cells plated. The loss of cells during the wash steps that was found in this study may or may not reflect events occurring during direct intravenous infusion commonly used clinically.

Donaldson et al. studied the recovery of $CD34^+$ cells cryopreserved in varying concentrations of DMSO and HES and reported an increase in $CD34^+$ cell recovery from 12.2 ± 10.0% (mean ± standard deviation) to 85.4 ± 28.4% as the concentration of DMSO was increased from 2.5% to 5.0%, but no further improvement with further increase in concentration to 10% [HES concentration kept constant at 4% weight by volume (w/v)] [49]. Varying the concentration of HES in the presence of 5% (v/v) DMSO had no effect on $CD34^+$ cell recovery.

HES

HES is a polymeric substance containing chains of different molecular weights. Initially explored as a cryoprotectant for red blood cells, it was found also to be an effective cryoprotectant for a variety of other cells [21,50]. Macromolecular cryoprotectants may be used as single agents, but the major focus in the study of HC cryopreservation using extracellular cryoprotectants has been their use in combination with penetrating cryoprotectants. In one early study, the addition of polyvinylpyrrolidone, another macromolecular cryoprotectant, to glycerol or DMSO improved the cryopreservation of murine cells compared with the use of a penetrating agent alone [51]. Stiff et al. froze human cells in a combination of 5% DMSO, 6% HES, and 4% human serum albumin, and reported improved progenitor cell survival as determined using in vitro cultures [28]. They subsequently successfully used this mixture of cryoprotectants to cryopreserve the marrow of 60 patients [43]. No engraftment failure was attributed to this technique, or after the cryopreservation of peripheral blood-derived HCs [44]. A single-blind phase III study of 294 patients undergoing autologous PBHC transplantation using either DMSO alone or DMSO in combination with HES, found 1-day differences in the times to achieve a white blood cell count of 1 × 10^9/L and an absolute neutrophil count (ANC) of 0.5 × 10^9/L, and time to discontinuation of antibiotics for recipients of cells frozen with the combination cryoprotectant solution [40]. No differences were found for times to achieve endpoints of platelet engraftment, indicating that this effect was lineage specific. Furthermore, the effect was more pronounced for recipients who received more than the median number of $CD34^+$ cells, suggesting a specific effect on a limited cell population (Table 42.4).

Table 42.4 Engraftment kinetics of patients receiving peripheral blood hematopoietic cells frozen in dimethlysulfoxide (DMSO) or DMSO and hydroxyethyl starch (HES)

Outcome	**Patients below the median***			**Patients above the median***		
	DMSO	**DMSO/HES**	***p* value**	**DMSO**	**DMSO/HES**	***p* value**
Granulocyte engraftment						
WBC ≥ 1.0 ×10^9/L	11	11	0.71	11	10	0.02
ANC ≥ 0.1 × 10^9/L	10	10	0.86	10	9	0.03
ANC ≥ 0.5 × 10^9/L	11	11	0.87	11	10	0.01
ANC ≥ 1.0 × 10^9/L	13	12	0.87	12	11	0.02
Platelet engraftment						
Platelets ≥ 20 × 10^9/L	11	12	0.25	9	9	0.36
Platelets ≥ 50 × 10^9/L	14	15	0.10	12	12	0.28
Last transfusion	9	9	0.67	8	8	0.75

ANC, absolute neutrophil count; WBC, white blood cell count.
*Median times to achieve the designated outcome for patients classified by cryoprotectant used and stratified by quartile of $CD34^+$ cell dose/kg (first and second quartiles combined versus third and fourth quartiles combined). The median cell doses for patients receiving products frozen with DMSO alone was 6.4 × 10^6/kg, and for those receiving cells frozen with DMSO/HES was 6.5 × 10^6/kg. *p* values are based on weighted log rank test, which compares the two estimated recovery curves.
Source: Adapted with permission from Rowley et al. 2003 [40]. Reproduced with permission of Nature Publishing Group.

Protein

Plasma proteins exert cryoprotectant effects, possibly by modifying the viscosity or the glass-transition temperature of the cryoprotectant solution. Lymphocytes can be cryopreserved using serum alone [31,52]. The addition of serum proteins to the cryoprotectant solution appears to improve HC survival. In one study using human marrow cells, myeloid progenitor cell survival, which was 41.1 ± 8.0% in the absence of serum, increased significantly to 64.8 ± 14.2% with 15% serum and 75.4 ± 14.8% with 50% serum [27]. Similarly, recovery of murine spleen colony-forming cells (CFU-S) increased from 18.2% to 100.5% when frozen in 10% DMSO with 10% serum added [53]. In this second study, cryopreservation with 10% fetal bovine serum was less effective (25.4% recovery) than that with homologous serum, which may reflect the low protein content that is a characteristic of fetal sources of serum. The effect of protein content or source on engraftment speed has not been defined, but anecdotal experience would suggest that the presence of protein is important. Engraftment will occur for $CD34^+$ cells frozen without the addition of protein [54]. The 33 days (median) to achieve an ANC of over 500/μL and 46 days to achieve a platelet count of over 20,000/μL in the study that did not use protein in the cryoprotectant solution, however, were much slower than the 23 days and 22 days for ANC and platelet engraftment reported by Shpall et al. using a similar marrow-processing technique but different cryoprotectant solution containing protein [55].

Virtually all cryopreservation solutions currently in use contain plasma proteins added as either part of the cryopreservation solution or during cell processing. The range in concentration and source of protein varies among the various transplant groups; the source of protein is similarly diverse, although fetal bovine serum (used in some early trials of marrow transplantation) is no longer used. The use of albumin solutions is appealing because of the ability to achieve high, uniform concentrations of protein while avoiding the marrow fat, cellular debris, anticoagulants, and risk of cryoglobulins associated with the use of autologous plasma collected during initial processing of the HC product.

A unique group of proteins are the antifreeze glycoproteins found in some species adapted to survival in cold environments. Some of these proteins are extremely efficient at preventing the recrystallization of ice and have been shown to enhance the cryopreservation of pig and murine embryos [56]. In studies of red cell cryopreservation, this effect was limited to a narrow concentration range, with higher concentrations actually increasing the growth of extracellular ice, with concomitant cell damage [57]. It cannot be assumed that the addition of these proteins will improve HC cryopreservation survival.

Salt and sugar content

Metabolically inactive cryopreserved cells do not require the complex medium preparations used for ex vivo growth of HCs. Successful cryopreservation has been achieved using commercially available pharmacologic salt solutions. However, the solution in which the cells are suspended should not be viewed as inert. For example, many tissue culture media contain compounds that may increase the sensitivity of cells to freezing (cryosensitizers) [33]. Also, as noted in the second equation cited earlier, the fraction of unfrozen water is determined by the initial osmolality of the solution. At least for red cells, cryopreservation in hypotonic solutions increases post-thaw hemolysis [58].

Various sugars may function as cryoprotectants. Leibo et al. found 50% survival of CFU-S after cryopreservation of murine marrow cells in 0.35 M sucrose without protein or other cryoprotectants [59]. Optimal rates of cooling, as with other extracellular cryoprotectants, were between 16 and 70 °C/min. Cooling at slower rates (<10 °C/min) was associated with much poorer cryosurvival. Using a cell line derived from human kidney, Vos and Kaalen showed cryoprotectant properties for a number of sugars, including glucose, mannitol, and sorbitol, at concentrations greater than 0.1 M [31]. The amount of glucose in tissue culture media is in the millimolar range, which is negligible compared with the concentration shown by Vos and Kaalen to be an effective cryoprotectant in their system. These sugars do not freely penetrate the cell membrane, and function as extracellular cryoprotectants. Sugars may serve to stabilize the cell membrane during freezing or dehydration [35,36]. It has also been suggested that glucose may protect against the cytotoxicity observed with high concentrations of DMSO [60].

Cryopreservation technique

Evaluation of cryopreservation efficacy

A major impediment to the study of HC cryobiology is the inability to culture primitive HCs in vitro. The only "assay" for the cryosurvival of these cells is engraftment after transplantation, the rate of which depends more on patient diagnosis and marrow function, quantity of cells harvested, and any ex vivo treatment performed than on the cryosurvival of HCs as reflected by in vitro cultures [2]. Experimental models include engraftment of human cells in immunodeficient NOD/SCID mice [61]. Initial studies of blood and HC cryobiology used either metabolic assays or dye exclusion assays [62]. These assays are irrelevant, if only because the rarity of HCs in the HC product containing mostly mature blood cells precludes a correlation between cell metabolism or dye uptake and HC cryosurvival. Techniques that measure the viability of the whole population of cells are of no use in determining HC viability in that a complete kill of HCs would change the proportion of cells with vital dye uptake by less than 1%. HCs cannot be identified morphologically, and moreover, metabolic and dye exclusion tests do not measure the proliferative capacity of these cells (Table 42.5) [62].

Progenitor cell assays such as culture for CFU-GMs after thawing can be predictive of engraftment [1,2], but the validity of these assays must be determined for each group of patients and the assay used. DMSO must not be present in concentrations greater than 0.1% in in vitro cultures [63], so some technique of dilution must be undertaken before cell culture. Rapid dilution of DMSO after thawing exposes the cells to osmotic shock. Some authors have reported satisfactory recovery of HCs after rapid dilution of thawed cells [1,64]. Others, however, have demonstrated a distinct advantage to serial dilution [27,65]. Deoxyribonuclease (DNAse) can be added to lyse clumps that form. The addition of sorbitol or other macromolecular substances after thawing may be of value to help prevent osmotic shock [66,67]. These techniques, of course, differ from those of direct intravenous infusion.

Table 42.5 Protective effect of various additives for murine marrow cryopreservation: viability of bone marrow cells cryopreserved with various additives as shown by vital dye stains and the ability of these cells to rescue an animal from irradiation

Cryoprotectant	Viability by vital stain (%)	Animal survival (%)
Dimethylsulfoxide (15%)	91	70
Glycerol (15%)	75	70
Serum (30%)	90	0
Bovine serum albumin (15%)	100	19
Dextran (15%)	62	24
Saline	3	0

Source: Rowe 1966 [62]. Reproduced with permission of Elsevier.

Some centers routinely freeze "test vials" containing small aliquots of cells alongside the greater quantity of cells contained in freezing bags. The different cooling properties of vials compared with bags diminishes the reliability of these small aliquots for determining cryosurvival [45,68]. If an attempt is made to use small aliquots for clinical decisions, a system of cooling, thawing, washing, and culture that correlates with engraftment kinetics must first be developed.

Processing of HC products for cryopreservation

One of the basic concepts of clinical HC cryopreservation is the heterogeneity of BM and blood cell populations. HC cryopreservation involves the cryobiology of not only HCs, but also the mature blood cells contained in the harvested product. HCs comprise a very small portion (generally <1%) of the BMHC, PBHC, or CBHC. Cryopreservation techniques that are optimal for HCs will not preserve mature blood cells and the presence of mature blood cells affects HC cryopreservation in at least three ways. First, the large proportion of mature blood cells collected may hinder the laboratory processing if clumping before freezing or after thawing is induced by damaged granulocytes or platelets. Second, damaged cells may cause infusion-related toxicity. The infusion of marrow that was frozen without depletion of red blood cells was associated with acute renal failure in three of 33 patients in one study [69]. This problem presumably resulted from massive hemolysis of the poorly preserved red blood cells. Third, if the cells are frozen at a set concentration, the presence of large numbers of mature blood cells requires that the cells be frozen in large volumes. Patients receiving such products are at considerable risk for serious infusion-related toxicity from the large quantities of cryoprotectant used. In addition, the concentration of HCs before storage reduces the space required, a logistical consideration for the management of large banks of cells.

Therefore, cryopreservation of HCs can be facilitated by the prefreeze depletion of mature blood elements. A number of apheresis or cell-washing devices are capable of processing the large quantities of cells harvested. Some apheresis devices provide enrichment of mononuclear cells relative to granulocytes, providing a "cleaner" preparation for processing and cryopreservation. At the extreme of prefreeze processing is the extensive purification of HCs by isolation of $CD34^+$ cells, which reduces the quantity of mature blood cells and the amount of cryoprotectant infused, leading to a reduction in infusion-related toxicity. Collection of buffy coat cells with depletion of red blood cells is the minimum processing required for cryopreservation of marrow. PBHCs collected by apheresis contain small proportions of red cells and generally do not require further separation, although volume reduction will decrease the amount of cryoprotectant used. Cord blood cells are processed to reduce the storage volume required for the large banks of cells being stored. [67].

Cell concentration

The cell concentrations used in most protocols are often driven by such practical considerations as the desire to freeze more than one bag, or to minimize the total volume and number of bags of product stored. PBHCs differ from BM products because of their much larger nucleated cell quantity that must be processed by the laboratory. Although many protocols set limits for the concentration of nucleated cells, few also define the maximum or minimum concentrations or quantities of red cells, granulocytes, or platelets. The effect of these cells on HC cryopreservation is not defined, but a potential effect is illustrated by a report of poor post-thaw recovery of red blood cells frozen at high cell concentrations, which may relate to the limited space in channels of non-frozen water between the growing ice crystals during cooling and increased packing of cells [70].

Most studies involving cryopreservation of HCs at high cell concentrations were not designed to detect subtle damage from this practice. The cryopreservation of PBHCs at an average 3.7×10^8 nucleated cells/mL (range $0.4–8.0 \times 10^8$/mL) with no obvious correlation between cell concentration and engraftment kinetics has been reported [71]. Although there also was no correlation between cell concentration during freezing and the post-thaw recovery of $CD34^+$ cells, the recovery of CFU-GMs appeared to decrease at higher cell concentrations. Although cell cryopreservation at high concentrations of nucleated cells is feasible, Rowley et al. suggested that infusion of PBHCs frozen at high concentrations resulted in increased risk for neurologic events during or shortly after cell infusion [72].

The development of techniques that enrich for $CD34^+$ cells presents a different challenge to the cryopreservation laboratory. These techniques typically recover about 1% of the nucleated cells initially present. Cryopreservation of these cells in the same volume used for unselected cells would result in very low cell concentrations. Dicke et al. cite one study of murine marrow cryopreservation that showed stable CFU-S cryosurvival at cell concentrations between 5×10^6 and 2×10^8 cells/mL [65]. At cell concentrations of 1×10^6 cells/mL and below, however, CFU-S cryosurvival dropped precipitously. This decline may be related to non-specific losses such as adhesion to surfaces and post-thaw wash steps. $CD34^+$ cell-enriched products may be frozen in small vials or bags with adjustment of the cooling technique for the smaller volume.

Rates of cooling and warming

Different cells have different optimal cooling rates, and the optimal cooling rate is also dependent on the type and concentration of cryoprotectant used. In general, the higher the concentration of a colligative cryoprotectant, the slower the optimal cooling rate (Table 42.6). The optimal cooling rate when using a colligative cryoprotectant also falls within a narrow range. In contrast, optimal rates for cooling when using extracellular cryoprotectants are generally more rapid, and the cells tolerate a broader range (Table 42.6). Ma et al. used in vitro cultures of human bone marrow to show optimal cooling at 1 °C/min for human hematopoietic progenitor cells suspended in 10% DMSO [73]. The recovery of colony-forming cells fell at rates slower than 1 °C/min or faster than 3 °C/min. Lewis et al. studied the impact of cooling rates before and after the plateau phase (immediately after the "heat of fusion"), and of the duration of the plateau phase on the recovery of murine CFU-S frozen in 12% glycerol [74]. No difference was found for preplateau cooling rates ranging from 0.8 °C/min to 4 °C/min. Increasing the duration of the plateau phase from 0 to 16 minutes resulted in a drop in spleen colonies from 16.7 to 12.0. Increasing the rate of the post-plateau cooling similarly decreased CFU-S recovery. Clinically, Gorin et al. associated a rapid cooling rate with delayed engraftment for eight recipients of autologous marrow [75].

Table 42.6 Effect of cryoprotectant on optimal cooling rate for murine colony-forming units – spleen (CFU-S)

Cryoprotectant	Optimal cooling rate	CFU-S survival
0.4 M glycerol	100 °C/min	18%
0.8 M glycerol	18 °C/min	40%
1.25 M glycerol	1.8 °C/min	65%
0.35 M sucrose	16–70 °C/min	54%

Source: Adapted with permission from Leibo et al. 1970 [59]. Reproduced with permission of Elsivier.

Virtually all the reports regarding the optimal rate of cooling for HCs do not discuss the temperature at which time rate becomes irrelevant. This author has routinely increased the rate to 10 °C/min when the product reached −40 °C and transferred the bags to the storage refrigerators when a temperature of −80 °C has been reached [40]. At −40 °C, water will spontaneously nucleate so supercooled water cannot exist below this temperature [76]. Leibo et al. reported that most cell damage in their murine model occurred at temperatures between −10 °C and −45 °C [59]. Therefore, HCs may be tolerant of higher cooling rates after initial freezing of water.

The warming rate is more critical when rapid rates of cooling are used because of the intracellular ice nucleation that occurs with rapid cooling. If warming is slow during the thawing process, growth of these ice crystals by recrystallization can occur. Cooling at slow rates limits intracellular ice nucleation, and mechanical disruption of the cell from ice recrystallization during warming is less likely. Leibo et al. found no difference in CFU-S recovery for cells in 1.25 M glycerol cooled at 1.7 °C/min and warmed at either 1.8 °C/min or 910 °C/min [59]. Therefore, rapid warming is appropriate.

Storage of cryopreserved cells

Most laboratories store HCs below −120 °C in mechanical (electrical) refrigerators or in either the vapor or liquid phase of nitrogen. The rationale for this method is the possible progressive growth of ice crystals at warmer temperatures as water migrates from smaller to larger crystals (recrystallization), a process that does not occur in pure water at temperatures below about −130 ° to −139 °C [25,26], or in cryopreservation solutions cooled below the phase-transition temperatures of the mixture [25].

Critical phase-transition temperatures for HC cryoprotectant solutions containing DMSO or DMSO/HES have not been fully explored. Nor has the effect of storage or intermittent warming above these phase-transition temperatures on HC viability been determined. Initial attempts of autologous transplantation infused cells stored at −80 °C, although the mild, probably marrow-sparing, pretransplant conditioning regimens used in those initial trials hinder interpretation of the results [77]. In other, preclinical studies, however, progressive loss of HCs was demonstrated for cryopreserved cells stored at relatively warm temperatures. BM cells stored for 25 weeks in 10% DMSO at −30 °C failed to rescue mice from marrow-lethal irradiation, compared to 80% survival after 22 weeks storage at −70 °C and 72% survival after storage for 26 weeks in liquid nitrogen [78].

Illustrative of the need to maintain stable storage temperatures for long-term HC cryopreservation is the report by Appelbaum et al., using a dog model of autologous transplantation, of the failure of engraftment in 30%, 66%, and 100% of recipients of 0.5×10^8 marrow cells/kg stored in the vapor phase of nitrogen for 9, 12, and 18 months, respectively [79]. Storage at ultralow temperatures is not a requirement for successful engraftment if adequate numbers of HCs are harvested and if the duration of storage is limited. Under such conditions, moderate losses of HCs may occur without obvious delay in engraftment kinetics. The engraftment failure observed by Appelbaum et al. can be explained by their intentional cryopreservation and transplantation of borderline quantities of marrow cells and the probable instability of the storage temperature of their cells.

Nitrogen vapor-phase refrigerators are noted for the temperature gradient formed. Temperatures as warm as −100 °C may exist at the top of the refrigerator. Cells stored at this location will be exposed to additional warming upon opening of the refrigerator or lifting of the rack to gain access to the storage cassettes. Over a prolonged storage time, cells may be repetitively exposed to warming during normal operation of the refrigerator, and this warming may be progressively damaging to HCs. This temperature gradient in the vapor phase can be minimized by constructing frames from metals with better heat conductivity, such as aluminum. Rowley and Byrne found a gradient of only 5.9 °C at 22 inches (56 cm) above the liquid when using an aluminum racking system compared with a gradient of 86 °C for a similar refrigerator containing a racking system constructed of steel [80]. This design achieved stable low-temperature storage throughout the refrigerator.

In addition to the issue of temperature gradients in vapor-phase refrigerators, many laboratories perceive storage in the liquid phase to be safer because of the larger quantity of nitrogen present. However, liquid nitrogen can serve as a reservoir for viruses, which may be of clinical importance for high-potency pathogens that require only a few organisms contaminating the bag port for transmission of disease. This problem was dramatically illustrated by the transmission of hepatitis B infection to at least three patients whose cells were immersed in the same liquid nitrogen refrigerator as those of the index case [81]. Molecular typing of the virus and its isolation from the detritus of the refrigerator on subsequent investigation confirmed this mode of transmission.

It is possible that different cryoprotectant solutions may permit storage at warmer temperatures, although this has not been prospectively tested using appropriate models of engraftment. Many centers have adopted storage at −80 °C because of its simplicity for short-term storage for patients expected to undergo transplantation within a few weeks or months of cell harvest. Mostly, this storage is used in conjunction with DMSO/HES cryoprotection. Stiff reported successful transplantation of products stored for up to 22 months at this temperature [82]. Whether this accomplishment results from the addition of HES to the cryoprotectant solution, thereby affecting the stability of the solution at this relatively warm temperature, or because enough HCs were harvested to allow progressive loss was not analyzed. Galmes et al. reported a progressive loss of CFU-GMs and BFU-Es from PBHC products stored at −80 °C in either 5% or 10% DMSO (without HES) and recommended that duration of storage at this temperature be limited [83].

The duration of storage may be indefinite if adequate temperatures are maintained and appropriate cryopreservation techniques are used. Evidence supporting this premise is found in both laboratory and clinical experience. Parker et al. found no loss of colony-forming units culture (CFU-Cs) after storage of human marrow in vapor-phase nitrogen for a median of 42 months [84]. Cord blood stem cells maintain proliferative capacity and ability to engraft in a NOD/SCID murine model for at least 15 years [85]. Furthermore, limited numbers of patients have received HC products stored for prolonged periods of time. One report of experience from multiple centers reported transplantation of cells stored for up to 11 years [86]. In that study of 33 patients, however, the median duration of storage was only 2.8 years, the authors did not correlate duration of storage with engraftment kinetics or other markers of HC survival, and some products were treated ex vivo with mafosfamide, which is known to delay engraftment. Some patients experienced markedly prolonged post-transplant aplasia (up to 119 days), and two patients failed to engraft. Another study reported the outcome of transplantation for 36 patients who received marrow stored for 2–7.8 years, and found, in retrospective comparison to a control group who received cells stored for less than 2 years, no differences in success or speed of engraftment [87]. The products in that report were stored either in nitrogen vapor-phase refrigerators or mechanical freezers at −135 °C.

The temperature of storage should be maintained during transportation of marrow. The availability of "dry shippers" in which the nitrogen is absorbed into the wall of the container simplifies the transportation of frozen cells. These "shippers" can maintain nitrogen vapor-phase temperatures of about −180 °C for periods of 7–10 days (if stored upright). The amount of residual nitrogen at any time after filling is determined by weighing the container.

Cells may be stored either in bags or vials constructed of plastics tolerant to cryogenic temperatures. The advantage of vials relates to the ease with which samples may be thawed for analysis. The large numbers of vials required for storage, the risk of explosion on warming from nitrogen seepage into poorly sealed vials, and the higher risk of microbial contamination during handling are reasons that favor bags.

Post-thaw manipulation

DMSO and HES need not be removed before infusion if consideration is given to the potential toxicities of these agents. Most products may be frozen at sufficiently high cell concentrations that the total cryoprotectant dose is held within tolerable limits. Clumping of damaged cells may occur after thawing, especially if the cells are manipulated. For these reasons, most centers infuse cells within a few minutes after thawing and without any post-thaw processing other than filtration through a blood administration set.

Most DMSO-associated toxicities are related to the quantity of DMSO infused, which can be reduced by washing the cells after thawing. The techniques described generally involve serial dilution to avoid osmotic shock, and re-suspension in a protein-containing medium [88,89]. Beaujean et al. reported an average 73.9 ± 6.4% CFU-GM recovery (± standard error; range 20.5–158.2%) for 50 density-gradient separated marrows after thawing and washing [88]. The recovery for 12 peripheral blood products was 93.9 ± 7.0% (range 67.4–135.6%). Twelve of the marrow bags were stored at room temperature for 4 hours after washing, with an additional 20–25% loss of cells and CFU-GMs. These authors did not describe the speed of engraftment, compare engraftment kinetics to similar patients receiving unmanipulated cells, or correlate engraftment speed with either total cell or CFU-GM recoveries. There is no unequivocal evidence published to date that dilution of DMSO from HC products significantly affects engraftment kinetics.

The risk of post-thaw clumping can be diminished by removing granulocytes and platelets from the cell product before freezing, or by the addition of acid–citrate–dextrose (ACD). Symptomatic hypocalcemia occurs with the infusion of citrate anticoagulants, which may predispose the patient to adverse neurologic events during the infusion [72]. ACD will not lyse clumps that have already formed. Thawed cells should be infused through a standard blood administration set with an in-line filter of 170 μm pore size. It is possible to process HC products extensively after thawing, including the separation of CD34$^+$ cells from cryopreserved PBHC products [90]. Cell clumping during the processing is, as stated, a complication that appears related to the quantity of mature blood cells contained in the product.

Cryoprotectant toxicity

Toxicity to HCs

High concentrations of cryoprotectants may incur direct toxicity to the cells being cryopreserved (similar to the effects of vitrification solutions on murine embryos previously discussed). In general, cells are more tolerant to cryoprotectants at reduced temperatures. For example, DMSO is also concentrated during the formation of ice. Optimal cryopreservation requires a balance between protection from freeze damage and the occurrence of cryoprotectant-induced toxicity. Toxicity of DMSO to HCs has been described, and most laboratories minimize the time of exposure to DMSO of cells before and after cryopreservation. Using in vitro cultures, Douay et al. described a 23.5% recovery of CFU-Cs after 60 minutes' exposure (at 4 °C, without cryopreservation) and a 15% recovery after 120 minutes [91]. However, Rowley and Anderson were unable to confirm their findings of DMSO-induced progenitor cell loss in similar studies, and surmised that the difference between these reports might relate to the purity of the DMSO used [63]. In the latter study, which used pharmaceutical-grade DMSO, the recoveries of nucleated cells, CFU-GMs, and BFU-Es were virtually 100% after exposure to 10% DMSO at either 4 °C or 37 °C. Only at 20% concentration of DMSO did cell clumping cause a decrease in cell recovery to 21.7% (and total CFU-GM quantity to 27.1%). The numbers of myeloid and erythroid progenitors per 5×10^4 cells plated dropped only after exposure to 40% DMSO (3.3% of control after 60 minutes' incubation). Similarly, they found no loss of progenitor cells after thawing if the removal of DMSO was delayed even up to 60 minutes. Others have also reported no toxicity to HCs with short exposure to DMSO [73], although the direct addition of DMSO into the culture medium at 1% or greater concentration is toxic to cell culture [53].

Toxicity to the HCT recipient

In contrast, a high incidence of generally mild, infusion-related morbidity with the reinfusion of either marrow or PBHCs has been reported by several centers [91–94]. DMSO itself has a variety of pharmacologic effects, which may be compounded by the presence of lysed blood cells, foreign proteins from tumor-cell purging procedures or contaminants from non-pharmaceutical grades of reagents used in the processing. The LD_{50} values (amount of DMSO required to kill 50% of test animals) reported for intravenous infusion of DMSO are 3.1–9.2 g/kg for mice and 2.5 g/kg for dogs [47]. The acute toxic dose of DMSO for humans has not been determined. If a large amount of cryopreserved material is to be infused, the infusion can be separated over 2 days to avoid complications from the infusion of excessive amounts of DMSO.

The most dramatic toxicity is the rare anaphylactic reaction occurring during the initial administration of thawed cells. This appears to be an allergic reaction to DMSO, contaminants of DMSO (DMSO is a potent solvent, easily contaminated) or products of tissue culture medium once used for cryopreservation. Treatment of this complication is the same as for anaphylactic reactions to other medications and, after resuscitation of the recipient, the remainder of the cells may be administered cautiously. Non-allergic, profound hypotension may result from the intravenous infusion of DMSO, presumably from histamine-induced vasodilatation. Skin flushing, dyspnea, abdominal cramping, nausea, and diarrhea, reported to varying degrees after HC infusion, can also all be attributed to DMSO-induced histamine release. These complaints resolve over a few hours and are treated symptomatically.

DMSO has a variety of cardiovascular effects. In a series of 82 patients who were premedicated with diphenhydramine, Davis et al. observed increased blood pressure and decreased heart rate that were maximal about 1 hour after the completion of the marrow infusion [92]. A number of authors have noted cardiac arrest or high-degree heart block occurring during or immediately after the infusion of cryopreserved marrow or PBHCs [95–97]. In two series, the incidence of bradycardia (heart rate <60 beats/min) was 48.8% and 65%, that of second-degree heart block was 9.7% and 24%, and of complete (third-degree) heart block was 4.8% and 5.9% [96,97]. In both reports, the median time of onset was about 3 hours after the completion of the infusion. In one series, the authors noted that the heart block was often episodic, occurring with episodes of emesis [96]. In both series, the cardiac rhythm abnormalities resolved spontaneously within 24 hours of infusion. In contrast, Lopez-Jimenez et al. found no cardiac rhythm changes in a prospective series of 29 patients [98]. A break of 20 minutes was allowed between bags of thawed cells in this last series, and it may be the slower overall infusion rate that accounted for the lack of rhythm changes. Alessandrino et al. noted bradycardia for recipients of BM but not PBHCs, describing a higher risk of this complication for recipients of products containing higher quantities of red blood cells in the product [99].

Although headache in up to 70% of recipients of cryopreserved cells has been reported [94], other central nervous system complications are rare and generally related to the amount of DMSO infused. Two recipients who received HC products containing a total of 225 mL and 120 mL, respectively, of DMSO developed reversible encephalopathy [100]. The first patient underwent plasmapheresis, with a prompt improvement in mental status; the second patient recovered over 5 days without specific treatment. The weights of the patients were not cited in this report, but both patients probably received over 2 g DMSO/kg body weight.

In an attempt to reduce the volume of DMSO used during the cryopreservation of PBHCs, Rowley et al. concentrated cells to very high concentrations, averaging over 3.7×10^8 nucleated cells/mL [71]. They subsequently reported several patients who experienced seizures during the administration of these cells [72]. The average cell concentration for these patients was 6.9×10^8 nucleated cells/mL (range 0.8–12.9×10^8 cells/mL), but the maximum amount of DMSO administered was only 0.6 g/kg body weight. Administration of similar quantities of DMSO during the infusion of BM frozen at much lower cell concentrations has not been associated with seizures, so it is likely that these events are related to the cell concentration, cell quantity or types of cell cryopreserved in a

PBHC product. This laboratory previously added ACD (formula A) after thawing to prevent clumping. This practice was stopped after a high incidence of citrate toxicity, including occasional seizures, was observed.

Calmeis et al. reported a relationship between the quantity of non-HCs in the product and infusional adverse events for recipients of products from which DMSO had been removed before infusion [101]. Similarly, Milone et al. noted a correlation of infusion rate with cardiovascular changes, but a relationship between the non-mononuclear cells in the products and non-cardiovascular adverse events [102]. Marrows frozen in 10% DMSO (which itself is 1.4 *m*) have an average osmolality of 1794 mOsm/kg H_2O after thawing, and infusion through a central venous catheter is preferred. Painful irritation may occur if thawed cells are infused through a peripheral vein.

Despite the hyperosmolality of the products, serum osmolality is not greatly affected for patients receiving less than about 1 g DMSO/kg body weight**.** Davis et al. did not detect significant hemolysis in their clinical study, as reflected by a lack of major change in hematocrit [92]. Hemoglobinuria occurs frequently with BMHC infusions [94], presumably resulting from cryopreservation-induced hemolysis of red cells within the HC inoculum. In anticipation of the infusion of hemolyzed red blood cells, many centers manage patients with urinary alkylinization and mannitol diuresis using a strategy recommended for the treatment of acute hemolytic reaction to red cell infusion. This author discontinued this practice (but maintained prophylactic mediation with an antihistaminic agent and a corticosteroid) without an increase in postinfusion renal failure [40]. The infusion of large quantities of red blood cells in marrow products that were not red cell depleted before cryopreservation was associated with the development of acute renal failure in three of 33 patients at one transplant center [69].

In contrast to the extensive studies of DMSO-induced toxicity, little attention has been given to the toxicity of non-penetrating cryoprotectants. HES toxicity to HCs has not been reported. HES is widely used as a blood volume expander during surgery and to enhance the collection of granulocytes from healthy donors by apheresis methodology, and significant systemic toxicity from the infusion of HES is rare.

Special considerations

Cryopreservation of HCs intended for allogeneic transplantation

Cryopreserved HC products are not commonly used in allogeneic PBHC or BMHC transplantation. The availability of a volunteer donor at time of transplantation obviates the cost and risks inherent with the cryopreservation of cells. However, when concerns about the availability of the donor arise (e.g., foreign nationals, prisoners, substance abusers, or overly apprehensive donors), the collection and cryopreservation of cells before initiation of a myeloablative conditioning regimen may be justified. Obviously, the only practical method of storage of CBHCs involves cryopreservation, potentially for decades.

Limited experience in the allogeneic transplantation of cryopreserved marrow from related or unrelated donors has been published [103–106]. The probability and rate of engraftment were similar for recipients of cryopreserved cells and retrospective control groups of patients in the two studies that performed this analysis (Table 42.7). Long-term survival of these patients also appeared similar. Eckardt et al., however, described a significantly lower incidence of acute graft-versus-host disease (GVHD) for the recipients of cryopreserved cells [103]. Stockschlader et al. reported a similar study that found no difference in the incidence of either acute or chronic GVHD [104]. Both studies used total body irradiation or busulfan-based conditioning regimens, and cyclosporine and methotrexate for prophylaxis against GVHD for most patients. Stockschlader et al. subsequently published their experience with cryopreserved cells from unrelated donors (Table 42.7). The incidence of severe acute GVHD for recipients of cryopreserved marrow was 75% [106], which is similar to the reported experience of transplantation of non-cryopreserved cells from unrelated donors transplanted at a number of centers.

Table 42.7 Engraftment and outcome for transplantation of cryopreserved marrow from allogeneic donors

	Study 1		**Study 2**		**Study 3**
	Fresh	**Frozen**	**Fresh**	**Frozen**	**Frozen**
Number of patients	33	10	19	18	10
Days to absolute neutrophil count >500/μL	16	19	17	18	21
Days to platelet count >50,000/μL	28	23	ND	ND	ND
Acute GVHD ≥II	57.5%	20%*	64%	78%	6/8
Chronic GVHD	ND	ND	38%	55%	1/5
Day 100 mortality	39.4%	30%	32%	26%	4/9

GVHD, graft-versus-host disease; ND, no data.

*Difference between fresh and frozen samples significant at $p = 0.037$.

Source: Adapted with permission from Eckardt et al. 1993 [103], Stockschlader et al. 1995 [104], and Stockschlader et al. 1996 [106]. Reproduced with permission of Nature Publishing Group.

Recipients of CBHCs experience slower engraftment compared with recipients of either marrow or PBHCs. It is likely that these observations result from the limited cell dose available in this source of HCs, and not from the cryopreservation process. The observation that granulocyte engraftment kinetics could be hastened by the post-thaw removal of DMSO for recipients of CBHC products could also be attributed to changes in the post-transplant immunosuppressive regimen (discontinuation of post-transplant methotrexate) or other modifications of the transplant regimen for the small number of patients reported [107].

Tumor cell purging

Some investigators have suggested that cryopreservation provides a purging mechanism that will reduce the risk of relapse after autologous HCT [108,109]. In one study, which used a rat model of acute myeloid leukemia (AML), Hagenbeek and Martens demonstrated a 30% recovery of normal CFU-Ss but only a 1.4% survival of splenic colonies derived from the leukemic cell line. Allieri et al. cloned leukemia progenitor cells (AML-CFUs) from the peripheral blood of five patients with AML before and after cryopreservation and compared the recovery of these cells with the recovery of CFU-GMs and BFU-Es from marrow specimens from healthy donors. The percentage recovery of AML-CFUs was always less than 50% of the percentage recovery of the normal cells in a series of cryopreservation experiments. Questions about the relevance of the models studied by these investigators can be raised, and whether cryopreservation of cells reduces the risk of relapse after autologous transplantation is not answered by these two studies. The demonstration using genetically marked cells that cryopreserved marrow products can be a source of relapse in patients treated for AML, neuroblastoma, and chronic myeloid leukemia suggest that cryopreservation is not a highly efficient purging technique [110–112].

Summary

The cryopreservation of HCs was explored from the earliest attempts to understand HCT. It should be obvious from this discussion that current cryopreservation techniques are satisfactory for the treatment of many patients, but no study has accurately quantified the HC losses resulting from cell freezing and the impact of these losses on engraftment kinetics after HCT. Virtually all centers freeze cells with DMSO alone or in combination with HES. The similarities between the techniques used by different transplant centers are much greater than the differences. Current cryopreservation techniques may reduce the HC content of the product, risking a potential delay in engraftment if inadequate quantities of HCs are harvested and stored. Moreover, there is considerable but generally minor toxicity from the currently used cryoprotectants, and the equipment and processing techniques are expensive.

The ideal cryopreservation solution is one that will achieve reproducible high cell recoveries, allow rapid cooling to minimize laboratory processing times, and be free of the toxicities associated with DMSO. More recent studies are focused on developing an understanding of apoptosis as a result of cell cooling, as opposed to cell necrosis from ice crystal formation, which may lead to modifications of cryopreservation solutions. These modifications, possibly the simple addition of inhibitors of apoptosis, may enhance engraftment kinetics with considerable savings in patient-care costs.

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CHAPTER 43

Use of Recombinant Growth Factors after Hematopoietic Cell Transplantation

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Introduction

The field of autologous and allogeneic hematopoietic cell transplantation (HCT) has changed considerably during the last decade due to the availability of growth factor mobilized peripheral blood hematopoietic cells as a graft source. Despite the more rapid hematopoietic reconstitution compared to the use of marrow-derived grafts, high-dose chemotherapy preceding HCT still induces a period of severe marrow aplasia with the risk of infections and bleeding, requiring prophylactic and therapeutic interventions. Sepsis during the time of neutropenia represents a major problem and may result in transplant-related death.

Hematopoietic growth factors and other cytokines contribute to the regulation of cellular growth and differentiation of the lympho-hematopoietic system. Early-acting growth factors contribute to the differentiation of pluripotent stem cells, capable of self-renewal, into multipotent and committed progenitor cells. Late-acting growth factors lead to further differentiation, eventually giving rise to mature cell elements with distinct function. However, almost all growth factors have multiple biologic activities, partly overlapping or synergistic with other hematopoietic growth factors, and can act on more than one cell type (Table 43.1). It has been demonstrated in murine models that in addition to very primitive long-term hematopoietic cells, other progenitor cells with a lower proliferative potential and restricted differentiation capabilities contribute to the hematopoietic reconstitution after HCT [1,2,3]. Kinetic as well as cell purification studies suggest that hematopoietic reconstitution in humans is dominated by different types of short- and long-term repopulating cells after HCT [4,5]. Myeloid-restricted short-term repopulating cells play a major role during the first few months after transplantation and are followed by a second type of long-term repopulating cells able to regenerate myeloid as well as lymphoid lineages [6].

After the discovery and clinical development of hematopoietic growth factor, for example G-CSF between 1984 and 1991 [7], the clinical impact on regeneration of hematopoiesis after chemotherapy has been extensively studied, demonstrating that engraftment kinetics after HCT can be influenced by the use of recombinant hematopoietic growth factors. Numerous studies, including randomized trials, have established the clinical role of specific growth factors after HCT. In the setting of allogeneic transplantation, not only the regeneration of the hematopoietic system but also the restoration of the immune system and modulation of GVHD continue to be of the utmost importance.

Erythropoietin

Erythropoietin (EPO) was the first growth factor to be identified experimentally and to be made available as a recombinant protein. EPO is widely used for the successful treatment of anemia associated with renal failure. The anemia encountered in HCT recipients is partly due to relative erythropoietin deficiency. Frequently, patients are already anemic prior to transplantation and present with elevated serum EPO levels [8]. Inappropriately low EPO levels in relation to the severity of anemia during the period following HCT is attributed to causes such as toxic effects of high-dose therapy and drugs like cyclosporine on EPO-producing cells of the kidney, as well as inhibitory effects of inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) on hypoxia-induced EPO production.

Recombinant human EPO after autologous HCT

Several trials have addressed the value of recombinant human (rHu) EPO after transplantation to accelerate red cell regeneration and to decrease the need for red blood cell (RBC) transfusions. After autologous bone marrow transplantation (BMT), EPO has been used in phase II as well randomized phase III trials alone or in combination with granulocyte colony-stimulating factor (G-CSF) or granulocyte–macrophage colony-stimulating factor (GM-CSF) [9,10]. Although EPO was tolerated without side effects and an increase in reticulocyte counts was attained, no obvious benefit regarding transfusional requirements, engraftment or treatment outcome was observed.

In a small case series of 28 patients with religous objection to blood products a combination of EPO, G-CSF, intravenous iron, and epsilon aminocaproic acid was given after autologous peripheral blood hematopoietic cell transplantation (PBHCT). With two treatment-related deaths (8%) and four bleeding complications the median fall of hemoglobin level was 4.7 g/% and the median total number of days with platelet count <10,000/μL was 4 days. This approach was judged to be safe without the use of any blood products post autologous PBHCT [11].

rHuEPO after allogeneic HCT

After allogeneic marrow transplantation EPO enhanced reticulocytosis, increased hemoglobin levels and reduced time to transfusion independence, resulting in the need for fewer RBC transfusions.

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Table 43.1 Growth factors available for clinical use

Growth factor	Gene localization	Naturally induced by	Main effector lineage	Generic name
Erythropoietin	7q21	Hypoxia	Erythroid	Epoetin-alpha, -beta, darbepoetin-alpha
G-CSF	17q11.2–q12	IL-1, TNF-α, endotoxin	Myeloid	Filgrastim, pegfilgrastim, lenograstim, biosimilars
GM-CSF	5q31.1	TNF-α, lipopolysaccharide	Myeloid/macrophage	Molgramostin, sargramostim
IL-3	5q31.1	Activation	Progenitor/myeloid/lymphoid	–
SCF	12q22–24	Constitutive	Myeloid/lymphoid	Ancestim
MGDF/TPO	3q2–28	Constitutive (thrombocytopenia)	Megakaryocytic/progenitor	–
IL-11	19q13.3–13.4	IL-1	Lymphoid/myeloid/megakaryocytic	Oprelvekin
IL-2	4q26–28	Activated T lymphocytes	T/NK/B lymphocytes	Aldesleukin
KGF	15q15–21.1	Tissue damage, PDGF, TGF	Epithelial cells	Palifermin

G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; KGF, keratinocyte growth factor; MGDF, megakaryocyte growth and differentiation factor; PDGF, platelet-derived growth factor; SCF, stem cell factor; TGF, transforming growth factor; TNF-α, tumor necrosis factor alpha; TPO, thrombopoietin.

In a large multicenter, placebo-controlled, randomized trial, 106 patients were treated with EPO after allogeneic BMT and 109 patients with placebo [12]. Patients received either 150 IU/kg/day EPO or placebo as continuous intravenous (IV) infusion. Therapy started after BMT and was continued until independence from RBC transfusions for 7 consecutive days with stable hemoglobin levels greater or equal to 90 g/L or until day 41 after BMT. The reticulocyte counts were significantly higher with EPO from day 21 to day 42 after BMT. The median time to RBC transfusion independence was 19 days (range, 16.3 to 21.6) with EPO and 27 days (range, 22.3 to >42) with placebo ($p < 0.003$). A multivariate analysis showed that acute GVHD, major ABO blood group incompatibility, age above 35 years, and hemorrhage significantly increased the need for transfusions. After day 20, EPO significantly reduced the number of RBC transfusions in these patient groups [12].

In another phase III trial in allogeneic BMT, 50 patients were randomized to treatment with EPO ($n = 25$) or placebo ($n = 25$) [8]. EPO was given at 200 U/kg daily for 4 weeks and 200 U/kg twice weekly for a further 4 weeks. There were no differences between the two groups regarding time to engraftment, fever, hospitalization, GVHD, infections, hemorrhages, transplant-related mortality, relapse, and survival. However, more patients in the control group had an elevated serum creatinine (43% vs. 14%; $p = 0.04$). RBC transfusion requirements for the first 2 months after BMT were significantly lower in the EPO group compared with the control group (5 units vs. 10; $p = 0.04$). Time to unsupported Hb >70 g/L was shorter in patients treated with EPO (14 days vs. 24; $p = 0.03$). No effect was seen on platelet engraftment or the number of transfused platelet units [8].

Ninety-one patients between the ages of 17 and 58 years undergoing allogeneic transplants from sibling donors were entered into a double-blind randomized trial to evaluate the effect of EPO at a dose of 300 U/kg/day given thrice weekly by intravenous injection. Treatment ended when the hemoglobin exceeded 120 g/L and recommenced if hemoglobin fell below 120 g/L, at 150 U/kg/day. Patients received two units of erythrocytes when hemoglobin dropped below 85 g/L. In a multivariate analysis, the administration of EPO was associated with an 18% reduction in RBC transfusion requirement when other variables were taken into account [13].

Allogeneic HCT with major ABO blood group incompatibility may be associated with a markedly prolonged time to red blood cell engraftment, immune hemolysis or pure red cell aplasia. Reports suggested a curative effect of EPO for these patients [14,15]. A 32-year-old AML patient developed pure red cell aplasia after major ABO-incompatible BMT. After receiving rHuEPO and methylprednisolone she developed reticulocytosis and hemolysis. She promptly recovered from hemolysis and pure red cell aplasia after plasmapheresis [15]. In the era of reduced-intensity conditioning using fludarabine-based conditioning regimens, a higher incidence of pure red cell aplasia may be expected in patients with high antidonor isoagglutinins in comparison to myeloablative regimens [16].

The role of EPO was also examined in the context of RIC. A phase II trial addressed the role of EPO given either from day 0 (19 patients), or starting day 28 (27 patients) after reduced-intensity conditioning and allogeneic HCT. EPO was administered subcutaneously once weekly at a dose of 500 U/kg/week and 14 patients receiving no EPO served as control group. Up to 6 months post-transplant, Hb values were significantly higher in patients receiving EPO compared to those receiving no EPO, but transfusion requirements were decreased only in the first month in patients receiving EPO from day 0. Interestingly, a reduced T-cell chimerism below 60% negatively influenced Hb levels post-transplant [17].The frequency of and risk factors for persistent anemia and inadequate EPO secretion were investigated after allogeneic stem cell transplantation (SCT) [18]. Of 83 evaluable allo-HCT patients, 63 (76%) developed persistent anemia at a median of 34 (range: 30–244) days after allo-SCT. Forty-one (49%) patients had anemia considered as primary, and in all of them inadequate serum EPO levels (median 43.3, range: 2.5–134 mU/mL) were found. A high creatinine level during the first month after allo-HCT was associated with primary anemia [relative risk (RR) 2.5; $p = 0.01$]. Of the 41 patients, 35 received erythropoiesis-stimulating proteins (ESP). Transfusion independence and an Hb level higher than 10 g/dL was achieved in 29 of 30 (97%) evaluable patients.

Summary: clinical use of EPO after HCT

In summary, EPO stimulates erythroid engraftment after BMT resulting in a small but significant reduction of RBC transfusion requirements. Whereas trials after autologous transplantation showed marginal benefits, the beneficial effect after allogeneic HCT was more prominent. Anemia associated with impaired EPO secretion after allo-SCT is more frequent than generally recognized and it is associated with early post-transplantation renal damage. Late after allogeneic HCT individual patients with delayed or inadequate erythropoiesis with inappropriate reticulocyte counts and inadequately low serum EPO levels, for example in patients with renal impairment, chronic inflammation, or chronic GVHD, are likely to benefit from the application of EPO.

Granulocyte colony-stimulating factor

Granulocyte colony-stimulating factor (G-CSF) is produced by many cell types and is rapidly induced by inflammatory stimuli. In vivo studies demonstrated that G-CSF is a potent myeloid growth and differentiation factor. The dose-dependent increase of peripheral blood neutrophils is caused by expansion of the myeloid compartment in the marrow, an accelerated production of mature neutrophils and a rapid shift of mature neutrophils from the marrow sinusoids into the peripheral blood [7].

Side effects of rHuG-CSF administration are usually mild and include bone pain, occasionally low grade fever, and weight gain. After high-dose therapy and HCT, transient pulmonary infiltrates that cause dyspnea, especially during the early phase of hematopoietic engraftment, can occasionally be observed radiologically and clinically. High-dose corticosteroids and diuretics are the treatment of choice for this complication. The entity of the clinical findings of fever, capillary leak, and pulmonary infiltrates, frequently also including a skin rash, observed during engraftment after HCT, has been termed "engraftment syndrome" and post-transplant G-CSF increased the incidence of this syndrome [19].

Two recombinant preparations of G-CSF are commonly in use, filgrastim produced in *E. coli* and the glycosylated form, lenograstim, produced in a Chinese hamster ovary cell line. More recently, also various biosimilar generic G-CSF products have attained clinical approval. The difference between the various G-CSF products appears to be minor. Filgrastim is also available in a pegylated formula, pegfilgrastim, for single-dose use.. The term "G-CSF" is generally used for the various recombinant products, unless the pegylated variant is specified.

G-CSF after autologous HCT

G-CSF after autologous BMT

Early data suggested that, after autologous BMT, neutrophil recovery was accelerated in G-CSF-treated patients compared to historical controls. Neutrophil counts exceeded 0.5×10^9/L on the G-CSF arm at a mean of 11 days after marrow infusion compared with 20 days for controls, a significant difference. This reduction led to significantly fewer days of parenteral antibiotic therapy, 11 versus 18 days in controls, and less protective isolation in reverse-barrier nursing, 10 versus 18 days [20]. Similar data were obtained after autologous BMT in lymphoma patients [21].

Several large randomized trials have confirmed the significantly accelerated neutrophil engraftment after autologous BMT compared to placebo (Table 43.2). In the first, G-CSF was given to 315 patients after autologous or allogeneic BMT in a prospective randomized placebo-controlled multicenter trial [22]. One day after bone marrow infusion, 163 patients received lenograstim 5 μg/kg/day by 30-min infusion, and 152 patients received placebo daily for 28 days or until neutrophil recovery. Neutrophil recovery to above 10^9/L for 3 consecutive days was seen earlier in G-CSF-treated patients (16 vs. 27 days; $p < 0.001$). Time to neutrophil recovery above 0.5×10^9/L was reduced (14 vs. 20 days; $p < 0.001$). The difference was significant both in autograft (20 vs. 14 days; $p < 0.001$) and in allograft (20 vs. 14 days; $p < 0.01$) patients, in children (20 vs. 13 days; $p < 0.001$), and in adults. G-CSF-treated patients had fewer days of infection, of antibiotic administration, and also spent less time in hospital. However, the incidence of clinical and microbiologic sepsis was similar in both groups. There was no significant toxicity ascribed to G-CSF. Survival was similar between groups at both days, 100 and 365, after transplantation [22].

Table 43.2 Randomized placebo controlled trials of G-CSF following HCT

Number of patients enrolled	Type of transplant	G-CSF given from day	ANC >500/μl (day after transplantation)		*p* value	Reference/ Comment
			G-CSF	Placebo		
315	Auto/Allo BMT	+1	14	20	0.001	[22] Placebo controlled
43	Auto BMT	+1	10	18	0.0001	[23] Placebo controlled
41	Auto PBHCT ± BMT	+1	10	18	0.0001	[24] Placebo controlled
54	Auto BMT	+1	12 (14)	20	0.0004	[25] Placebo controlled
38	Auto PBHCT	+1	10	14	0.0001	[27] Placebo controlled
62	Auto PBHCT	+1	9	12,5	0.0001	[28] Placebo controlled
62	Auto PBHCT	+5	10	12	0.0008	[30] Placebo controlled
54	Allo PBHCT	±0	11	15	0.0082	[45] Placebo controlled
42	Allo PBHCT	+1	12	15	0.002	[46] Placebo controlled

Auto, autologous; Allo, allogeneic.

Patients with lymphoma were treated in a randomized, open-label trial to study the use of G-CSF as an adjunct to high-dose chemotherapy and autologous BMT [23]. Of 43 patients, 19 were randomized to receive filgrastim by continuous subcutaneous infusion at a dose of 10 μg/kg/day, 10 to filgrastim 20 μg/kg/day, and 14 to a parallel control group that received no filgrastim. The median time to neutrophil recovery greater or equal to 0.5×10^9/L after the day of autologous BMT was significantly accelerated to 10 days in the combined G-CSF groups compared with 18 days in control patients. The median number of platelet transfusions was identical in both groups. Clinical parameters, including the median number of days with fever (1 vs. 4) and neutropenic fever (5 vs. 13.5) were significantly improved in the G-CSF group compared to the control group. For patients treated with the two different dose levels of G-CSF, the neutrophil recovery and clinical results were similar [23].

Forty-one patients undergoing high-dose therapy followed by infusion of autologous PBHCT with or without bone marrow were randomized to receive G-CSF 5 μg/kg/day beginning on day + 1, or no G-CSF. [24]. The median time to a neutrophil count greater or equal to 500/μL was significantly shorter with 10.5 days in the G-CSF group compared to 16 days in the control group. G-CSF was associated with statistically significant reductions in the time to neutrophil engraftment among patients who received PBHC alone (11 vs. 17 days) and in patients who received PBHC in conjunction with bone marrow (10 vs. 14 days) [24].

In 54 patients with malignant lymphoma, hematopoietic recovery after high-dose chemotherapy and autologous BMT was compared between patients randomized to receive 10 or 30 μg/kg/day of filgrastim or no growth factor [25]. After high-dose chemotherapy with cyclophosphamide, VP-16, BCNU (CVB) or a BCNU, etoposide, cytosine arabinoside, melphalan (BEAM) regimen followed by autologous BMT, filgrastim was administered by continuous intravenous infusion from the first day after autologous BMT until neutrophil recovery. When the filgrastim groups were compared with the control group the major findings were: the median time to reach an ANC greater or equal 0.5×10^9/L was 20 days in the control group and 12 and 14 days, respectively, in the filgrastim groups ($p = 0.0004$). The duration of neutropenia (ANC $< 0.5 \times 10^9$/L) was reduced from

27 days in the control group to 11 and 13 days in the G-CSF groups ($p = 0.0001$). In addition, fewer days of febrile neutropenia were observed in the G-CSF groups (5 and 6 days) than in the control group (10 days; $p = 0.036$). No significant effects on the total number of days with fever were observed [25]. An accelerated neutrophil engraftment was also observed in 41 consecutive children undergoing autologous BMT for hematologic malignancies in comparison with a similar historical control group of 38 children who did not receive G-CSF after autologous BMT. Their ages ranged from 2 to 16 years (mean 7.2 years). G-CSF was given at a dose of 10 μg/kg/day IV in a 2-h infusion from day +1 until +28 or until the ANC was $>1 \times 10^9$/L [26].

G-CSF after autologous PBHCT

The results obtained after autologous BMT were essentially confirmed in several phase II and III trials (Table 43.2) addressing the role of G-CSF after myeloablative chemotherapy and autologous cytokine mobilized PBHCT [27–30]. Of note, since engraftment after PBHCT is generally accelerated compared to unstimulated BMT, the potential benefit of G-CSF given post PBHCT is likely to be smaller.

Thirty-eight patients with lymphoproliferative disorders were randomized to receive low-dose filgrastim (19 patients) or placebo (19 patients) beginning on the first day after HCT [27]. All patients received more than 2.5×10^6 CD34$^+$ cells/kg, which had been mobilized with chemotherapy and filgrastim 300 μg from day 5. Neutrophil engraftment was significantly more rapid in patients who received G-CSF, with a median number of days until ANC greater than 0.5×10^9/L of 10 (9 to 13) versus 14 (9 to 19). The time to reach an ANC greater than 1×10^9/L was 12 (9 to 14) versus 16 days (10 to 25). The total number of patients who required intravenous antibiotic therapy was lower in the G-CSF-treated group (68%) compared with the placebo group (89%) [27].

In another prospective study, 34 patients had been randomized to receive lenograstim and 28 to no growth factor [28]. The median time to ANC $>0.5 \times 10^9$/L was 9 days in the G-CSF arm versus 12.5 days in the no-G-CSF arm ($p = 0.0001$). Median days to platelet independence, platelet transfusions, incidence of infection, and RBC transfusion were the same in both arms [28].

Factors influencing hematopoietic engraftment were analyzed within the French multicenter LNH93-3 trial treating patients with aggressive high-grade NHL with intensive chemotherapy including BEAM and autologous PBHCT [29]. In the patient population treated with the same first-line regimen, bone marrow involvement and infusion of fewer CD34+ cells delayed platelet recovery. Administration of G-CSF after PBHCT significantly reduced neutropenia [29].

The effect on accelerated neutrophil engraftment by G-CSF appears to be preserved when the start of application is delayed to day +5 after HCT. Sixty-two adult patients were randomized to receive filgrastim after PBSC infusion ($n = 30$) and 32 patients, the control group, received no cytokines [30]. G-CSF was administered subcutaneously from day +5 in the treated group at a dose of 5 μg/kg body weight/day. Faster granulocyte engraftment was evident in the treated group (mean of 10 vs. 12 days to achieve $>0.5 \times 10^9$/L granulocytes; $p = 0.0008$), without differences in incidence and severity of infections, days of fever, or duration of antibiotic treatment between groups.

Several randomized trials showed no delayed neutrophil engraftment when G-CSF was not given before days +3, +5, +6, or +7 after PBHCT [31–33]. Early (day +1) or delayed (day +7) G-CSF after immunoselected CD34+ autologous PBHCT significantly accelerated ANC recovery but did not reduce the amount of supportive treatment or the duration of hospitalization in 21 consecutive patients with hematologic malignancies [34].

A randomized trial compared pegfilgrastim with filgrastim after high-dose melphalan and autologous PBHCT in 37 multiple myeloma patients receiving a single 6 mg dose of pegfilgrastim on day 1 post-transplant versus daily subcutaneous injections of filgrastim 5 μg/kg starting on day 5 post-transplant. The median duration of grade 4 neutropenia in the pegfilgrastim and filgrastim groups was 5 and 6 days, respectively (p = not significant). The results for the two groups were also not significantly different for time to neutrophil and platelet recovery, but incidence of febrile neutropenia (61.1% vs. 100%; $p = 0.003$) and duration of febrile neutropenia (1.5 vs. 4 days; $p = 0.005$), were lower in the pegfilgrastim arm demonstrating the safety and efficacy of this approach [35].

A single dose of 6 mg of PEG-filgrastim on day +5 was compared to G-CSF, given from day +2 until neutrophil recovery following high-dose melphalan and PBHCT for patients with multiple myeloma (MM) in an outpatient setting [36]. Overall, a second hospital admission was required in 36 procedures out of 161 (32%). Febrile neutropenia (FN) and severe mucositis were the most frequent causes of hospitalization. In the PEG group, readmission was needed in 6 out of 48 autografts (12%) as opposed to 30 out of 113 (26%) in the G-CSF subgroup ($p = 0.06$). The median time of hospital stay for readmitted patients was identical for the two subgroups (9 vs. 9 days; $p = 0.94$). One case of transplant-related mortality occurred in the whole patient series (0.6%). The authors concluded that autologous HCT on an outpatient basis is feasible and safe in patients with MM, the majority of whom are manageable at home. The administration of a single-dose PEG-filgrastim resulted in no different outcome in terms of safety and efficacy as compared to 8 days of G-CSF.

In a phase II study in patients receiving autologous HCT for MM and lymphoma 40 patients received pegfilgrastim 6 mg on day +1 and 40 were treated with daily filgrastim starting at median day +7 (5–7). Duration of neutropenia, intravenous antibiotic use, and hospitalization favored pegfilgrastim, while platelet engraftment, transfusion requirement, and fever duration were equivalent in both groups [37].

In a randomized study, 101 patients who were eligible for PBHCT and aged >18 years were identified before high-dose chemotherapy, after the harvesting and cryopreservation of peripheral blood progenitor cells [38] (Table 43.3). Eligible patients received either standard carmustine/etoposide/cytarabine/melphalan (BEAM) or carmustine/etoposide/cytarabine/cyclophosphamide (BEAC) high-dose chemotherapy. Before high-dose chemotherapy, patients were randomly assigned to receive pegfilgrastim 6 mg on day 1, or weight-based dose-adjusted filgrastim beginning on day 1 after transplantation until neutrophil engraftment. In this randomized trial, pegfilgrastim appeared equivalent to multiple daily doses of filgrastim [38].

In a double-blinded, placebo-controlled trial compared the efficacy, costs, and safety of single-dose pegfilgrastim (single 6 mg dose) versus daily filgrastim (5 μg/kg/day) 78 patients were enrolled [39]. Cytokines were started on day +1 post-transplant and continued to an absolute neutrophil count (ANC) of 5×10^9/L for 3 days or 10×10^9/L for 1 day. The median time to neutrophil engraftment (ANC $>1.5 \times 10^9$/L for 3 days or 5×10^9/L for 1 day) was the same in both groups (12 days). No differences in platelet engraftment (11 vs. 13 days), number of platelet transfusions (5 vs. 4), percent with positive cultures for bacterial pathogens (23% vs. 15%), days of

Table 43.3 Randomized trials of pegfilgrastim versus filgrastim following HCT

Number of patients enrolled	Type of transplant	CSF given from day	ANC >500/μL (day after transplantation)		*p* value	Reference
			Pegfilgrastim	Filgrastim		
78	Auto PBHCT	+1	12	12	n.s.	[39]
80	Auto PBHCT	+1	10.8	11.5	n.s.	[98]
151	Auto PBHCT	+5	FN 3.07	FN 3.29	n.s.	[99]
101	Auto PBHCT	+1	9.3	9.8	n.s.	[38]

Auto, autologous; FN, febrile neutropenia; n.s., not significant.

fever (1 vs. 2), deaths prior to engraftment (1 vs. 1), or duration of hospital stay (19 vs. 19 days) were seen between the pegfilgrastim and filgrastim groups, respectively, demonstrating the clinical equivalence of pegfilgrastim and filgrastim when given post-autologous PBHCT [39].

To address the question of the optimal application time 53 patients were randomly assigned to pegfilgrastim on day + 1 or day + 4 after autologous HCT [40]. Median time to neutrophil and platelet recovery was 10 days in both treatment arms and there was no difference regarding the incidence (67% vs. 60%; $p = 0.77$) or duration of febrile neutropenia episodes in both groups.

G-CSF after allogeneic HCT

Initial concerns over potential aggravation of GVHD or an increase of relapse in patients with myeloid leukemias tempered the use of growth factors after allogeneic BMT. Essentially, after allogeneic BMT the same effects as after autologous transplantation can be observed. Schriber et al. reported the experience with 50 patients treated at a single institution using G-CSF after allogeneic sibling ($n = 30$) and matched unrelated ($n = 20$) BMT [41]. The time to an ANC greater or equal 500/μL was significantly faster in patients who received G-CSF and cyclosporine and prednisone for GVHD prophylaxis when compared to historical control patients receiving the same GVHD prophylaxis without G-CSF (10 vs. 13 days; $p < 0.01$). A similar accelerated myeloid engraftment was observed for patients who received additional methotrexate for GVHD prophylaxis when compared to historical control patients receiving the same GVHD prophylaxis regimen (16 vs. 19 days; $p < 0.05$) [41]. Similar results were obtained in children [42].

Delayed treatment with G-CSF resulted in a reduction of G-CSF treatment from 19 days to 14 days ($p = 0.0017$) in 38 patients randomly assigned to receive G-CSF starting day +1 or +6 after allogeneic BMT [43]. Reducing the length of treatment by 5 days lowered G-CSF treatment costs by 26.3% and postponing treatment with G-CSF had no influence on the hematologic recovery after allogeneic BMT [43]. Similar results were obtained in 69 patients after unrelated donor BMT randomly assigned to G-CSF starting day 0, +5, or +10 resulting in equivalent rapid neutrophil engraftment [44].

A randomized, double-blind, placebo-controlled study was performed to determine the effects of G-CSF on hematopoietic recovery after allogeneic PBHCT from related donors [45]. Fifty-four patients were randomly assigned to receive daily G-CSF at 10 μg/kg or placebo starting on the day of transplantation. The median time to achieve an ANC greater than 0.5×10^9/L was 11 days (range, 9–20 days) for patients who received filgrastim compared with 15 days (range, 10–22 days) for patients who received placebo ($p = 0.0082$). The median time to achieve a platelet count exceeding 20×10^9/L was 13 days (range: 8–35 days) for patients who received G-CSF compared with 15.5 days (range: 8–42 days) for patients who received placebo ($p = 0.79$). There were no significant differences for RBC transfusion independence, in the incidence of acute GVHD, or 100-day mortality between the groups. The authors concluded that the administration of G-CSF appears to be a safe and effective supportive care measure following allogeneic peripheral blood HCT [45].

Forty-two adult recipients of allogeneic PBHCT from HLA-matched related donors were randomized to receive G-CSF 10 μg/kg/day SC from day 1 through neutrophil recovery, or no growth factor support after transplantation [46]. There was no significant difference between the two groups in the number of CD34+ cells infused (median, 4.8 vs. 4.3×10^6/kg). GVHD prophylaxis consisted of tacrolimus and steroids for nine patients and tacrolimus and mini-methotrexate for 33 patients. The group receiving G-CSF had a shorter time to neutrophil levels above 0.5×10^9/L (day 12 vs. day 15; $p = 0.002$) and to neutrophil levels over 1.0×10^9/L (day 12 vs. day 16; $p = 0.01$) [46].

After full haplotype-mismatched HCT in humans, the application of G-CSF after transplantation markedly delayed T-lymphocyte reconstitution and omitting G-CSF led to a more rapid increase of T cells and improved anti-infectious defense in this specific situation [47].

A retrospective analysis of the EBMT addressed the use of G-CSF after allogeneic HCT in 1789 patients with acute leukemia receiving BMT and 434 patients receiving PBHCT from HLA-identical siblings from 1992 to 2002 [48]. Among the BMT and PBHCT patients, 501 (28%) and 175 (40%), respectively, were treated with G-CSF during the first 14 days after transplantation. BMT and PBHCT patients treated with G-CSF had a faster engraftment of absolute neutrophils, but platelet engraftment was slower. In the BMT patients, GVHD grades II–IV was 50% in the G-CSF group versus 39% in the controls (RR, 1.33; $p = 0.007$, in the multivariate analysis). The incidence of chronic GVHD was also increased (RR, 1.29; $p = 0.03$). G-CSF was associated with an increase in TRM (RR, 1.73; $p = 0.00016$) and had no effect on relapse but reduced survival (RR, 0.59; $p < 0.0001$) and leukemia-free survival rates (LFS; RR, 0.64; $p = 0.0003$). No such effects of G-CSF were seen in patients receiving PBHC [48].

In a single center retrospective analysis the question was asked whether G-CSF might contribute to the incidence of GVHD [49]. Two hundred and sixty patients given G-CSF prophylaxis after allogeneic hematopoietic stem-cell transplantation were compared with 205 controls transplanted between 1993 and 2003. G-CSF hastened the engraftment of neutrophils, but that of platelets was delayed ($p < 0.0001$). The proportion of acute GVHD of grades II–IV was 29% in the G-CSF group and 19% in the controls ($p < 0.01$) and that of chronic GVHD was 54% and 43%, respectively ($p = 0.019$). G-CSF increased acute and chronic GVHD in patients preferentially conditioned with chemotherapy. Unexpectedly, it exacerbated acute GVHD in recipients of peripheral blood stem cells and enhanced chronic GVHD in bone marrow recipients. A multivariate analysis showed that both acute and chronic GVHD were associated with G-CSF. There was no significant difference between study groups regarding non-relapse mortality, relapse, or survival.

This question was again addressed in a more recent retrospective analysis of the EBMT studying the effects of growth factor therapy on outcome after allogeneic HCT with >9 years of follow-up [50]. Of 1887 adult patients with acute leukemia who received bone marrow from sibling donors and were treated with myeloablative conditioning, 459 (24%) were treated with growth factor. Growth factor hastened engraftment of neutrophils ($p < 0.0001$), but reduced platelet counts ($p = 0.0002$). GVHD-free survival (no acute GVHD grade II–IV or chronic GVHD) at 10 years was 12 ± 2% (±SE) in the growth factor group, as opposed to 17 ± 2% in the controls and this effect was independent of conditioning with or withour irradiation. Non-relapse mortality (NRM) was higher in the growth factor group. Both groups had similar probabilities of leukemic relapse. Leukemia-free survival (LFS) at 10 years was 35 ± 2% in those receiving growth factor prophylaxis, as opposed to 44 ± 1% in the controls. Prophylaxis with growth factor increased the risk of GVHD, did not affect relapse, increased NRM and reduced LFS >10 years after HSCT, regardless of conditioning with TBI [50].

Summary: clinical use of G-CSF after HCT

In summary, G-CSF application after allogeneic or autologous bone marrow or peripheral blood HCT significantly accelerates the time to neutrophil engraftment. Non-randomized retrospective studies suggest that growth factor therapy may negatively influence other parameters like platelet engraftment or GVHD. This benefical effect is more prominent after BMT and is preserved when the start of G-CSF application is delayed from day +1 to +5 or to +7 after HCT. The same applies to a single injection of pegfilgastrim: responses are no different whether the drug is given on day + 1 or day + 5. Whether the long-term impact of pegfilgrastim on GVHD may be different from filgrastim or lenograstim remains to be determined, similarly whether there might be a difference between unglycosylated and glycosylated variants of the drug and whether the new biosimilars may again exhibit subtle differences. All randomized trials have failed to show a clear beneficial effect on the outcome of patients after HCT.

A meta-analysis based on 34 randomized controlled trials of prophylactic G-CSF and GM-CSF after autologous and allogeneic HCT demonstrated a reduced risk of documented infections and duration of parenteral antibiotics but not in infection-related mortality [51]. The absolute decrease in the risk of documented infection was 8% and 13 patients would need to be treated with CSFs in order to prevent one infection. Subgroup analyses regarding autologous and allogeneic HCT, the use of G-CSF and GM-CSF, or PBHCT and BMT showed similar results.

In contrast to autologous HCT, the clinical situation is more complex after allogeneic HCT and the present data do not allow definite conclusions regarding specific patient situations. Despite the retrospective character of the EBMT studies mentioned earlier, its results showing a negative influence of G-CSF after allogeneic HCT remain a caveat. The potential immune modulating effects of G-CSF on mobilized stem cell grafts as well as G-CSF application to the recipient and a possible aggravation of an "engraftment syndrome" should be kept in mind when treating patients with G-CSF after allogeneic HCT. The use of G-CSF post allogeneic transplantation is associated with increased risk of GVHD and in subgroups NRM.

Granulocyte–macrophage colony-stimulating factor

Granulocyte–macrophage colony-stimulating factor (GM-CSF) can be produced by many different cell types, particularly activated T cells. In vitro, GM-CSF promotes the growth and expansion of granulocytic and monocytic, and, in combination with EPO, also multilineage colony-forming units. Multiple functions of mature neutrophils and macrophages, such as tumoricidal activity, superoxide production, antibody-dependent cell-mediated cytotoxicity, phagocytic activity, microbial killing, and secretion of other cytokines are stimulated [52,53]. Furthermore, GM-CSF, when used in vitro, induces the differentiation of malignant antigen-presenting cells with potent T-cell stimulatory capacity [54].

In humans, GM-CSF causes a dose-dependent increase mainly in blood neutrophils and eosinophils, but also macrophages and lymphocytes. There is no effect on the erythroid or megakaryocytic lineage. After application, patients may experience low-grade fever, fatigue, myalgias, and dyspnea. After higher doses, significant fluid retention, pericarditis, pleuritis, and a capillary leak syndrome may develop [55,56].

Commercially available recombinant preparations of GM-CSF (molgramostin and sargramostim) differ in their state of glycosylation. However, this difference does not appear to be clinically relevant.

While all earlier trials have focused on time to neutrophil recovery, this objective is today almost exclusively pursued by application of G-CSF. More recent trials have almost solely addressed the effects of GM-CSF in the context of immunotherapeutic approaches.

GM-CSF after autologous HCT

In a randomized, double-blind, placebo-controlled trial, 128 patients undergoing autologous BMT for lymphoid malignancies were enrolled [57]. Fifty-six patients received GM-CSF in a 2-hour IV infusion daily for 21 days, starting within 4 hours of marrow infusion, and 63 patients received placebo. The patients given GM-CSF had a recovery of the neutrophil count to 500×10^6/L 7 days earlier than the patients who received placebo (19 vs. 26 days; $p < 0.001$), had fewer infections, required 3 fewer days of antibiotic administration (24 vs. 27 days; $p = 0.009$), and required 6 fewer days of initial hospitalization (median, 27 vs. 33 days; $p = 0.01$). There was no difference in the survival rate at day +100 after HCT [57].

Another study comparing the use of G-CSF (5 μg/kg/day) with sargramostim (GM-CSF) 500 μg/day from day 0 until neutrophil recovery (ANC > 1500/μL) in patients with breast cancer or myeloma who underwent PBHCT showed faster neutrophil recovery with G-CSF in comparison to GM-CSF [58].

GM-CSF after allogeneic HCT

GM-CSF was also tested in randomized trials after allogeneic transplantation. In a randomized double-blind trial in patients undergoing HCT for leukemia, 20 received GM-CSF and 20 received placebo, for 14 days after allogeneic, matched sibling BMT [59]. The neutrophil count recovered to 0.5×10^9/L 3 days earlier in the GM-CSF group than in the placebo group (not significant). No difference in GVHD or relapse was reported.

In a prospective randomized placebo controlled trial involving 57 patients receiving T-cell-depleted marrow transplants, GM-CSF led to higher neutrophil and monocyte counts for 6 to 10 days during the time period of 2 to 3 weeks after transplantation resulting in fewer pneumonias [60].

Long delay in hematologic recovery after BMT, especially graft failure, can extend and amplify the risks of infection and hemorrhage, compromise patients' survival, and increase the duration and cost of hospitalization. GM-CSF was evaluated in 37 patients with marrow graft failure after allogeneic ($n = 15$), autologous ($n = 21$), or syngeneic ($n = 1$) bone marrow transplantation [61]. GM-CSF was administered by 2-hour infusion at doses between 60 and 1000 μg/m^2/day for 14 or 21 days. At doses of less than 500 μg/m^2, GM-CSF was well tolerated and did not exacerbate GVHD in allogeneic transplant recipients. No patient with myelogenous leukemia relapsed while receiving GM-CSF. Twenty-one patients reached an ANC equal to or above 0.5×10^9/L within 2 weeks of starting therapy while 16 did not. None of seven patients who received chemically purged autologous marrow grafts responded to GM-CSF. The survival rates of GM-CSF-treated patients were significantly better than those of a historical control group [61].

Summary: clinical use of GM-CSF after HCT

In summary, GM-CSF accelerates neutrophil engraftment after autologous and allogeneic transplantation. However, due to a lack of advantage over G-CSF, GM-CSF is not routinely used for this purpose. GM-CSF has been used effectively in bone marrow failure states. The immunostimulating effects of GM-CSF with its capacity to differentiate antigen-presenting cells are employed with or without concomitant donor lymphocyte infusions for relapse of leukemia after allogeneic transplantation and a beneficial role of the combination of alpha-interferon and GM-CSF is suggested [62]. Furthermore GM-CSF may have a place as an adjunct in post-transplant vaccination trials and in anti-infectious strategies, for example in treating patients with invasive fungal infections by augmenting the phagocytic capacities of monocytes and macrophages. However, at this point the clinical use of GM-CSF is primarily in clinical studies in the context of post-transplant immunotherapy.

Other hematopoietic growth factors and cytokines

While many growth factors and cytokines have effects on hematopoiesis and its niche, only a few have been investigated in the context of hematopoietic cell transplantation. After initial promising preclinical results, most have been withdrawn from further clinical development because of untoward effects and lack of efficacy. The results for some of these agents are summarized here.

Interleukin-3

Interleukin-3 (IL-3), also known as multi-CSF, is a multilineage hematopoietic growth factor produced by T lymphocytes and mast cells that acts on early and committed cell populations of all lineages. In vivo, IL-3 administration is often accompanied by severe side effects with fever, malaise, chills, headache, arthralgias, and urticaria [63]. Because of these side effects, a dose-escalating study using IL-3 alone had to be stopped at 2 μg/kg/day [63]. Used alone, IL-3 did not enhance neutrophil engraftment compared to the use of GM-CSF [64].

In summary, due to the side effects and only modest positive impact on hematopoiesis, IL-3 or its modifications have not been licensed for clinical use after HCT.

Stem cell factor

Stem cell factor (SCF), also known as steel factor, kit-ligand, or mast cell growth factor promotes the proliferation and differentiation of the most primitive hematopoietic progenitor cells into committed progenitor cells. Recombinant SCF (rHuSCF, ancestim) has been developed for clinical use in combination with G-CSF to optimize stem cell mobilization from the marrow into the peripheral blood for apheresis and to provide a sustained increase in the number of HSC capable of engraftment [65]. When used for hematopoietic cell mobilization, a considerable number of patients reacted with delayed-type hypersensitivity like skin rash, dyspnea, angioedema, and cardiovascular symptoms.

No trials with the use of SCF after HCT have been published. Presently, there is no proven indication for SCF after transplantation and there is no licensed product available for clinical use.

Thrombopoietin/megakaryocyte growth factor

Thrombopoietin (TPO) stimulates the growth and differentiation of megakaryocytes and proplatelets via the receptor c-mpl. It is predominantly produced by hepatocytes and serum levels are inversely correlated with platelet counts.

Two ligands for the mpl receptor with broad in vitro megakaryocytic proliferative, maturational, and differentiation capacity have been developed and tested in clinical trials. One is a modified (PEGylated) form of a recombinant truncated mpl ligand, called megakaryocyte growth and development factor (MGDF) [66]. The other is the recombinant glycosylated version of the naturally occurring thrombopoietin (TPO) molecule [67].

In a randomized trial, 47 patients with stage II, III, or IV breast cancer undergoing autologous HCT were allocated to placebo ($n = 13$) or to one of five sequential dose cohorts of PEG-rHuMGDF ($n = 34$) resulting in a median time to platelet recovery of 11 and 12 days for the placebo and combined PEG-rHuMGDF groups, respectively [68].

The role of recombinant megakaryocyte growth factors after HCT has not been thoroughly tested and remains unclear. Furthermore, due to reports on the development of neutralizing antibodies and thrombophilia in vivo, the above-mentioned recombinant products have not been licensed for clinical use.

After the experience of these first-generation thrombopoietic growth factors, second-generation thrombopoietic factors have been developed as summarized in a review by Kuter [69]. AMG 531 is a TPO-mimetic peptide linked to human IgG fragments and weekly subcutaneous applications in healthy volunteers and patients with immune thrombocytopenia resulted in elevated platelet counts without major side effects apart from mild headache at the day of injection. This agent is now licensed under the name "romiplostin" for treatment of immune thrombocytopenia or thrombocytopenia induced by chronic liver disease, as well as the orally available small molecules eltrombopag with TPO agonistic properties [70,71].

Romiplostin was successfully used for the treatment of acute GVHD-associated thrombocytopenia [72] or prolonged thrombocytopenia after HCT [73].

Interleukin-11

Interleukin-11 (IL-11) is a naturally occurring cytokine produced by fibroblasts and bone marrow stromal cells and is a growth factor with pleiotropic effects overlapping with other growth factors. In vivo, IL-11 administration stimulates megakaryopoiesis and increases peripheral platelet and neutrophil counts [74], an effect that can be enhanced by combination with IL-3. A recombinant preparation of IL-11, oprelvekin, produced in *E. coli*, is licensed for clinical use for the prevention of severe thrombocytopenia and the reduction of the need for platelet transfusions following myelosuppressive chemotherapy. Major side effects include fluid retention and edema.

A placebo-controlled randomized trial with 80 breast cancer patients tested the effects of recombinant IL-11 on platelet recovery after high-dose chemotherapy and autologous transplantation with peripheral blood hematopoietic cell support. The results did not demonstrate that rhIL-11 treatment significantly decreased platelet transfusion [75].

In a randomized double-blind pilot study, IL-11 was administered with cyclosporine/MTX prophylaxis after cyclophosphamide/TBI conditioning and allogeneic stem cell transplantation for hematologic malignancies [76]. Patients received IL-11, 50 μg/kg/day SC or placebo. Only 13 patients (10 IL-11, three placebo) were enrolled because the early stopping rule for mortality was enforced. Of 10 evaluable patients who received IL-11, four died by day 40 and one died on day 85. Deaths were attributable to transplant-related toxicity. Patients receiving IL-11 had severe fluid retention and early mortality, making it impossible to determine whether IL-11 given in this schedule can reduce the rate of GVHD. Grade II–IV acute GVHD occurred in two of eight evaluable patients on IL-11 and one of three patients on placebo. The primary adverse events of the study were severe fluid retention resistant to diuresis (average weight gain 9 +/– 4%) and multiorgan failure in five of 10 evaluable patients. The authors concluded that IL-11 as administered in this trial for GVHD prophylaxis in allogeneic transplantation cannot be recommended [76].

In view of these marginal benefits and the serious side effects of IL-11, no further trials have been reported in recent years.

Cytokines and growth factors for immunotherapy after transplantation

While many cytokines and growth factors for immunotherapy after transplantation have been studied in preclinical settings, only a few have entered the clinical stage.

Preclinical studies and in some cases early clinical studies have shown that exogenous keratinocyte growth factor (KGF), IL-7, Flt-3 ligand (Flt3L), and growth hormone (GH), IGF-1, IL-7, IL-15, and IL-12 may protect the thymus, enhance thymic cellularity, and enhance post-transplant recovery of T cells in various experimental settings. Of these, only KGF has been approved by the US FDA for the prophylaxis of mucositis in recipients of high-dose chemotherapy (including HCT).

GM-CSF for immunotherapy after transplantation

A variety of early clinical trials in patients with various hematologic malignancies and solid tumors has investigated the post-transplant application of GM-CSF, frequently in combination with IL-2 as well as monoclonal antibodies directed against cancer cells. All studies demonstrated feasibility with acceptable toxicity, enhanced in vitro immune responses, for example in cytotoxicity assays, but failed to demonstrate a clinical benefit.

Interleukin-2

Interleukin-2 (IL-2) is produced by activated T lymphocytes and is an important regulator of the immune system acting on T-, B-, and NK cells. IL-2 knockout mice develop T cells normally, but they suffer from generalized fatal immunoproliferative disorders and loss of self-tolerance.

Recombinant IL-2, aldesleukin, is licensed for the treatment of metastatic renal cell carcinoma. Several phase II trials that used IL-2 after autologous HCT for patients with lymphoma or AML as an immunomodulator to reduce relapse rates demonstrated feasibility; however, without large randomized trials the role of IL-2 in the context of autologous HCT remains unclear.

In a pilot trial the effects of low-dose IL-2 treatment in 12 patients with metastatic cancer and 9 patients with chronic myeloid leukemia after allogeneic HCT was examined [77]. Overall, IL-2 treatment resulted in a 1.9-median-fold increase in the frequency of $CD4^+CD25^+$ cells in peripheral blood as well as a 9.7-median-fold increase in FOXP3 expression in $CD3^+$ T cells [77]. Successful treatment with IL-2 0.5 mIU/m^2/day continuous IV of a patient with progressive multifocal leukencephalopathy developing after allogeneic HCT was reported [78], highlighting the role of IL-2 as a potent immunomodulator after allogeneic HCT. As discussed earlier, the stimulation of APC by G-CSF and expansion of T effector cells by IL-2 is being investigated in several early clinical trials. IL-2 is also used to enhance engraftment and expansion of adoptively transferred T and NK cells [79].

IL-2 was combined with donor lymphocyte infusion (DLI) after chemotherapy in patients with relapsing leukemia after allogeneic HCT [80]. Seventeen patients received DLI (1×10^8 CD3/kg for patients with related donors, and 0.1×10^8 CD3/kg for those with unrelated donors) and an escalating dose of induction IL-2 [1.0, 2.0, or 3.0×10^6 IU/m^2/day representing levels I ($n = 7$), Ia ($n = 9$), and II ($n = 1$)] for 5 days followed by maintenance (1.0×10^6 IU/m^2/day) for 10 days as a continuous intravenous infusion. Unacceptable IL-2-related toxicities developed in one patient at level I, two at level Ia, and one at level II. Grades III–IV acute graft-versus-host disease (aGVHD) developed in five patients, and extensive chronic GVHD (cGVHD) developed in eight. Eight patients had a complete remission after chemotherapy prior to DLI, and two additional patients had a complete remission after DLI plus IL-2 therapy. The authors concluded, that the maximal tolerated induction dose of IL-2 combined with DLI appears to be 1.0×10^6IU/m^2/day, and IL-2 administration after DLI may increase the incidence of cGVHD [80].

Another strategy for administration of IL-2 at low doses is the attempt to restore the balance of the immune system by expanding regulatory T cells [81]. In an observational cohort study, patients with cGVHD that was refractory to glucocorticoid therapy received daily low-dose subcutaneous IL-2 (0.3×10^6, 1×10^6, or 3×10^6IU per square meter of body-surface area) for 8 weeks. After a 4-week hiatus, patients with a response could receive IL-2 for an extended period. A total of 29 patients were enrolled. None had progression of chronic GVHD or relapse of a hematologic cancer. The maximum tolerated dose of IL-2 was 1×10^6IU per square meter. The highest dose level induced unacceptable constitutional symptoms. Of the 23 patients who could be evaluated for response, 12 had major responses involving multiple sites. The numbers of CD4+ Treg cells were preferentially increased in all patients, with a peak median value, at 4 weeks, that was more than eight times the baseline value ($p < 0.001$), without affecting $CD4^+$ conventional T (Tcon) cells. The Treg : Tcon ratio increased to a median of more than five times the baseline value ($p < 0.001$). The Treg cell count and Treg : Tcon ratio remained elevated at 8 weeks ($p < 0.001$ for both comparisons with baseline values), then declined when the patients were not receiving IL-2. The increased numbers of Treg cells expressed the transcription factor forkhead box P3 (FOXP3) and could inhibit autologous Tcon cells. Immunologic and clinical responses were sustained in patients who received IL-2 for an extended period, permitting the glucocorticoid dose to be tapered by a mean of 60% (range, 25 to 100). The authors concluded, that daily low-dose IL-2 was associated with preferential, sustained Treg cell expansion in vivo and amelioration of the manifestations of chronic GVHD in a substantial proportion of patients [81].

Keratinocyte growth factor

Keratinocyte growth factor (KGF), also called fibroblast growth factor 7 (FGF-7), is a mediator of epithelial cell proliferation and a growth factor for hepatocytes and pneumocytes. KGF is naturally produced in mesenchymal cells and acts on a wide variety of epithelial cells. KGF has been shown to be protective in radiation- or chemotherapy-induced organ damage. In murine models of allogeneic transplantation it protected the gut from lethal GVHD [82]. Furthermore, pharmacologic doses of KGF allow regeneration of GVHD-induced thymic damage and therefore KGF appears to exert a potent effect on thymic epithelial cell function, which in turn allows for normal T lymphopoiesis to occur during acute GVHD [83].

Palifermin, the N-truncated form of recombinant human keratinocyte growth factor-1, is available for the prevention of mucosal damage after intensive chemo- or radiation therapy [84]. Several studies have shown the beneficial effects on oral and intestinal mucositis [85].

An interesting case report also suggests a therapeutic benefit in the treatment of post-transplant cystitis [86].

KGF in autologous HCT

In a case series of patients treated prophylactically with KGF during high-dose chemotherapy and autologous HCT, reduction of severe oral mucositis and related symptoms but not infections, dietary intake, time to engraftment, or cumulative dose and duration of narcotic administration compared to patients without KGF treatment, was observed [87].

KGF proved to significantly reduce duration and severity of oral mucositis after high-dose TBI-containing therapy in randomized trials [88,89]. In one trial KGF 60 μg/kg/day was given for 3 days prior to TBI and at days 0, 1, and 2 after transplantation and the incidence of oral mucositis of World Health Organization (WHO) grade 3 or 4 was 63% in the KGF group and 98% in the placebo group ($p < 0.001$) The median duration of oral mucositis of WHO grade 3 or 4 was 3 days (range, 0 to 22) in the KGF group and 9 days (range, 0 to 27) in the placebo group ($p < 0.001$) and patients reported less soreness and use of opioid analgesics in the KGF group [89]. Side effects were skin rash, pruritus, erythema, and taste alteration with mild to moderate severity, and were transient.

In all clinical studies in autologous HCT a beneficial effect on mucositis has been observed. However, no survival benefit was seen in any of these studies [90–92].

KGF in allogeneic HCT

KGF was tested in a randomized, double-blind, placebo-controlled, dose-escalation study regarding safety, engraftment, and influence on GVHD ($n = 69$ patients) as compared to placebo ($n = 31$) in patients conditioned with Cy/TBI or Bu/Cy and given methotrexate along with a calcineurin inhibitor (cyclosporine, tacrolimus) for GVHD prophylaxis. All patients received three doses before conditioning and either three (cohort 1), six (cohort 2), or nine (cohort 3) doses after HCT. Palifermin doses were 40 μg/kg/day (cohort 1 only) or 60 μg/kg/day (all cohorts). Six patients (two on placebo, four on palifermin) experienced a total of 11 dose-limiting toxicities (most often skin, respiratory, or oral mucositis). The most common adverse events included edema, infection, skin pain, or rash. Palifermin was associated with reduced incidence and severity of mucositis in patients conditioned with Cy/TBI but not Bu/Cy. KGF was regarded as safe in allogeneic HCT but had no significant effect on engraftment, acute GVHD, or survival in this trial [93]. With longer follow-up there were no additional differences between the palifermin-treated and placebo cohorts with respect to CMV or invasive fungal infections, chronic GVHD, or long-term survival between cohorts [94].

The question of immune reconstitution after allogeneic HCT was addressed by a follow-up analysis [95]. It was investigated whether palifermin-treated patients ($n = 69$) had improved absolute lymphocyte counts (ALCs) at days 30, 60, and 100 after transplant compared to placebo-treated patients ($n = 31$). No statistically significant difference in ALC was noted at these time points. Additionally, there was no difference in ALC between patients who received low- (240 μg/kg) versus high- (720 μg/kg) dose palifermin. Patients with a day 30 ALC of $>600 \times 10^6$/L had a trend towards improved progression-free survival (49% vs. 29%; $p = 0.07$), lower transplant-related mortality (18% vs. 35%; $p = 0.1$), and grade II–IV aGVHD (31% vs. 47%; $p = 0.14$), but this was not influenced by palifermin therapy. In contrast to animal studies, peri-transplant palifermin did not accelerate early lymphocyte recovery following myeloablation and a T-replete allogeneic graft.

In a multicenter study, 30 patients undergoing matched related or unrelated allogeneic HCT for leukemia were treated with palifermin, and retrospectively compared to a matched control group [96]. Palifermin recipients transplanted with an unrelated donor showed a significant reduction of severity, incidence, and duration of oral mucositis WHO grades 2–4. In addition, in the palifermin group the use of opioid analgesics and the duration of total parenteral nutrition decreased, whether stem cells were used from matched related or unrelated donors. No beneficial influence of palifermin on the incidence and severity of aGVHD was apparent. The incidence and duration of febrile neutropenia, documented infections, hematopoietic recovery, or overall survival (OS) remained unchanged. The most common adverse effects included rash or erythema, generally mild and transient in appearance. In a multicenter, non-randomized, matched-control study no difference for early OS, incidence and severity of acute and chronic GVHD was observed in 36 patients with hematologic diseases treated with allogeneic HCT and palifermin [97].

The benefits of palifermin appear primarily to be limited to ameliorating mucotoxicity when given to allogeneic HCT recipients with no additional benefits on morbidity or survival.

Conclusion

In several situations hematopoietic growth factors have a defined role after HCT. In particular, randomized trials documented faster erythroid engraftment with EPO after allogeneic BMT, and G-CSF resulted in accelerated neutrophil engraftment after allogeneic and autologous marrow as well as peripheral blood hematopoietic cell transplantation. Regarding other endpoints the results are equivocal. The value of these growth factors given after HCT has to be seen in the light of pros and cons of the individual patient and specific transplant situation. Faster engraftment is only one aspect and other factors, such as days in hospital or in isolation, transfusion requirements, possible cytokine-induced engraftment syndromes, or influence on the immune system, especially increased rates of GvHD after allogeneic HCT, as well as costs have to be taken into consideration. Studies using other cytokines are continuing. The benefits of all agents appear to be in the area of supportive care, while survival benefits have yet to be demonstrated.

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CHAPTER 44

Hematopoietic Cell Transplantation from Human Leukocyte Antigen Partially Matched Related Donors

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Introduction

Hematopoietic cell transplantation (HCT) from human leukocyte antigen (HLA)-matched siblings has become the treatment of choice for many hematologic diseases, but fewer than 40% of patients have an HLA-matched sibling [1]. Registries of HLA-typed volunteers have been established worldwide to provide HLA-matched unrelated donors for HCT (see Chapter 45). The chance of finding an unrelated donor matched for HLA-A, -B, -C, and -DR depends on the HLA diversity of the population and varies with race, ranging from 65% in Caucasians to less than 20% for US ethnic minorities [2,3]. Umbilical cord blood offers the advantages of easy procurement, no risk to the donors, low risk of transmissible infections, and immediate availability of the cryopreserved graft (see Chapter 39). However, engraftment is a major concern when the harvested cord blood contains a low number of mononuclear cells. Older patient age and HLA disparity are poor risk factors for engraftment and survival after transplantation of unrelated cord blood hematopoietic cells [4,5].

An alternative source of hematopoietic cells is from relatives who are partially matched for HLA antigens. Almost all patients have at least one HLA partially matched family member, parent, sibling, or child, who is immediately available as donor. Transplants of T-replete marrow or growth factor-mobilized peripheral blood progenitor cells (PBPCs) from relatives mismatched for a single HLA-A, -B, or -DR antigen have met with acceptable success rates using standard protocols commonly employed for transplants from HLA-identical siblings [6,7]. Conversely, transplants of T-replete marrow from donors mismatched for two or three HLA-A, -B, and -DR antigens have resulted in an extremely high incidence of severe acute graft-versus-host disease (GVHD) when patients receive postgrafting immunosuppression with methotrexate alone or in combination with calcineurin inhibitors [6,8]. A variety of approaches for ex vivo or in vivo T cell depletion has decreased the incidence of acute and chronic GVHD and has permitted long-term survival after HCT from highly mismatched relatives [9–12].

In this chapter, we first present principles for the selection of related donors with the least degree of HLA disparity, and later present more recent developments in the field of GVHD prevention after transplants from incompatible relatives.

Donor selection

Function and polymorphism of HLA

The HLA system (described in detail in Chapter 10) includes at least 12 genetic sites, named HLA loci, located on the short arm of human chromosome 6. Each HLA locus is highly polymorphic because it is occupied by multiple alternative forms of an HLA gene, designated HLA alleles, any of which may be carried by a given individual. HLA alleles encode class I HLA-A, -B, and -C antigens, and class II HLA-DR, -DQ, and -DP antigens. Each of these genes regulates transplantation responses [6,13–16]. Class I HLA antigens are expressed on the surface of all nucleated cells in the body, while class II HLA antigens are expressed on the surface of antigen-presenting cells (APCs) such as dendritic cells, monocytes, B cells, and activated T cells. It is the function of HLA molecules to bind antigenic peptides and activate the immune response in a specific manner. In order to accommodate the need for the binding of ever-changing environmental and microbial antigens, HLA molecules have evolved through gene duplication, gene conversion, recombination, and point mutation to acquire an enormous degree of polymorphism, most evident across different ethnic groups.

Class I HLA molecules present antigenic peptides and activate cytotoxic CD8 T cells, while class II HLA molecules present antigenic peptides and activate helper CD4 T cells. Both classes of HLA molecule lead to T-cell activation through the binding of specific T-cell antigen receptors. In the case of HLA-incompatible HCT, donor and recipient differ not only for one or more types of HLA molecule, but also for the thousands of antigenic peptides that each mismatched HLA molecule can bind and present to foreign T cells [17]. By this process, HCT leads to the activation of an enormous number of donor T cells that mount a response against the host and vice versa. The strength of the immune response depends on the degree of HLA mismatch. T-cell recognition of antigenic peptides presented by mismatched HLA molecules can result both in GVHD and graft rejection. Low levels of T-cell recognition define "permissible" mismatches, and these have been associated with improved transplantation outcomes when compared to "non-permissible" mismatches [15,18,19].

HLA class I molecules also bind specific receptors on natural killer (NK) cells. Recent developments on the role of NK cells in transplantation are presented later in this chapter, in the section on T-depleted grafts, where the effects of NK cell function have become apparent. HLA class I and II molecules also function as antigens by eliciting antibody responses by B cells. It is thought that

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anti-HLA antibodies can mediate a hyperacute rejection of allogeneic HCTs [20].

HLA haplotypes and segregation in families

The HLA antigens inherited together from one chromosome of each parent are referred to as an HLA haplotype (Figure 44.1). When a family study is performed in search of an HLA-identical sibling donor, parents should also be typed for HLA-A, -B, and -DR, and each of the four parental HLA haplotypes identified. Parental haplotypes can segregate among the offspring in four different combinations, and the chance that any one sibling is HLA identical with another is 25%. If an HLA-identical sibling is not found, it is possible to find family members who are partially matched for HLA, even though they have not inherited the same two haplotypes, because certain HLA antigens and haplotypes are frequent. The parental haplotypes should be reviewed for homozygosity or sharing of antigens. The parents, siblings and children, and other relatives who share a haplotype with the patient may have a second haplotype that is partially matched with the patient.

Figure 44.1(a) illustrates a family in which the parents have matching haplotypes, "b" and "c." The patient (sib 1, "a/c") has a unique HLA type, and none of the siblings is HLA identical with the patient. However, the patient and the father ("a/b") share the paternal "a" haplotype and are matched for the HLA-A, -B, and -DR antigens of their unshared haplotypes ("b" and "c"). Figure 44.1(b) illustrates a family in which the mother ("c/d") is HLA homozygous. The patient (sib 1, "a/c") and sib 2 ("a/d") share the paternal "a" haplotype and each has inherited one of the two maternal haplotypes ("c" and "d"). Because of the serendipitous similarity of the maternal "c" and "d" haplotypes, sib 1 and sib 2 are HLA matched.

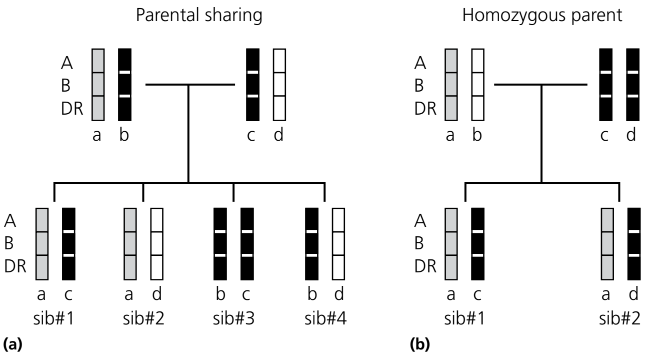


Figure 44.1 Segregation of human leukocyte antigen (HLA) haplotypes and partial sharing of HLA antigens within a family. (a) The two parental haplotypes "b" and "c" are identical, so that sibling 1 (haplotypes "a" and "c") is compatible with one of these parents (haplotypes "a" and "b"). (b) One parent is HLA homozygous (haplotype "c" and "d" being identical), so that sibling 1 (haplotypes "a" and "c") and sibling 2 (haplotypes "a" and "d") are compatible.

In haploidentical sibling HCT, the mismatched haplotype can originate from either of the parents. In Figure 45.1(a), the patient (sib 1, "a/c") is mismatched with sib 2 ("a/d") for non-inherited paternal antigens (NIPA) on haplotypes "c" versus "d," while another patient ("a/d" that is not shown) is mismatched with sib 4 ("b/d") for non-inherited maternal antigens (NIMA) on haplotype "a" versus "b." Donor exposure to non-inherited maternal HLA antigens benefits patients undergoing bone marrow transplantation. In one study, sibling transplantations mismatched for NIMA had similar rates of graft failure but lower rates of acute GVHD ($p < 0.02$) than those mismatched for NIPA [21]. Mother-to-child transplantations were associated with improved outcomes than father-to-child transplantations in some but not all studies [21–23].

Donor and recipient matching

Potential donors are those relatives who share by inheritance one HLA haplotype with the patient and are variably mismatched for 0, 1, 2, or 3 HLA-A, -B, and -DR loci of the unshared haplotype. A more precise classification of matching, useful in predicting the risk of rejection and GVHD after HLA-incompatible T-replete transplants, takes into consideration the vector of incompatibility in situations where donor or recipients are homozygous at one of the mismatched loci (Table 44.1) [8]. If recipient and donor are overall incompatible for one HLA locus but the recipient is homozygous at the mismatched locus, the mismatch does not contribute a risk for GVHD [8,24]. Because HLA-homozygous recipients are matched with their donors in the direction of GVHD, transplants incompatible for one HLA locus in homozygous recipients are associated with lower incidence of acute GVHD than in heterozygous recipients (Figure 44.2) [8]. The incidence of acute GVHD for homozygous recipients of one-locus-incompatible transplants is similar to that seen in HLA-matched transplants. Conversely, if recipient and donor are overall incompatible for one HLA locus but the donor is homozygous at the mismatched locus, the mismatch does not contribute a risk for graft rejection [20,25].

DNA sequencing can distinguish thousands of distinct HLA alleles, each designated from a letter that represent the locus followed by an asterisk and a unique number with two doublets separated by colons (i.e., HLA-A*02:01). Many alleles are expressed with a unique

Table 44.1 Mismatching according to vector of genetic disparity for human leukocyte antigen (HLA)-A, -B, and -DR

Example	Haplotype	Recipient	Donor*	Vector†		
				Overall	Rejection	Graft-versus-host disease
Heterozygous recipient and donor	Mismatched	**A2**, **B44**, **DR7**	**A3**, **B7**, **DR2**	3	3	3
	Shared*	A1, B8, DR3	A1, B8, DR3			
Homozygous recipient	Mismatched	A1, B35, DR1	**A3**, B35, DR1	1	1	0
	Shared	A1, B8, DR3	A1, B8, DR3			
Homozygous donor	Mismatched	**A2**, **B44**, DR4	A1, B8, DR4	2	0	2
	Shared	A1, B8, DR3	A1, B8, DR3			

Incompatible antigens are represented in bold.
*Recipient and donor are related and share HLA haplotype.
†Number of mismatches at HLA-A, B or DR loci.

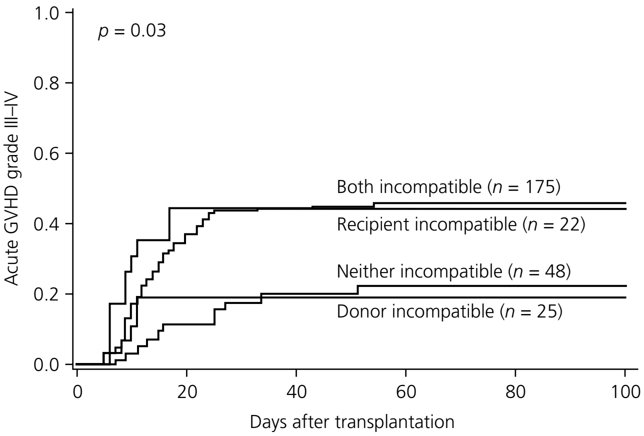


Figure 44.2 Effect of the vector of human leukocyte antigen (HLA) mismatch on acute graft-versus-host disease (GVHD). Probability of grade III–IV acute GVHD in patients who received a marrow transplant from a haploidentical relative. Transplants incompatible for one HLA-A, -B or -D/DRB1 locus are classified as "donor incompatible" in the case of homozygous recipients where mismatch is only in the direction of graft-versus-host, as "recipient incompatible" in the case of homozygous donors where mismatch is only in the direction of host-versus-graft, and as "both incompatible" in the case where donor and recipient are both heterozygous. Transplants from a haploidentical relative whose unshared haplotype is matched to that of the patient are classified as "neither incompatible."

amino acid sequence (i.e., HLA-A*02:01 vs. A*02:02) (see http://www.ebi.ac.uk/ipd/imgt/hla for the updated list of alleles; accessed February 3, 2015) [26]. Some HLA alleles have synonymous substitutions that result in expression of the same amino acid sequence and are indicated by a third doublet (i.e., HLA-A*02:03:01 vs. A*02:03:03) and therefore are matched for the purpose of donor selection. Some other unique HLA-A*02 alleles are not expressed as proteins (e.g., A*02:53N), they are instead defined as "null" and are immunologically silent. Once a null allele is identified, it should be ignored for donor selection. Distinction between alleles with highly similar sequence is important because those disparities are functionally relevant: for example, seven of the B*27 alleles can be distinguished by specific cytotoxic T-cell clones [27].

The relevance of HLA incompatibility between donor and recipient has been analyzed in patients with hematologic disorders who received HCTs of T-replete marrow grafts and an identical regimen of postgrafting immune suppression. The patient and related donor shared one HLA haplotype, hence haploidentical, but differed to a variable degree for the HLA-A, -B, and -DR antigens of the unshared haplotype. The results of these transplants have shown the effect of HLA incompatibility on GVHD, graft failure and survival, formally demonstrating that HLA antigens constitute the major histocompatibility complex (MHC) in humans. The clinical importance of subtle HLA-A, -B, -C, and -DRB1 allelic differences was demonstrated in studies of unrelated donor HCT where such mismatching (i.e., HLA-B*27:02 vs. B*27:03) was associated with increased GVHD and worse survival [14,28,29]. Increasing evidence has related DPB1 mismatching with graft failure, acute GVHD, protection from leukemia relapse, and overall mortality [15,30–34]. The relevance of donor disparity for DQB1 to GVHD has been confirmed, but its relevance to the post-transplant survival of patients with malignancy remains disputed [16,34,35].

Donor-specific antibodies

Patients may be alloimmunized by pregnancy or blood transfusions, and sensitization to donor alloantigens increases the risk of graft failure [20,36–38]. Donor-specific antibodies denote that the recipient has been sensitized to HLA antigens of the donor, and is indicated before the selection of an HLA-mismatched donor for HCT. Standard assays for crossmatching the patient's serum with donor lymphocytes include complement-dependent microcytotoxicity and multicolor flow microfluorimetry [39,40]. If complete patient and donor HLA genotyping is available, HLA-specific antibodies can be detected by enzyme-linked immunosorbent assay, flow cytometry or Luminex technologies that are more sensitive and have replaced the more cumbersome direct crossmatch [41–43]. Prior patient immunization to donor HLA antigens has a profound effect on engraftment. In a study of marrow transplants from HLA-incompatible relatives, graft failure occurred in 13 of 21 (62%) patients with a positive pre-transplant crossmatch of patient serum reactive against donor T or B lymphocytes, compared with 31 of 501 (7%) patients with a negative crossmatch ($p = 7.8$, E-10) [20,44]. Alloimmunized patients who tested positive for anti-HLA antibodies by screening against a random cell panel but were negative for donor-specific antibodies were not at increased risk of graft failure [40]. Ottinger et al. [36] also found that donor-specific antibodies predict graft failure and poor survival after peripheral blood HCT from an HLA-mismatched donor. In unrelated donor transplantation, flow cytometry and Luminex technology can detect patient antibodies specific against the donor mismatched HLA alleles and predict for graft failure [37,38]. In the presence of donor-specific antibodies, graft failure is likely the result of alloimmune rejection mediated both by sensitized radioresistant host T cells and by antibody-dependent cell-mediated killing. Removal of antidonor antibody through a plasma exchange followed by cyclophosphamide (CY) (120 mg/kg) and total body irradiation (TBI) (1200–1575 cGy), the addition of total lymphoid irradiation (600 cGy) or antithymocyte globulin (ATG) (60 mg/kg) to the standard regimen of CY and TBI has not resulted in consistent marrow engraftment [20,44]. Other more promising approaches tested include the administration of platelet transfusions, intravenous immunoglobulin, and post-transplant cyclophosphamide followed by tacrolimus and mycophenolate mofetil, but their efficacy remains to be validated [45,46]. If a patient has antibodies against an HLA-incompatible family donor, there is no consistently effective measure to offset the risk of graft rejection, and the best option for engraftment is to identify an HLA-matched unrelated donor, or a related or unrelated donor that is mismatched for an HLA antigen against which the patient is not sensitized.

Probability of identifying an HLA partially compatible family donor

One report from Germany found that the probability of identifying an HLA-matched sibling was 40.7%, a matched relative 4.0%, a one HLA-A, -B, or -DR locus-mismatched sibling 3.1%, and a one-locus mismatched relative 10.4% [1]. The best chance of finding a closely matched donor for patients without an HLA-matched or a one-locus-mismatched sibling is an unrelated donor search, because this is currently successful in up to 65% of cases [2,3]. If the initial search identifies one or more HLA-A, -B, and -DR matched unrelated donors, there is no obvious justification to undertake an extended family search beyond siblings, because not only would the probability of finding a suitable donor be lower, but also the donor typing effort and the cost of finding donors would be greater [1]. Computer programs and registry search algorithms have been developed that calculate the probability of finding a suitable related or unrelated donor based on patient typing [1,2,47,48], and the results of such programs can be utilized to define the best search strategy for an individual patient. Candidates for allogeneic HCT with no suitably matched donor and a short life expectancy might be better served by immediate transplantation from a relative mismatched for one entire HLA haplotype, or by a partially matched unrelated umbilical cord blood transplant, than by a lengthy search for a completely matched donor.

Transplantation of T-replete marrow grafts

GVHD prophylaxis with methotrexate, calcineurin inhibitors, or both

After HCT from partially matched relatives, the incidence and severity of acute GVHD correlates with the degree of HLA incompatibility for HLA-A, -B, and -DR. When patients received methotrexate (MTX) as single agent for GVHD prophylaxis, the incidence of grades II–IV GVHD was 34% for recipients of HLA-identical sibling marrow and increased progressively up to 84% for recipients of HLA-A, -B, and -DR locus-incompatible marrow [6]. Acute GVHD occurs early after HLA-incompatible transplants. The median onset of acute GVHD was 14 days after HLA-incompatible transplants, compared with 22 days after HLA-identical sibling transplants [6]. The use of cyclosporine (CSP) alone as GVHD prophylaxis after HLA-incompatible transplants was associated with a hyperacute syndrome characterized by fever 7–10 days after transplantation, fluid retention, low central venous pressure, low serum albumin, pulmonary edema, and renal failure (see Chapter 83). If other immunosuppressive agents could not reverse the syndrome, patients died from respiratory failure and other complications [49]. When it became apparent that the combination of CSP and MTX was superior to either agent alone in HLA sibling transplants, the same regimen was adopted for HLA partially matched transplants. The risk of severe acute GVHD of grade III–IV was significantly less and the time of onset was delayed by the use of CSP and MTX [relative risk (RR) 0.35; $p < 0.0001$]. The incidence of severe grade III–IV acute GVHD was decreased from 53% to 28% in one-locus-incompatible recipients and from 63% to 47% in two-locus-incompatible recipients. In a multivariate analysis of 474 patients who received CSP and MTX or MTX alone and achieved sustained engraftment, HLA incompatibility remained a significant risk factor for acute GVHD (RR 1.95 per HLA locus; $p < 0.0001$) [8,44]. Substituting CSP with tacrolimus was not adequate to prevent GVHD after transplantation from HLA-DR-incompatible relatives [50]. Post-transplant immunosuppression with CSP and MTX compared with MTX alone has not resulted in improved outcome after transplants incompatible at two or three HLA loci [8]. Therefore, despite post-transplant immunosuppression with CSP and MTX, HLA incompatibility has remained an important risk factor for GVHD (Figure 44.3) [8].

Marrow transplants from HLA partially matched family members have been associated with a higher probability for developing clinical extensive chronic GVHD (49% vs. 33%) and an earlier median day of onset (159 vs. 201 days) compared with transplants from HLA-identical siblings [51]. Consistent results have been found by the CIBMTR [52]. The duration of immunosuppressive treatment for chronic GVHD is prolonged after HLA disparate-donor transplants, with a hazard ratio of 0.8 (95% CI 0.7–0.9) per mismatched HLA locus ($p = 0.007$) [53].

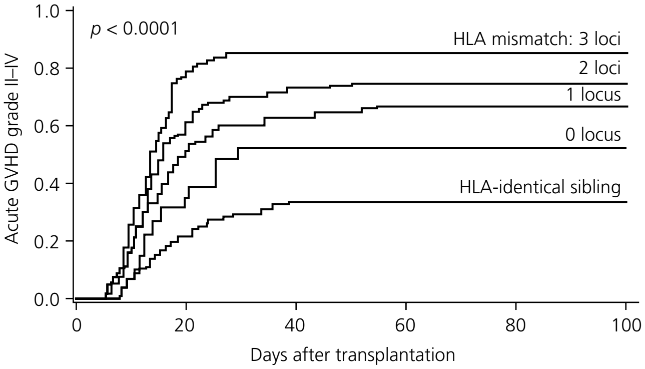


Figure 44.3 Effect of donor and recipient human leukocyte antigen (HLA) mismatch on acute graft-versus-host disease (GVHD). Probability of grade II–IV acute GVHD according to the degree of matching for HLA-A, -B, and -D/DRB1 in patients transplanted from a haploidentical relative or an HLA genotype-identical sibling. Patients received unmodified marrow and methotrexate plus cyclosporine for GVHD prophylaxis.

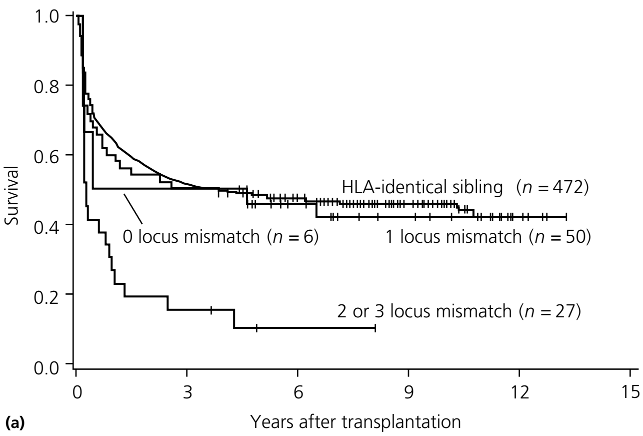
Survival in patients receiving post-transplant immunosuppression with MTX alone was similar after transplantation from an HLA-identical sibling or a one-HLA antigen-incompatible family donor. However, survival was lower in patients transplanted from a family donor incompatible for two or three HLA loci [6]. Subsequent studies of patients whose post-transplant immunosuppression consisted of CSP and MTX also showed that the degree of overall HLA incompatibility was inversely correlated with the probability of survival (Figure 44.4). Survival was predicted more precisely by matching classified according to the degree of overall incompatibility than to the incompatibility for each vector alone, because the degree of overall incompatibility best reflects the risks of both graft rejection and GVHD. In a multivariate proportional hazard regression analysis, the factors associated with lower survival were leukemia in marrow relapse at the time of transplant, and the degree of overall HLA incompatibility between donor and recipient. Post-transplant immunosuppression with CSP and MTX compared with MTX alone did not have a beneficial effect on survival.

Pre-transplantation alemtuzumab

Humanized anti-CD52 antibody alemtuzumab (100 mg over 5 days) was added to fludarabine (120 mg/m^2 over 4 days) and CY (2000 mg/m^2 over 4 days) to develop an immune-suppressive regimen that does not produce myeloablation. With T-replete PBPC transplantation from HLA partially matched related donors, primary engraftment was achieved in 46 of 49 (94%) patients, and secondary graft failure occurred in four (8%) patients, so overall sustained engraftment was 86% [54]. The incidence of acute GVHD of grades II–IV was 16%, suggesting that a large fraction of donor T cells infused in the graft were depleted by alemtuzumab in vivo [54]. The benefits of a reduced-intensity conditioning regimen were the relatively low (31%) non-relapse mortality and low (16%) incidence of grades II–IV acute GVHD.

Pre-transplantation polyclonal anti-T-cell therapy, PBPC, and post-transplantation mycophenolate mofetil

Huang and other investigators from Peking University (Beijing, China) utilized rabbit ATG (Thymoglobulin) 10 mg/kg over 4 days, or porcine ATG 80 mg/kg over 4 days before the transplant of T-replete marrow cells plus PBPCs from HLA partially matched



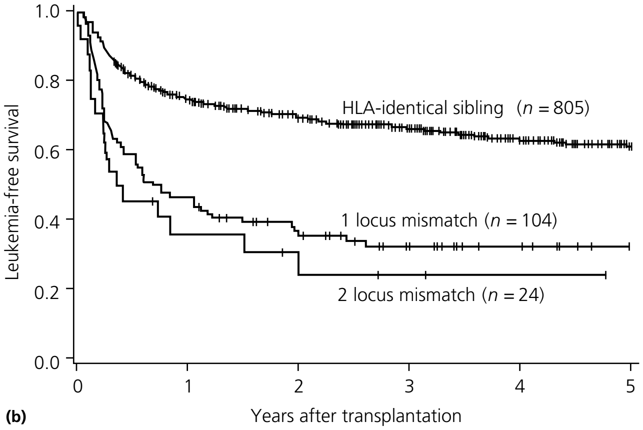


Figure 44.4 Effect of donor and recipient human leukocyte antigen (HLA) mismatch on survival after hematopoietic cell transplantation. Probability of survival in patients with chronic myeloid leukemia in chronic phase, acute myeloid leukemia in first remission or acute lymphoblastic leukemia in first or second remission, according to the degree of donor and recipient HLA incompatibility. (a) Results from a single-center study. Source: Anasetti et al. 1990 [8]. Reproduced with permission of Elsevier. (b) Results from a study of the International Bone Marrow Transplant Registry. Source: Szydlo et al. 1997 [52].

related donors, and post-transplant immune suppression with MTX/CSP plus mycophenolate mofetil [55]. All 171 patients achieved full donor chimerism and hematopoietic reconstitution. Acute GVHD grade II–IV and III–IV was observed in 55% and 23% of patients, respectively, with no suggestion for an association between degree of HLA disparity and risk of acute GVHD. Intriguing results were reported by Lu and collaborators using ATG treatment before T-cell-replete marrow or PBPC transplantation from HLA partially matched relatives. The 2-year incidence of chronic GVHD was 55% (95% CI 46–64%) after mismatched HCT with ATG compared with 56% (95% CI 47–64%) after HLA-identical sibling HCT with no ATG (p = 0.9) [56]. A separate report from the same institution compared the outcome of HLA partially matched transplants in patients pretreated with Thymoglobulin with the outcome of HLA-matched sibling transplants, without Thymoglobulin. All patients received the same postgrafting immune suppression with MTX/CSP plus mycophenolate mofetil. The cumulative incidences of acute GVHD grade II–IV were 32% (95% CI 25–39%) versus 40% (95% CI 32–48%) in the matched and mismatched cohorts, respectively (p = 0.13). The risk of acute GVHD grade II–IV was significantly higher, and GVHD had a more rapid onset after HLA-mismatched transplants. Clinical manifestation of grade III–IV GVHD after mismatched transplants included severe (44%) and bloody diarrhea (23%), hepatic dysfunction (41%), skin rash (31%), and non-cardiac edema (18%) [56]. While the relative contribution of pre-transplant ATG and post-transplant mycophenolate mofetil to GVHD prevention cannot be discerned from these studies, the data indicate that the administration of ATG just before HCT can deplete T cells in vivo and diminish the risk of GVHD.

The outcomes of HLA-mismatched related HCT (n = 135) for leukemia were compared with those of HLA-identical sibling HCT (n = 158) performed during the same time period in Beijing, China by Lu et al. [56]. The donor was mismatched for one (n = 21), two (n = 62), or three (n = 52) HLA-A, -B or -DR loci. All patients received conditioning with BU/CY. In addition, ATG was administered before mismatched HCT only. Unmanipulated marrow and/or PBPCs were used for transplantation. All patients received postgrafting immune suppression with MTX/CSP plus mycophenolate mofetil. All patients achieved full engraftment. The cumulative incidences of grade II–IV acute GVHD in the matched and mismatched cohorts were 32% versus 40%, respectively (multivariate p = 0.02), while chronic GVHD did not differ between the cohorts (p = 0.97) (Figure 44.5). Two-year incidences of treatment-related mortality and relapse for matched versus mismatched were 14% (95% CI 9–20%) versus 22% (95% CI 15–29%) (p = 0.10), and 13% (range 8–19%) versus 18% (range 10–27%) (p = 0.40), respectively. Two-year adjusted overall survival and leukemia-free survival were 72% (95% CI 64–79%) versus 71% (95% CI 62–77%) (p = 0.72), and 71% (95% CI 63–78%) versus 64% (95% CI 54–73%) (p = 0.27), respectively (Figure 44.5). Multivariate analyses showed that advanced disease stage and a diagnosis of acute leukemia had an increased risk of relapse, treatment failure, and overall mortality, whereas donor HLA mismatch did not affect these outcomes.

A long-term follow-up of HLA-mismatched related HCT using the Beijing regimen extended the favorable initial findings of Huang and Lu on more than 700 patients [11]. In addition, HLA-mismatched related HCT proved superior to chemotherapy alone as post-remission treatment for patients with acute myeloid leukemia in first complete remission [57]. Results from two additional trials from Lee et al. in Korea, and Di Bartolomeo et al. from Italy largely reproduced the data from Huang and Lu [58,59]. Whereas the effectiveness of the Beijing regimen was verified by these two external trials, it remains unclear whether each of its components is key to success. A large controlled trial by Finke et al. demonstrated that administration of polyclonal T-cell specific antibodies before HCT from HLA-matched unrelated donors is effective in preventing acute and chronic GVHD [60,61]. Antibody preparations vary in potency and safety, but all data from randomized trials taken together indicate that ATG preparations are biologically active in the prevention of GVHD [62]. Therefore, ATG is very likely contributing to the prevention of GVHD after HLA-mismatched HCT.

G-CSF mobilized blood stem cells are rich in both hematopoietic progenitors and T cells and are more consistent in achieving engraftment compared to bone marrow in unrelated donor transplantation [63]. Furthermore pre-transplant ATG is effective in reducing the burden of GVHD associated with the use of mobilized blood stem cells [64]. An adequately sized controlled trial from Canada has recently demonstrated that marrows from G-CSF-treated donors lead to similar acute or chronic GVHD compared to mobilized blood stem cells [65]. Therefore, it remains speculative

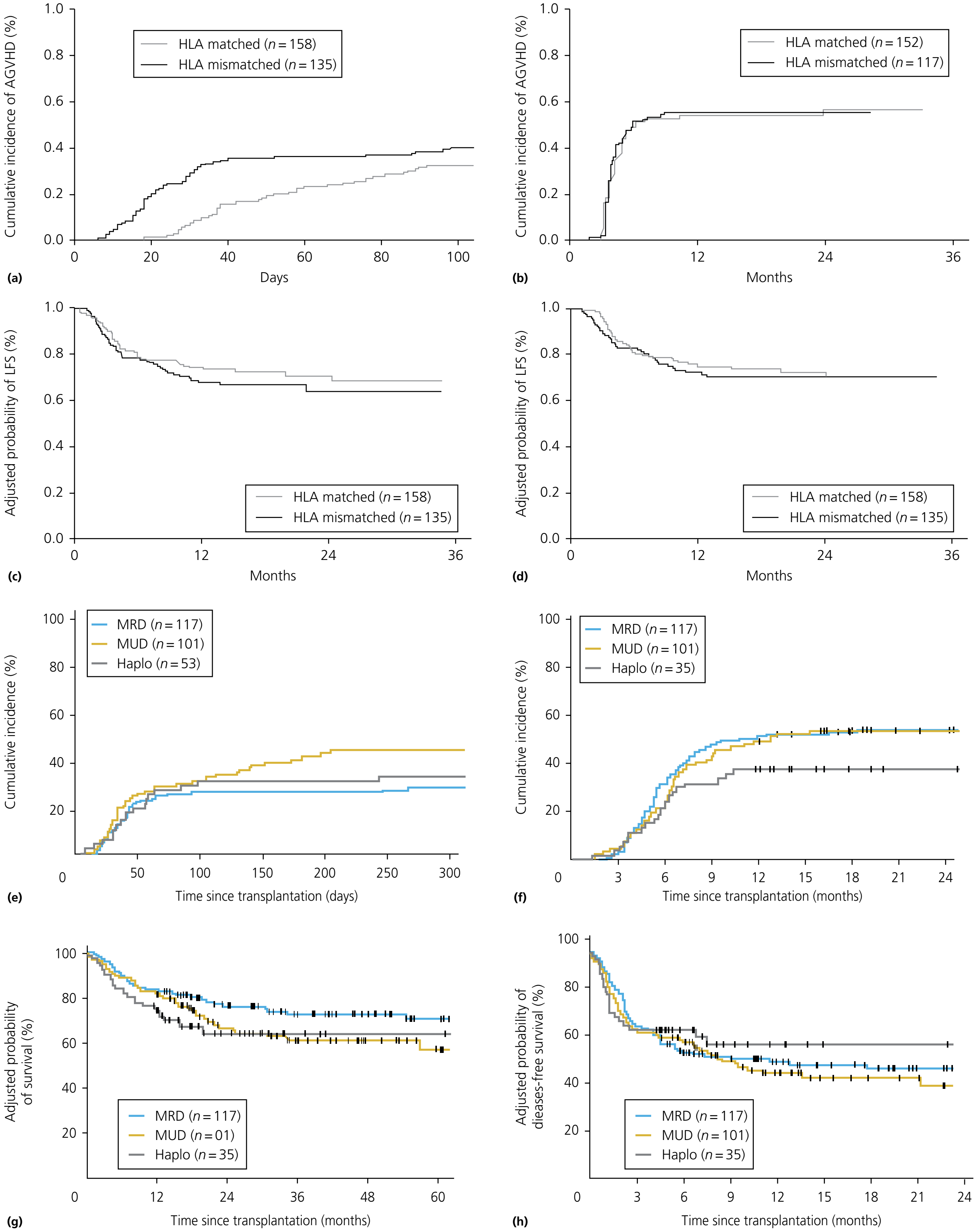


Figure 44.5 Effect of novel GVHD prevention regimens on outcomes. (a) Pre-transplantation ATG and post-transplantation mycophenolate mofetil: cumulative incidence of acute GVHD (A), chronic GVHD (B), disease-free survival (C), and survival (D) after HLA-identical sibling or HLA-mismatched related transplantations with (p = 0.13 and p = 0.97, respectively). Source: Lu et al. 2006 [56]. (b) Post-transplantation cyclophosphamide: cumulative incidence of acute GVHD (E), chronic GVHD (F), survival (G), and disease-free survival (H) by donor type. Haplo, haploidentical donor; MRD, matched related donor; MUD, matched unrelated donor. Source: Bashey et al. 2013 [12].

whether the addition of bone marrow cells to a mobilized blood stem cell graft helps in securing engraftment while ameliorating GVHD.

Mycophenolate mofetil was almost as effective as methotrexate in the prevention of GVHD in HLA-matched HCT in controlled trials, when used in combination with calcineurin inhibitors [66,67]. Since mycophenolate mofetil and methotrexate have distinct mechanism of action, it is likely that their combination is additive in preventing GVHD. Controlled trial evidence for this interaction, however, is lacking. These data suggest that G-CSF mobilized stem cells contribute to overcoming the risk of graft rejection, while the addition of pre-transplant ATG and post-transplant mycophenolate mofetil to standard regimens may offset the risks of GVHD associated with donor HLA-mismatching.

Post-transplantation, high-dose CY

Data from experimental animals indicate that post-transplantation CY depletes at least in part alloreactive effector T cells, while sparing regulatory T cells that are important for induction of transplantation tolerance [68]. Two independent clinical trials from Johns Hopkins and Seattle evaluated the safety and efficacy of high-dose, post-transplantation CY to prevent graft rejection and GVHD after outpatient reduced-intensity conditioning and T-cell-replete bone marrow transplantation from partially HLA-mismatched related donors [69]. Patients with advanced hematologic malignancies ($n = 87$) or paroxysmal nocturnal hemoglobinuria ($n = 1$) were treated with a reduced-intensity regimen of fludarabine 150 mg/m^2, CY 29 mg/kg, and TBI 200 cGy before marrow transplant, and post-transplantation CY (50 or 100 mg/kg), tacrolimus, and mycophenolate mofetil after the transplant [69,70]. Graft failure occurred in 15 (18%) of the 84 evaluable patients, and was fatal in two. The cumulative incidences of grade II–IV and grade III–IV acute GVHD by day 200 were 35% and 10%, respectively, and that of chronic GVHD at 1 year was 22%. The cumulative incidences of non-relapse mortality and relapse at 1 year were 19% and 50%, respectively. Overall and event-free survivals at 2 years after transplantation were 35% and 24%, respectively. These data strongly suggest that post-transplantation CY can decrease the risk of graft rejection, GVHD, and mortality associated with donor HLA disparity. A prospective, multicenter trial by the US Blood and Marrow Transplant Clinical Trials Network confirmed this observation [71].

Engraftment and GVHD are enhanced by more intense pre-transplantation conditioning therapy that ablates recipient immunity responsible for graft resistance. At least four independent teams have reported that post-transplantation CY is effective in controlling the risk of acute and chronic GVHD after myeloablative regimens [72–75]. In each of these trials, CY was followed by maintenance immunosuppression with tacrolimus and mycophenolate mofetil. There is theoretical concern about the risk of myelodysplasia secondary to hematopoietic stem cell exposure to the alkylating effects of CY, and to offset such a risk it is feasible to administer selected donor CD34 cells after CY therapy [75]. An analysis of post-transplantation CY from Northside Hospital in Atlanta, USA, found increased acute but not chronic GVHD and similar survival in patients treated with reduced-intensity or ablative regimens, and marrow grafts from HLA-mismatched related compared to HLA-matched related or unrelated donors (Figure 44.5) [12]. Prospective comparison of post-transplantation CY and ex vivo or in vivo T-cell depletion using T-cell-specific antibodies has not yet been performed in HLA-mismatched transplantations.

Graft failure with T-cell-replete marrow

Diagnosis of graft failure and second transplants

Primary graft failure is likely to occur if severe granulocytopenia persists for 21 days after transplantation of marrow or PBPCs, but secondary graft failure may also occur after initial engraftment (see Chapter 77) [10]. Studies of chimerism (see Chapter 24) are necessary to assess the presence of donor cells. Functional studies in patients with graft failure have demonstrated that residual host T lymphocytes are cytotoxic against donor alloantigens, and the patient's serum may be active in the antibody-dependent cell-mediated cytotoxicity test against donor cells [76,77]. These findings have indicated that alloimmune-mediated rejection is a mechanism for graft failure after HCT from HLA-incompatible donors. If a high-dose conditioning regimen is administered, either primary or secondary graft failure is usually associated with persistent aplasia. In certain patients, recovery of autologous myeloid cells can occur, especially if the patient is pretreated with reduced-intensity conditioning regimens and administered granulocyte–macrophage colony-stimulating factor (GM-CSF) or granulocyte colony-stimulating factor (G-CSF) after graft failure [78]. If there is no recovery of myeloid function despite growth factor therapy, a second transplant from the same or a different donor can be attempted, but the success of second transplants has been limited [79].

Risk of HLA disparity

The relevance of HLA compatibility to sustained engraftment was first analyzed in 269 patients with hematologic neoplasms who underwent T-replete marrow transplantation from a family member who shared one HLA haplotype with the patient, but differed to a variable degree for the HLA-A, -B, and -DR antigens of the unshared haplotype [10]. All patients were treated with cyclophosphamide (CY) and total body irradiation (TBI). The incidence of graft failure was 12.3% among recipients of marrow from an HLA partially matched donor compared with 2.0% among recipients of marrow from an HLA-matched sibling ($p = 0.0001$) (Table 44.2). The incidence of graft failure correlated with the degree of HLA incompatibility in the host-versus-graft direction.

Recipient HLA homozygosity

When the recipient is homozygous at a mismatched HLA locus, the degree of HLA disparity is greater in the direction of rejection than in the direction of GVHD (Table 44.1). Transplants in recipients homozygous at one or more mismatched loci had a significantly higher incidence of graft failure than transplants in heterozygous recipients. Graft failure after a transplant mismatched for one or more HLA loci occurred in 11 of 76 (15%) homozygous recipients compared with 22 of 350 (6%) heterozygous recipients. The effect of the vector of incompatibility on graft failure was significant in a multivariable analysis ($p = 0.005$). The association between recipient

Table 44.2 Effect of HLA incompatibility on marrow graft failure

	HLA-non-identical donor ($n = 269$) number of patients (%)	HLAgenotype-identical donor ($n = 930$) number of patients (%)	*p* value
Failure of engraftment	23 (8.5%)	15 (1.6%)	<0.0001
Late graft failure	10 (4.1%)	4 (0.4%)	<0.0001
All failures	33 (12.3%)	19 (2.0%)	<0.0001

Source: Modified with permission from Anasetti et al. 1989 [20].
HLA, human leukocyte antigen.

homozygosity for the mismatched locus and increased graft failure has been confirmed in a subsequent study of HLA-mismatched unrelated donor transplants [25]. These results are consistent with the concept that the graft-versus-host reaction protects from graft failure. Presumably, the graft-versus-host reaction is directed against residual immune cells of the recipient that are responsible for graft failure. In cases where the degree of disparity in the donor exceeds the degree of disparity in the host, the graft-versus-host reaction is less and the risk of graft failure is increased.

Immune competence

The risk of graft failure is low in patients who are immunodeficient by virtue of a congenital disorder or as the result of prior cytotoxic or immunosuppressive therapy provided as treatment for the underlying disease. Most patients with severe combined immunodeficiency lacking both T and NK cells have been engrafted with HLA-incompatible HCT despite receiving neither pre-transplant conditioning nor post-transplant immunosuppression [80]. Patients with leukocyte adhesion deficiency (see Chapter 73), a congenital defect resulting in failure to express the leukointegrin β2 chain on white cells including T and NK cells, demonstrate low resistance to engraftment with T-depleted transplants from HLA-incompatible donors [81]. Thus, β2 leukointegrins have a critical role in the rejection of HLA-incompatible HCTs. Patients with chronic myeloid leukemia (CML) have a higher risk of graft failure than patients with acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL). The incidence of graft failure was examined in non-T-depleted marrow grafts from HLA partially matched relatives in patients treated with the same conditioning regimen of CY (120 mg/kg) plus hyperfractionated TBI (1320–1440 cGy) followed by post-transplant immunosuppression with cyclosporine (CSP) and a short course of methotrexate (MTX). Graft failure occurred in 11 of 84 (13%) patients with CML compared with five of 133 (4%) patients with other diagnoses (odds ratio = 3.5; 95% CI, range 1.1–10.5; multivariate $p = 0.03$) [44]. Patients with acute leukemia may be at lower risk for graft failure because of the immunodeficiency induced by repeated treatment with chemotherapy agents [82].

Conditioning regimen intensity

Pre-transplant conditioning with CY (120 mg/kg over 2 days) and TBI (920–1575 cGy) allowed engraftment in 88% of 269 patients transplanted from HLA partially matched relatives [10]. Thus, a high-dose conditioning that includes TBI is not uniformly effective in facilitating engraftment of HLA-incompatible marrow or PBPC grafts. Increasing the immune-suppressive potency of the pre-transplant conditioning regimen can minimize the incidence of immune-mediated rejection. A more effective high-dose regimen containing TBI was developed for T-depleted transplants: CY was replaced by ATG, fludarabine, and thiotepa, agents that contributed immunosuppressive activity without additional toxicity (see later, under T-cell-depleted transplants) [83]. Pre-transplant immunosuppression with CY, fludarabine, and thiotepa was developed to facilitate engraftment of HLA-mismatched cord blood, and is very effective to facilitate engraftment after HLA-disparate related donor transplants [59,84]. Thalassemia patients are not only more immunocompetent than leukemia patients but are also sensitized to histocompatibility antigens by multiple blood cell transfusions. The addition of thiotepa to BU/CY decreased significantly the risk of graft failure in thalassemia patients transplanted from HLA partially compatible unrelated donors [85].

Graft cell dose

Cord blood transplantation provides the most convincing data demonstrating that low cell dose is a risk factor for graft failure after HLA-mismatched transplants [4,5]. Conversely, a controlled trial in unrelated donor transplantation formally demonstrated that PBPC lowers the risk of graft failure compared to marrow [63]. Protocols that use in vivo or ex vivo T-cell depletion have adopted PBPC to optimize engraftment and prevent graft failure [10,11].

Post-transplant immunosuppression

Post-transplant immunosuppression decreases the risk of graft failure. Patients transplanted from HLA partially matched donors had a graft failure rate of 5% when treated with CSP plus short-course MTX, but 9% when treated with MTX alone ($p = 0.03$) [44]. This observation is consistent with the report from the Center for International Blood and Marrow Transplant Research (CIBMTR) showing that, in T-depleted marrow transplants from HLA-identical sibling donors, the use of post-transplant immunosuppression with CSP plus MTX was associated with a lower risk of graft failure than CSP alone [86].

Immune reconstitution

After transplantation, new T cells are derived from donor hematopoietic stem cells (HSCs) following positive and negative selection within the host thymus. T lymphocytes recognize immunogenic peptides presented by self-HLA but not by mismatched allogeneic HLA molecules, a phenomenon defined as HLA restriction. Positive T-cell selection is facilitated by HLA molecules expressed on thymic epithelial cells, whereas negative selection is controlled predominantly by HLA molecules expressed on marrow-derived APC [87]. Thus, new T cells that are positively selected on thymic epithelium can optimally recognize antigen in the context of marrow-derived APCs in peripheral tissues only if there is sharing of HLA antigens between donor and recipient [88]. Mature T cells transplanted into an HLA-disparate recipient may not recognize antigens presented by host APCs, thereby failing to help immune reconstitution. These premises justify why donor and recipient matching for at least one HLA haplotype is the minimum requisite for donor selection.

Extrathymic pathways of immune reconstitution after transplantation are predominant in adults as thymus function begins to decline relatively early in life, usually before the age of 20 years. Therefore, T cells that repopulate adult transplant recipients are derived predominantly from the relatively small number of mature donor T cells infused with the hematopoietic cell inoculum [89]. Immunosuppressive regimens should be developed that are effective in preventing GVHD while sparing donor T cells that do not recognize host alloantigen but contribute to long-term immune reconstitution. Both acute and chronic GVHD also contribute to immunodeficiency. Because adult patients have very poor thymic function, T-cell-depletion has been associated with severe and prolonged postgrafting immunodeficiency [90]. Data on T-cell receptor excision circles as a marker for recent thymus emigrants has demonstrated that the human thymus continues to function at low levels until late in life, providing hope that effective treatment can be developed to restore immunity quickly in T-cell-deficient adults [91]. Initial data on interleukin-7 (IL-7) is promising for this application [92,93].

Graft-versus-leukemia effect

Relapse of leukemia after HLA-identical sibling transplants is less frequent in patients who develop clinically significant GVHD compared with syngeneic or allogeneic transplant recipients without GVHD (see Chapter 16). Patients with ALL after transplantation from HLA partially matched donors in whom GVHD developed also had a lower risk of relapse than patients without GVHD [8]. In one analysis, chronic but not acute GVHD was associated with a lower relapse rate in patients with AML or CML [8]. However, the probability of leukemic relapse in patients without clinically significant GVHD was the same whether the marrow donor was an HLA-identical sibling or an HLA partially matched relative. These data suggested that HLA disparity in absence of acute GVHD has no obvious antileukemic effect. Because recipients of one-HLA locus-incompatible transplants have a higher incidence of acute GVHD than recipients of HLA-identical sibling transplants, one would expect to see a lower incidence of leukemic relapse. A study from the CIBMTR comparing the outcome of 1222 HLA-identical sibling transplants to that of 238 transplants from one-HLA locus-incompatible donors failed to detect different rates of relapse [52]. The use of T-cell-replete grafts of G-CSF-mobilized PBPCs from one-HLA antigen-mismatched related or unrelated donors has been associated with a lower risk of post-transplant relapse in patients with CML [94]. The apparent increase in antitumor effect with PBPCs compared with marrow is likely related to the number of donor T cells transplanted [95].

Making a choice among stem cell sources

A large study from the CIBMTR compared the outcomes of marrow transplants from HLA-identical siblings, HLA partially matched related donors, and unrelated donors for treatment of leukemia [52]. The definition of donor and recipient histocompatibility was based exclusively on historical serologic data and not on cellular or DNA typing. The probability of leukemia-free survival for patients transplanted for early-stage leukemia, CML in chronic phase, AML or ALL in first remission is presented in Figure 44.4(b). A multivariable analysis of transplant-related mortality showed an increase in risk with the use of a one-HLA locus-mismatched relative compared with an HLA-identical sibling, and for the use of a two-HLA locus-incompatible relative compared with a one-HLA locus-incompatible relative. Results of matched unrelated donor transplants were similar to results of one-antigen-mismatched related donor transplants, while results for one-antigen-mismatched unrelated donor transplants were similar to results for two-antigen-mismatched related donor transplants [52]. Taken together, these data demonstrate that a limited degree of HLA disparity between donor and recipient can be tolerated with T-cell-replete marrow in transplants for hematologic malignancies, but a higher degree of donor HLA disparity reduces survival.

A subsequent CIBMTR study confirmed the original findings of similar survival after transplantation from one-locus incompatible related compared to unrelated donors that were well-matched using DNA typing technology [96]. Conversely, a report from the Japanese Marrow Donor Program showed inferior survival with one-locus-mismatch related donors compared to well-matched unrelated donors typed by high-resolution DNA typing technology [97]. The apparent discrepancy may be related to genetic heterogeneity between the predominantly Caucasian population in the CIBMTR report and the Japanese or the choice of transplant protocols that may offset the HLA effect.

A single-center study from Milwaukee compared transplantation outcomes in patients with hematologic malignancies who received marrow grafts from either HLA-matched unrelated, one-antigen-mismatched unrelated or highly mismatched family donors [98]. All patients received a standardized conditioning regimen and a uniform GVHD prophylaxis schedule – with the exception of mismatched related recipients, who received ATG as additional postgrafting immunosuppression. There was a higher probability of survival for matched unrelated transplants (58%) than either mismatched unrelated (34%; $p = 0.01$) or mismatched related transplants (21%; $p = 0.002$). This study supports the fact that patients lacking an HLA-matched family donor can be offered a matched unrelated donor, if available. Effective transplant protocols have challenged this conclusion by producing similar survival after transplantations from HLA-mismatched related donors, matched siblings or matched unrelated donors [10–12]. Only one prospective trial has been designed to address the effectiveness of transplantations from HLA-mismatched unrelated cord blood versus HLA-mismatched related donors for adult patients with hematologic malignancies, and it is ongoing in the United States within the Blood and Marrow Transplant Clinical Trials Network.

Transplantation of T-cell-depleted hematopoietic cell grafts

Risk of graft failure with T-cell-depleted marrow

Numerous clinical trials have demonstrated that extensive ex vivo depletion of T cells from the marrow graft without post-transplant immunosuppression prevents acute and chronic GVHD and associated morbidity (see Chapter 82). Unfortunately, marrow T-cell depletion is associated with an increased risk of graft failure that is proportional to the degree of donor HLA incompatibility. A study from the CIBMTR on transplantation from donors incompatible for two or three HLA-A, -B, and -DR antigens found an incidence of graft failure of 42% with T-cell-depleted marrow compared with 28% in non-T-cell-depleted marrow ($p < 0.03$) [9]. In a small series of patients with leukemia transplanted from a two- or three-HLA antigen-mismatched donor at Memorial Sloan Kettering Cancer Center, New York, the use of T-cell depletion was also associated with an incidence of graft failure approaching 50%. In contrast, T-cell-depleted marrow from parents incompatible for a full HLA haplotype engrafted almost all patients with severe combined immune deficiency [80].

In immune-competent hosts, resistance to engraftment is mediated primarily by host T lymphocytes [76]. We presume that with T-cell-depleted marrow grafts, the balance between competing host and donor T cells shifts in favor of the host, resulting in unopposed host-versus-graft reaction. In rodents, resistance to MHC-incompatible HCT can be overcome by adding BU, thiotepa, or anti-T-cell antibodies to the host-conditioning, or by increasing the number of donor T cells or hematopoietic cells in the donor graft [99]. In leukemia patients transplanted from HLA-mismatched donors, a regimen of TBI, CY, thiotepa, and ATG was inadequate for engraftment of T-cell-depleted marrow, but was adequate for engraftment of T-depleted PBPCs [10].

Partial marrow T-cell depletion

Since even a small number of T cells in the marrow inoculum can overcome allogeneic resistance, investigators have developed strategies for partial T-cell depletion. Removal of less than 2 logs T cells from the donor marrow requires, however, the administration of post-transplant immunosuppression for the prevention of GVHD. In 201 patients, conditioning comprised high doses of TBI, CY, cytarabine, and etoposide, while GVHD prophylaxis consisted of partial marrow T-cell depletion with either anti-CD3 murine monoclonal antibody OKT3 ($n = 143$) or T10B9 ($n = 58$), plus post-transplant immunosuppression with ATG, CSP, and glucocorticoids [100]. Engraftment occurred in 98% of patients, grade II–IV acute GVHD in 13%, and chronic GVHD in 15%. The 5-year transplant-related mortality, however, was 51% and was caused predominantly by opportunistic infections. The incidence of relapse was 31%, and the 5-year disease-free survival was 18%. Patient age greater than 15 years, active disease at transplant, donor age greater than 25 years, and a donor mismatched for three HLA-A, -B, and -DR antigens adversely affected outcome. This study demonstrated that partial T-cell depletion plus postgrafting immunosuppression can prevent GVHD after marrow transplantation from donors mismatched for one HLA haplotype, that the risk of graft failure is low, but that mortality from opportunistic infections is unacceptably high.

Hematopoietic cell dose and use of PBPCs

Preclinical studies

Animal models showed that high doses of T-cell-depleted marrow cells can overcome the MHC barrier without causing GVHD [99]. When purified HSCs were transplanted in irradiated mice, stable hematopoietic chimeras were generated in all cases, but 10–60 times the number of HSCs were required for the survival of mice transplanted across MHC disparities compared with no MHC disparities. Cell-dose escalation allowed full donor cell engraftment even in mice that had been presensitized by donor lymphocytes, in mice whose immune system had been partially reconstituted with the infusion of small numbers of host T cells before the allogeneic transplant, and in mice pretreated with sublethal doses of TBI low enough to spare recipient T lymphocytes. Human $CD34^+$ cells exhibited "veto" activity in vitro, that is, they neutralized allogeneic cytotoxic T-lymphocyte precursor cells directed against their antigens but not against a third party [101,102]. Early myeloid $CD33^+$ cells were also endowed with marked veto activity, which is not found in late myeloid cells expressing CD14 or CD11b. Therefore, soon after transplantation, infused $CD34^+$ cells and their $CD33^+$ progeny might inhibit residual antidonor cytotoxic T-lymphocyte precursors from the recipients through deletion mediated by tumor necrosis factor-alpha [103].

Transplant studies: the benchmark "Perugia" protocol

The hypothesis that escalation of the hematopoietic cell dose can facilitate engraftment of T-cell-depleted, HLA-mismatched transplants was first tested in patients with advanced leukemia. In 1993, the Perugia Bone Marrow Transplant Center developed a transplant protocol using the combination of G-CSF-mobilized T-depleted PBPCs and T-cell-depleted marrow cells for transplantation from HLA-mismatched relatives [10]. Donor and recipients were incompatible for two or three HLA-A, -B, and -DR antigens of the unshared haplotype. More than 3 log depletion was achieved and the T-cell content in the graft was a median of $1–2 \times 10^5$/kg recipient body weight, and $CD34^+$ cells showed a median of 10×10^6/kg recipient body weight. The average dose of GM-CFU was seven- to 10-fold greater in the combined product than in marrow alone. Conditioning included TBI (8 Gy at an instantaneous dose rate of 16 cGy per minute with the lungs shielded to receive 7 Gy), thiotepa (10 mg/kg over 2 days), CY (120 mg/kg over 2 days), and rabbit ATG (25 mg/kg over 5 days). All patients received G-CSF after transplantation until engraftment and no planned post-transplant immunosuppression. Sixteen of 17 patients with advanced leukemia achieved primary engraftment. Acute GVHD grade II–IV occurred in one patient, and there were no cases of chronic GVHD. Two patients relapsed, and nine died of transplant-related complications, which were mainly infections. Six of 17 patients survive [10].

After the first pilot study of T-cell-depleted PBPCs plus marrow, the Perugia team focused on a graft containing only PBPCs and on optimizing the graft-processing by means of automated positive $CD34^+$ cell selection. This approach ensured a median of 4.5 log T-cell depletion and a 3.2 log B-cell depletion, which helps prevent Epstein–Barr virus-associated lymphoproliferative disorders [104]. To minimize the extrahematologic toxicity of the conditioning regimen, fludarabine (40 mg/m^2/day for 5 days) replaced CY, based on data from a murine model where fludarabine/TBI provided similar immunosuppression to TBI/CY [105]. In addition, the dose of total lung irradiation was decreased from 7 to 4 Gy. The modified protocol was tested in 43 adult patients with high-risk acute leukemia. Primary sustained engraftment was achieved in 41 of 43 (95%) patients. Grade II–IV GVHD did not occur. The 2-year disease-free survival was 36% for 20 patients with AML, and 17% for 23 patients with ALL ($p = 0.05$), and all survivors recovered a normal performance status [46]. In a subsequent confirmatory series, primary engraftment was achieved in 94 of 101 patients, and six more engrafted after a second transplantation. Acute GVHD developed in eight patients, and chronic GVHD in five. Thirty-eight patients died of causes other than leukemia. Relapse occurred in nine of 66 patients receiving transplantation in remission, and in 17 of 38 receiving transplantation in relapse. Event-free survival at 1 year was 48% and 46%, respectively, for the 42 AML and 24 ALL patients receiving transplantation in remission, and 4% for the 38 patients with AML or ALL receiving transplantation in relapse [106].

An analysis of 255 high-risk acute leukemia patients treated in Perugia over almost 20 years showed a relapse rate of 18% with AML and 30% with ALL in any complete remission (CR) [107]. The intensely myeloablative conditioning regimen likely contributed to leukemia control. In the absence of post-transplant immunosuppression, the few T cells in the graft may have mounted a subclinical graft-versus-leukemia effect (GVLE), and donor-versus-recipient NK alloreactivity, a biologic effect unique to mismatched transplant, helped prevent relapse of AML. The cumulative-incidence of non-relapse mortality was 0.36 (95% CI, 0.29–0.53) for 145 patients in any CR at transplant and 0.58 (95% CI, 0.40–0.65) for 110 patients transplanted in relapse. Event-free survival (EFS) was 43% with AML and 30% with ALL in any CR, with no events occurring between 2 and 17 years of follow-up. Patients with advanced AML had a noticeable 18% EFS. All long-term survivors enjoy an excellent quality of life with no cGVHD.

A European Group for Blood and Marrow Transplantation retrospective study, analyzed the outcome of haploidentical transplantation in several European centers and reported similar results with a 48% one-year EFS for AML patients in first CR [108]. Encouraging results were also demonstrated in pediatric patients. A European multicenter analysis of 127 children with high-risk ALL reported a 5-year disease-free survival (DFS) of 27% for patients in CR [109]. A multivariate analysis detected a trend toward a center-related effect with better DFS in larger centers (39% vs. 15%). An improved outcome with less relapse was also described for patients receiving a higher $CD34^+$ cell dose.

These clinical trials demonstrated that a highly immune-suppressive conditioning regimen of TBI, fludarabine, thiotepa, and ATG combined with positively CD34-selected PBPC cells can overcome the engraftment barriers across HLA disparities in leukemia patients. In addition, $CD3^+$ cell doses up to 2×10^4/kg do not cause severe GVHD if ATG is administered as part of the conditioning regimen.

Effective GVHD prevention by CD34 positive selection hinders the antileukemia effects mediated by T cells against histocompatibility antigens on recipient leukemia [86], but donor NK-cell alloreactivity against the recipient contributed to control post-transplant relapse in AML patients (see the section on NK alloreactivity later).

T-cell negative selection from PBPCs and reduced-intensity conditioning

Handgretinger and collaborators modified the Perugia protocol by substituting OKT3 for ATG, and shifting from positive CD34^{+} cell selection to negative selection through the use of CD3- and CD19-coated CliniMACS microbeads, so as to infuse both CD34^{+} and CD34^{-} cells, including CD8^{+} T cells, NK cells, and other accessory cells in addition to hematopoietic precursor cells [110]. In contrast to the frequently used polyclonal ATG, OKT-3 spares incoming engraftment facilitating cells such as NK-cells. In children with leukemia or lymphoma, Lang et al. [111] compared positive HSC selection with CD34- ($n = 39$) or CD133-coated magnetic microbeads ($n = 14$) to T- and B-cell depletion using CD3- and CD19-coated microbeads ($n = 11$). Primary engraftment was 85% after CD34-positive selection, 72% after CD133-positive selection, and 91% after CD3/CD19-negative selection. The incidence of grade II–IV GVHD was similar after CD34- or CD133-positive selection (3% and 7%, respectively), but increased to 27% after CD3/CD19-depletion. Despite the rise in GVHD, the investigators reported a lower than 10% incidence of fatal viral infections, suggesting that rapid CD3^{+} cell recovery had occurred. Graft processing by negatively selecting T cells that express an alpha/beta T-cell receptor could exploit the principle that gamma/delta T cells do not cause GVHD and may have antileukemia effects [112]. It remains unclear whether CD3 or T-cell receptor α/β plus CD19-negative selection provides any advantage over positive CD34-selection, or whether OKT3 is as effective as ATG.

To reduce toxicity, Bethge et al. designed a conditioning regimen consisting solely of chemotherapy: fludarabine (150–200 mg/m^2), thiotepa (10 mg/kg), melphalan (120 mg/m^2), and OKT3 (5 mg/day, from day −5 to day +14) and no post-transplant immunosuppression [113]. Engraftment was prompt, with full donor chimerism after 2–4 weeks in all patients. The incidence of grade II–IV GVHD was 48%. In the first 100 days, six of 29 (20%) patients died of treatment-related complications. A prospective multicenter phase II study of haploidentical HSC transplantation using CD3/CD19-depleted grafts after reduced-intensity conditioning with fludarabine, thiotepa, melphalan and OKT3 extended encouraging results to adults, median age 46 years (range, 19–65) [114]. In addition to chemotherapy the anti-CD3 monoclonal antibody OKT3 was used in order to deplete remaining host-T-cells, avoiding graft rejection. Only 30 of the 61 patients were in complete remission. Grafts contained a median of 7.0×10^6 (range, 3.2–22) CD34^{+} cells/kg, 4.2×10^4 (range, 0.6–44) CD3^{+} T cells/kg and 2.7×10^7 (range, 0.00–37.3) CD56^{+} cells/kg. Engraftment was rapid with median of 12 (range, 9–50) days to >500 granulocytes/μL and 11 (range, 7–38) days to >20,000 platelets/μL. Incidence of grade III–V acute and chronic GVHD was 46% and 18%, respectively. The authors concluded that with this regimen doses $<5 \times 10^4$ CD3^{+} cells/kg seem safe even without GVHD prophylaxis, doses of $>5 \times 10^4$ CD3^{+} cells/kg require GVHD-prophylaxis such as MMF. Doses $>15 \times 10^4$ CD3^{+} cells/kg should be avoided. Non-relapse-mortality on day 100 was 23%, and 42% at 2 years. Cumulative incidence of relapse/progression at 2 years was 31%. The Kaplan–Meier estimates of EFS were 34% at 1 year and 25% at 2 years, and the survival estimates were 41% at 1 year and 28% at 2 years. Blood T-cells were a median of 191 (range, 38–799) cells/μL on day 100, and the subset of naïve T cells were slower to repopulate compared to memory T cells with a median of 28 (range 0–152) CD4^{+}CD45RA^{+} versus 79 (range 14–310) CD4^{+}CD45R0^{+} cells/μL at days 100 and 166 (range 21–2396) versus 237 (range 46–252) at 400 days after transplantation. T-cell repertoire was skewed with oligoclonal T-cell expansion to day 100 and tended to normalize after day 200. DLI was used in 24 patients for relapse or mixed chimerism and a faster recovery in T- and NK cells occurred in six of the patients. Although this approach demonstrated that megadoses of stem cells higher than 10×10^6/kg and full myeloablative conditioning were not required for successful engraftment in HCT from haploidentical donors, it was complicated with higher relapse rates, possibly because of reduced intensity of the conditioning and persistently delayed immune reconstitution.

Studies using conventional high-dose conditioning regimens

In Japan, a transplant trial of CD34^{+} cells selected with the Isolex device (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) enrolled 135 children, 64 of whom received two-HLA locus-mismatched grafts, and 43 three-locus-mismatched grafts [115]. Median CD34^{+} cell doses were 3.2×10^6/kg for patients receiving marrow only, 5.5×10^6/kg for those receiving PBPCs only, and 4.9×10^6/kg for those receiving marrow and PBPCs, and median T-cell doses were 6.0, 9.4, and 12.1×10^4/kg, respectively. The conditioning regimens before HCT included TBI in 77% of the cases and ATG in 52%; therefore they were in general less intense and immunosuppressive than those developed in Perugia. Patients needed post-transplant immunosuppression to prevent GVHD. Graft failure occurred in 13% of patients with hematologic malignancies, and 40% of patients with non-malignant disease. The incidence of GVHD was 10% when ATG was included in the conditioning and 27% when it was not included. Disease-free survival at 5 years was 39% in standard-risk patients and 5% in high-risk patients. These results confirm that CD34^{+} PBPCs do not ensure consistent engraftment of HLA-mismatched transplants unless an optimized conditioning regimen is administered, and that GVHD prevention requires postgrafting immune suppression if T-cell depletion is suboptimal.

Harnessing immunity to infections and leukemia

Several mechanisms are responsible for patient immunodeficiency after transplantation of T-cell-depleted hematopoietic cells from HLA-incompatible donors. In adults, early immune recovery stems from the expansion of mature T cells in the graft. However, in HLA-mismatched transplants with T-cell-depleted grafts, the number of T cells in the graft must be sufficiently low to prevent GVHD, and anti-T-cell antibodies administered in the conditioning regimen further deplete the few transplanted T cells. Intense conditioning regimens induce tissue damage that prevents T-cell homing to peripheral lymphoid tissues, where the generation and maintenance of T-cell memory take place [116,117]. Administration of G-CSF after transplantation is immunosuppressive as it blocks IL-12 production by APCs and decreases T-cell responses to pathogens in vitro and in vivo after transplantation [118]. Since the administration of G-CSF after transplantation was omitted in the Perugia trials, engraftment rates have remained unchanged, and recovery rates of immune parameters, including APC production of IL-12, have improved [118].

Several strategies are under investigation to hasten post-transplant immune recovery and decrease mortality from opportunistic infections without causing GVHD. Reinfusion of non-alloreactive T cells into patients previously transplanted with T-cell-depleted grafts from HLA-mismatched donors may lead to immune reconstitution without GVHD. Such approaches involve the co-incubation of donor T cells with recipient APCs in the presence of agents that can selectively eliminate or inactivate host-reactive T cells. In two studies, donor T cells were exposed ex vivo to recipient alloantigen and treated with an immunotoxin specific for the IL-2 receptor alpha chain [119,120]. This approach was effective in selectively eliminating alloreactive T cells without eliminating T cells reactive to third-party antigens. Based on preclinical data that antigen presentation in the absence of CD28 co-stimulation induces a state of T-cell unresponsiveness, one clinical trial employed host APCs and soluble cytotoxic T-lymphocyte antigen-4 immunoglobulin (Ig) to present host alloantigen to donor T cells while blocking the CD28 co-stimulatory pathways. This approach resulted in ex vivo donor T-cell unresponsiveness to the HLA-mismatched cells of the recipient [121]. The latter approach appears to have the potential to facilitate engraftment and perhaps immune reconstitution, but does not appear to eliminate GVHD.

To hasten reconstitutition of pathogen-specific immunity, Perruccio et al. generated T-cell clones specific for *Aspergillus* or cytomegalovirus (CMV) antigens, screened them for cross-reactivity against recipient alloantigens, and infused the non-alloreactive clones soon after HCT [122]. The maximum safe dose (1 million $CD3^+$ cells/kg recipient body weight) appeared clinically effective and did not cause GVHD. CMV reactivation and *Aspergillus* galactomannan antigenemia disappeared over time, while the frequencies of anti-*Aspergillus*- and anti-CMV-specific T-cell clones increased. Pathogen-specific T cells persisted over time, did not cause GVHD, and were associated with control of *Aspergillus* and CMV antigenemia and infectious mortality. In another study, CMV-specific T cells were generated by ex vivo stimulation with CMV pp65 and infused to control refractory CMV viremia or disease after HLA-matched unrelated donor or haploidentical HCT [123]. Similar approaches were used to prevent or control adenovirus or Epstein–Barr virus infections [124–126]. Although efficacious such approaches are time consuming and labor intensive and thus not broadly applicable.

For adoptive transfer of T cells with a wide T-cell receptor repertoire, polyclonal donor T cells were engineered to express suicide genes, for example the herpes simplex thymidine kinase (HSV-TK) gene, to guarantee engineered cell lysis if they triggered GVHD [127,128]. To date, 128 patients were treated with TK-engineered T cells in several phase I or II clinical trials, and follow-up of more than 228 persons/year has been accumulated [129]. No adverse event related to gene transfer has been observed [129]. Most of the patients who received TK-engineered T-cell add-back after haplotype mismatched HCT obtained a rapid and broad immune reconstitution, protective against pathogens and apparently benefited from a low rate of infection-related mortality. Notably, in clinical trial "TK007," only patients with TK-engineered T-cell engraftment progressed to full-term immune reconstitution, whereas in the absence of TK-engineered T cells, the immune recovery was dramatically slow, and similar to that reported after TCD transplants. Although TK-engineered T-cell engraftment appeared necessary and sufficient to promote a robust T-cell recovery, the immune reconstitution was progressively enriched by T lymphocytes negative for transgene expression and retroviral integration. Starting from these observations, Vago et al. recently reported that donor-derived transgene-negative T cells, observed in TK007, were enriched for recent thymic emigrants, denoting their de novo generation in the host thymus [130]. These data indicate that immune reconstitution after TK-cell infusion is supported by a thymic-dependent pathway and results in the maturation and differentiation of donor hematopoietic precursors in the recipient thymus. The low incidence of GVHD observed in the absence of any pharmacologic immunosuppressive treatment, suggest that the high numbers of T cells circulating in these patients had achieved central tolerance in the patient thymus, with negative selection of host-reactive cells. This was also confirmed by the fact that the activation of the suicide gene machinery, sufficient to control GVHD, did not affect immune reconstitution [130]. Di Stasi et al. devised an inducible T-cell safety switch based on the fusion of human caspase 9 to a modified human FK-binding protein, allowing conditional dimerization and cell suicide following administration of the small molecule dimerizing drug AP1903 [131]. Five leukemia patients were transplanted with HLA-haploidentical, CD34-selected hematopoietic stem cells followed by infusion of the genetically modified T cells. Four patients developed GVHD and were treated with AP1903; 90% of the modified T cells were eliminated within 30 minutes and the GVHD was terminated without recurrence.

Photodepletion using dibromorhodamine (TH9402) in a mixed lymphocyte reaction efficiently eliminates alloreactive donor T lymphocytes against the recipient, as it affords a thousand-fold decrease in the frequency of alloreactive T cells [132–134]. In a phase I clinical trial, Grade II acute GVHD developed in 4/19 patients and extensive chronic GVHD developed in five. Immunologic reconstitution was dose-dependent as infection and non-relapse mortality rates improved with the highest T cell doses [135].

Naturally occurring regulatory T cells (Treg) are a thymic-derived $CD4^+$ cells subpopulation which constitutively expresses the IL-2 receptor (CD25). They play a crucial role in the control of autoimmune processes in mice and humans. Like all other T cell-receptor alpha/beta expressing T lymphocytes, Tregs are antigen specific. Various animal models of mismatched HCT have shown that priming and robust expansion of alloantigen-specific Tregs occur in the peripheral lymphoid organs. These cells act in an alloantigen-specific manner during their effector phase in vivo thus controlling activation and expansion of alloreactive donor T lymphocytes via interaction with APCs at priming sites. Infusion of naturally occurring Tregs or ex vivo expanded Tregs, followed by conventional T lymphocytes (Tcons), prevents GVHD and favors immune reconstitution [136–139]. These insights from animal models prompted the Perugia group to evaluate the impact of post-transplant adoptive immunotherapy with freshly isolated donor Tregs and Tcons in the clinical setting of mismatched transplantation. Twenty-eight leukemia patients were conditioned with 8 Gy TBI, fludarabine, thiotepa, and CY. On day −4, 2×10^6/kg freshly isolated donor Tregs were infused, followed 4 days later, by 1×10^6 Tcons and 10×10^6 highly purified $CD34^+$cells/kg. Full donor engraftment was achieved in 26/28 patients. With no post-transplant pharmacologic immunosuppression, grade II–IV GVHD occurred in two cases and 13/26 evaluable patients died from non-relapse causes, including eight from infection with or without GVHD. The pattern of post-transplantation immune reconstitution featured rapid recovery of T-cell subpopulations, development of a wide T-cell repertoire, and high frequencies of pathogen-specific $CD4^+$ and $CD8^+$ lymphocytes. Indeed, patients had significantly fewer CMV reactivation episodes; none died of CMV disease [140].

These studies demonstrated the naturally occurring Tregs that are harvested from healthy donors efficiently control the alloreactivity of large numbers of otherwise lethal, conventional T cells. Evidently, activation of alloantigen-specific Tregs occurs efficiently in vivo and is not associated with long-term inhibition of general immunity to pathogens. In a different approach by Brunstein et al. on 23 patients, double-unit unrelated cord blood cell transplantation was followed by infusion of 0.1–30.0 × 10^5/kg third-party unrelated cord blood Tregs, which were ex vivo expanded using anti-CD3/anti-CD28 antibody-coated Dynabeads [141]. The incidence of Grade II–IV aGVHD was lower than in historical controls (43% vs. 61%; $p = 0.05$), with no significant differences in relapse and infection rates.

A crucial point about adoptive immunotherapy with Tregs is whether a GVLE is maintained in high-risk patients with acute leukemia since FoxP3$^+$ Tregs can also suppress antitumor responses. Studies in human beings have highlighted the increase in number of FoxP3$^+$ Tregs in tumor tissues and draining lymph nodes in several types of cancer. In contrast, a mouse model of mismatched transplantation showed that early infusion of Tregs protected the animals from GVHD and did not abrogate Tcon-mediated GVL effect against A20 leukemia cell line [142].

To address the issue of leukemia relapse, a subsequent study investigated whether Treg–Tcon adoptive immunotherapy provided a Tcon-mediated GVLE in the absence of GVHD [143]. The conditioning regimen included total body TBI, thiotepa, and fludarabine. The first 25 patients also received cyclophosphamide, while 18 others received alemtuzumab or thymoglobulin 21 days before transplant to avoid interference with the subsequent Treg–Tcon. All patients received freshly isolated donor Tregs on day −4, followed by a megadose of purified CD34+ cells and Tcons on day 0. The 4-day interval between Treg and Tcon infusions was in accordance with animal data indicating that early Treg administration provided greatest protection against GVHD. No pharmacologic post-transplant GVHD prophylaxis was given. Thirty-three out of 43 patients had AML (18 in 1st CR, 15 in 2nd or subsequent remission), 10 had ALL (7 in 1st CR; 3 in 2nd or subsequent remission). All patients in 1st remission were at high risk of relapse. All patients received a full haplotype-mismatched graft, which included CD34$^+$ cells (mean 9.7 × 10^6/kg ± 3.1; mean contaminating T cells: 0.8 × 10^4/kg ± 0.4), Tregs (mean 2.5 × 10^6/kg ± 1) and Tcons (mean 1.1 × 10^6/kg ± 0.6). Forty-one of 43 patients achieved primary, sustained full-donor type engraftment and 6/41 (15%) patients developed grade II or greater acute GVHD. In two patients, GVHD required a short course of immunosuppression and one patient developed chronic GVHD. Twenty-three out of 41 patients are alive with a DFS probability of 0.56 at 18 months minimum follow-up. Overall, the cumulative incidence of non-relapse mortality was 0.40 which fell to 0.21 in 18 patients who received anti-T antibodies in the conditioning. At a median follow-up of 46 months (range 18–65 months), 2/41 evaluable patients have relapsed. In the Treg–Tcon cohort the cumulative incidence of relapse was significantly lower than in historical controls (0.05 vs. 0.21; $p = 0.03$). Multivariate analysis identified Treg–Tcon adoptive immunotherapy as the only predictive factor associated with a reduced risk of relapse (RR 0.06; 95% CI, 0.02–0.35; $p = 0.02$). Tregs did not impact upon post-transplant generation of donor versus recipient alloreactive NK cells. This report showed for the first time that Treg/Tcon infusion was associated with a surprisingly low incidence of leukemia relapse while it efficiently prevented GVHD. One may hypothesize that the infusion of high numbers of mature T lymphocytes in the absence of any post-transplant immunosuppression exerted the apparently strong antileukemia effect.

Role of NK cell alloreactivity in HLA-incompatible HCT

NK-cell activation is regulated by a balance between inhibitory and activating receptors (see Chapter 11). In humans, currently 16 inhibitory killer Ig-like receptors (KIR) genes and pseudogenes are known to codify for inhibitory and activating KIRs. Inhibitory KIRs recognize amino acids in the COOH-terminal portion of the MHC class I $\alpha1$ helix [144–146]. They possess two (KIR2D) or three (KIR3D) extracellular C2-type Ig-like domains and a long cytoplasmic tail (L) containing immunoreceptor tyrosine-based inhibition motifs, which recruit and activate SHP-1 and SHP-2 phosphatases for inhibitory signal transduction. KIR2DL1 recognizes HLA-C alleles characterized by a Lys80 residue (HLA-Cw4 and related; "Group 2" alleles). KIR2DL2 and KIR2DL3 (which are allele variants) recognize HLA-C with an Asn80 residue (HLA-Cw3 and related; "Group 1" alleles). KIR3DL1 is the receptor for HLA-B alleles sharing the Bw4 supertypic specificity (Tables 44.3 and 44.4).

Another type of human NK-cell inhibitory receptor involved in HLA recognition is CD94-NKG2A. It binds to the non-conventional class I molecule HLA-E [147]. Several HLA class I alleles provide signal sequence peptides that bind HLA-E and allow its expression at the cell surface. Consequently, it is expressed in every individual.

Inhibitory KIRs, CD94/NKG2A, and HLA-class I genes determine individual NK-cell repertoires during development [148,149]. As they are located on different chromosomes, receptors and ligands segregate independently in human pedigrees. The HLA class I genotype selects a self-tolerant repertoire by dictating which KIR and/or NKG2A receptor combinations are to be used as inhibitory receptors for self-HLA class I. Consequently, every functional NK cell in the mature repertoire expresses at least one inhibitory receptor for self-HLA: coexpression of two or more receptors is less frequent.

Since inhibitory KIRs recognize specific groups of HLA class I molecule, that is, HLA-C group 1, HLA-C group 2, or HLA-Bw4 alleles, NK cells with the potential to exert allogeneic reactions use KIRs as inhibitory receptors for self-HLA class I [150–155]. NK cells that express, as their only inhibitory receptor for self-HLA, a KIR for the class I group that is absent on allogeneic targets sense the missing expression of the self-class I KIR ligands and mediate alloreactions ("missing self" recognition) (Figure 44.6).

Most individuals can exert allogeneic reactions as they possess a full complement of inhibitory KIR genes. The KIR2DL2 and/or

Table 44.3 Human leukocyte antigen (HLA) class I specificity of the main inhibitory killer immunoglobulin-like receptors (KIRs) expressed by human natural killer (NK) cells

KIR genes*	Encoded protein	HLA specificity†
KIR2DL1	P58.1 receptor	HLA-C group 2 i.e., Cw2, Cw4, Cw5, Cw6 Sequence: Asn77, Lys80
KIR2DL2/3	P58.2 receptor	HLA-C group 1 i.e., Cw1, Cw3, Cw7, Cw8 Sequence: Ser77, Asn80
KIR3DL1	P70/NKB1 receptor	HLA-Bw4-associated i.e., B27

*KIR2D refers to receptor molecules with two immunoglobulin-like domains, whereas KIR3D refers to those displaying three immunoglobulin-like domains. Receptors having a long inhibitory cytoplasmic tail are designated as L (long), whereas those having a short activating tail are termed S (short).

†Two groups of HLA-C alleles can be distinguished on the basis of alternative amino acid sequence motif at position 77 and 80 of the $\alpha1$ helix.

Table 44.4 Human leukocyte antigen (HLA)-C group 1, HLA-C group 2 and HLA-Bw4 group alleles

Group 1 HLA-C alleles	Group 2 HLA-C alleles	HLA-Bw4 alleles
(Ser77, Asn80)	(Asn77, Lys80)	B5
Cw1		B13
	Cw2	B17
Cw3 (except C*0307, C*0310,[†] and C*0315)	C*0307 and C*0215	B27
	Cw4	B37
	Cw5	B38
	Cw6	B44
Cw7 (except C*0707 and C*0709)	C*0707 and C*0709	B47
Cw8		B49
Cw12[‡] (except C*1204 and C*1205)	C*1204 and C*1205	B51
Cw13		B52
Cw14 (except C*1404[§])		B53
C*1507	Cw15 (except C*1507)	B57
Cw16 (except C*1602)	C*1602	B58
	Cw17	B59
	Cw18	B63
		B77
		B*1513
		B*1516
		B*1517
		B*1523
		B*1524

Each serologically defined group includes all alleles except where noted.

[†] C*0310 (Ser77, Lys80) belongs to both HLA-C groups 1 and 2 [142]. C*0310 blocks natural killer (NK) cells expressing all HLA-C-specific receptors, but does not block clones expressing the Bw4 receptor.

[‡] C*1207 (Gly77, Asn80) cannot be assigned to either group based on its amino acid sequence, and still needs to be tested functionally.

[§] C*1404 (Asn77, Asn80) does not belong to either HLA-C group 1 or 2 and does not block NK cells expressing any HLA-C-specific receptor [142]. Expression of C*1404 in a patient behaves with respect to NK-cell recognition as if the patient does not express that HLA-C allele.

KIR2DL3 receptors for HLA-C group 1 are present in all; the KIR2DL1 receptor for HLA-C group 2 is found in 97% and the KIR3DL1 receptor for HLA-Bw4 alleles is found in approximately 90% of people [90,113,114]. Individuals expressing group 1 HLA-C alleles possess the KIR specific for group 1 HLA-C alleles (KIR2DL2 and/or KIR2DL3) and are alloreactive against cells from individuals who do not express group 1 HLA-C alleles. Individuals who express group 2 HLA-C alleles and possess the KIR specific for group 2 HLA-C alleles (KIR2DL1) exert NK alloreactions against cells from individuals who do not express group 2 HLA-C alleles. HLA-Bw4$^+$ individuals who also express the Bw4-specific KIR3DL1 receptor may have NK cells that are alloreactive against Bw4$^-$ cells. These KIR ligand mismatches occur often in donor–recipient pairs that are disparate for one HLA haplotype (Table 44.5).

When exerted in the donor-versus-recipient direction, NK cell alloreactivity emerged as a crucial factor in improving outcomes of haploidentical transplantation. The opportunity for NK cell alloreactivity reduced the risk of leukemia relapse, did not cause GVHD, and markedly improved event-free survival in a series of haploidentical transplants (57 patients with AML, 20 of whom were transplanted from NK-alloreactive donors) [154].

In an updated analysis [155], 112 patients with AML received HLA-incompatible HCT from NK-alloreactive ($n = 51$) or non-NK-alloreactive donors ($n = 61$). For patients in remission at the time of HCT, the cumulative incidence of relapse was significantly lower after transplantation from NK-alloreactive donors (3% vs. 47%; $p < 0.003$) (Figure 44.7), and this translated into an improved event-free survival (67% vs. 18%; $p = 0.02$) (Figure 44.7). For patients in relapse at the time of HCT, donor

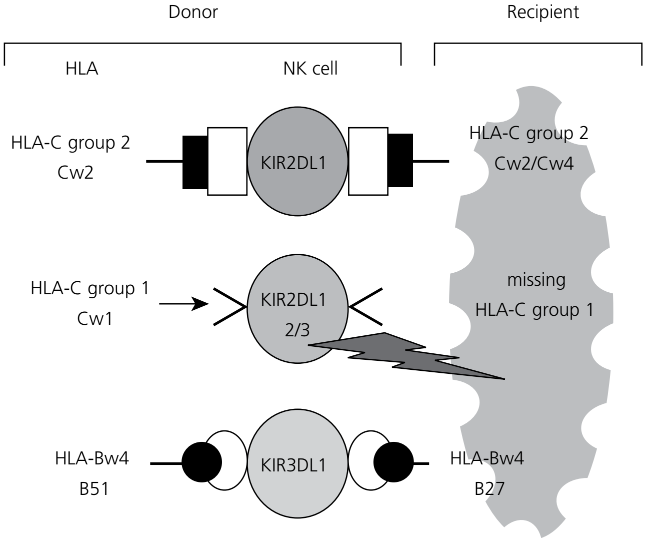


Figure 44.6 Donor-versus-recipient natural killer (NK) cell alloreactivity in human leukocyte antigen (HLA)-mismatched transplants. NK cell alloreactions are generated between donors and recipients who are killer immunoglobulin-like receptor (KIR) ligand-mismatched in the graft-versus-host direction. In these circumstances, donor NK cells expressing, as their only inhibitory receptor for self-HLA, a KIR for the class I group that is absent in the recipient sense the missing expression of the self-class I ligand on allogeneic targets and mediate alloreactions. This figure illustrates the example of a donor–recipient pair with a combination of mismatched HLA-C antigens, resulting in donor NK cell alloreactivity against recipient targets. The top donor NK cell expresses KIR2DL2/3 and is blocked by HLA-C group 1 antigens Cw1 on donor cells and Cw8 on recipient cells. The bottom donor NK cell expresses KIR3DLI that is blocked by Bw4 on both recipient and donor cells. The middle donor NK cell expresses KIR2DL1 that is blocked by donor (self)-HLA-Cw2 (an HLA-C group 2 antigen), but not by the recipient HLA-Cw3 (an HLA-C group 1 antigen). Consequently, this NK cell is alloreactive and lyses the recipient's cells (lightning bolt).

Table 44.5 Donor–recipient human leukocyte antigen (HLA) class I combinations associated with natural killer (NK) cell alloreactivity in the graft-versus-host direction

Recipient HLA type	HLA type of NK-alloreactive donor*
Group 1 HLA-C, Group 2 HLA-C, HLA-Bw4	No NK-alloreactive donor
Group 1 HLA-C, Group 2 HLA-C	HLA-Bw4
Group 1 HLA-C, HLA-Bw4	Group 2 HLA-C
Group 2 HLA-C, HLA-Bw4	Group 1 HLA-C
Group 1 HLA-C	Group 2 HLA-C and/or HLA-Bw4
Group 2 HLA-C	Group 1 HLA-C and/or HLA-Bw4

*Recipients who express class I alleles belonging to the three major class I groups (HLA-C group 1, HLA-C group 2, and HLA-Bw4 alleles) will block all NK cells from every donor. Donors may exert donor-versus-recipient NK cell alloreactivity when HLA-C and HLA-B typing shows KIR-ligand mismatches in the graft-versus-host direction, that is, the recipient does not possess one HLA-C allele group (C1 or C2) and/or the HLA-Bw4 group that are present in the donor. In the donor's HLA typing, the alleles listed are associated with the potential to exert NK cell alloreactions against the specific HLA type of the recipient, whatever other alleles may be present.

The HLA-C group 1 receptor genes (KIR2DL2 and/or KIR2DL3) are present in 100% of individuals. High-frequency alloreactive NK clones are detected in these individuals. The HLA-C group 2 receptor gene (KIR2DL1) is present in approximately 97% of donors. When the gene is present, high-frequency alloreactive NK clones are detected in donors. The KIR3DL1 HLA-Bw4 receptor gene is present in only around 90% of individuals. Even when the gene is present, alloreactive NK clones occur in highly variable frequencies and are detected in two-thirds of donors. Thus, functional assessment of the donor NK repertoire appears necessary.

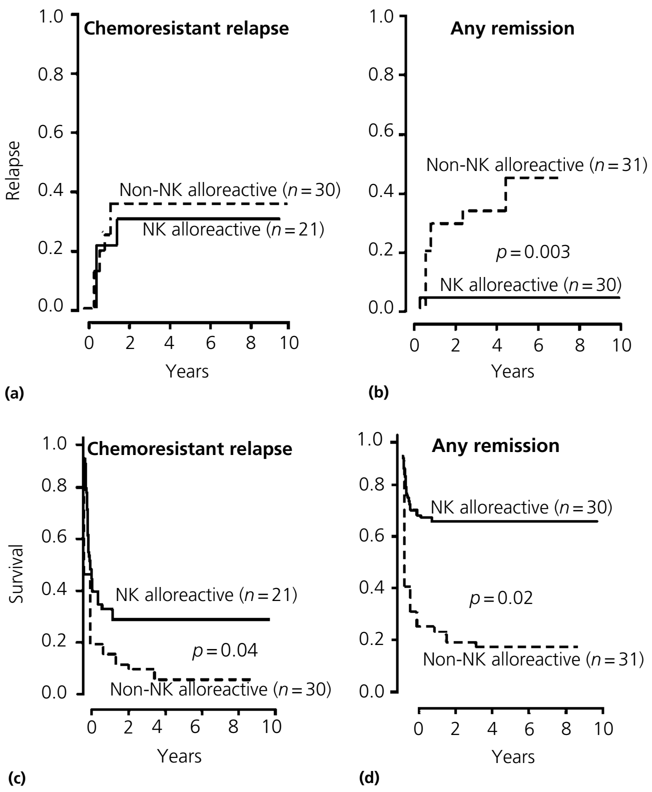


Figure 44.7 Transplantation from haploidentical natural killer (N-alloreactive donors controls relapse in acute myeloid leukemia and improves event-free survival. (a) Relapse in patients transplanted in chemoresistant relapse from NK-alloreactive versus non-NK-alloreactive donors. (b) Relapse in patients transplanted in any remission from NK-alloreactive versus non-NK-alloreactive donors. (c) Event-free survival in patients transplanted in relapse from NK-alloreactive versus non-NK-alloreactive donors. (d) Event-free survival in patients transplanted in complete remission from NK-alloreactive versus non-NK-alloreactive donors. Data source: Ruggeri et al. [155].

NK cell alloreactivity was associated with no difference in post-transplant relapse (32% vs. 37%; p = NS) (Figure 44.7), although it was associated with improved event-free survival (0.48%; 95% CI 0.29–0.78; $p < 0.001$) (Figure 44.7). Based on these data, donor NK cell alloreactivity against recipient cells has become a selection criterion for HLA-disparate donors when a T-cell-depleted protocol is employed.

The 67% probability of surviving event-free for AML patients transplanted in any remission from NK-alloreactive donors is in the range of best survival rates after transplantation from unrelated donors and cord blood units. The 34% event-free survival of patients transplanted in chemoresistant relapse from NK-alloreactive donors is also remarkable. Each of the three KIR ligand mismatches (C1, C2, Bw4) provided a survival advantage over non-NK-alloreactive transplants. NK cell alloreactivity provided better protection from leukemia relapse when exerted by maternal donor [22], presumably because of an additive effect provided by memory T cells against the child's paternal HLA haplotype as a result of maternal exposure to fetal antigens during pregnancy. Results from clinical trials showed NK cell alloreactivity was an effective form of immunotherapy in pediatric ALL as a lower incidence of ALL relapse was observed [156–158]. Several observations suggest alloreactive NK cells are responsible for favorable transplantation outcomes. Transfer of human alloreactive NK cells into NOD-SCID mice eradicated previously transplanted human AML cells [154]. KIR ligand mismatches correlated with the ability of donor NK cell clones to kill cryopreserved hematopoietic recipient cells, including leukemic cells [153–155]. Most importantly, engrafted HSCs give rise to an NK cell repertoire that includes donor-versus-recipient alloreactive NK clones that kill cryopreserved hematopoietic recipient cells, including leukemic cells [153]. Donor-versus-recipient alloreactive NK clones are detected in vivo in recipients for up to 1 year after transplant [155].

One immediate consequence of these clinical findings is exploitation of NK cell alloreactivity in haploidentical donor selection. The search for NK-alloreactive donors may require extension beyond the immediate family to aunts, uncles, cousins, etc. In the afore-mentioned clinical studies, NK-alloreactive donors were found for around 50% of patients, which approaches the maximum because one third of the population express class I alleles belonging to all three class I groups recognized by KIRs and block NK cells from every donor.

How is an NK-alloreactive donor selected? Recipients who express alleles belonging to one or two of the three class I allele groups recognized by KIRs may find NK alloreactive donors. Donors who are HLA-C group mismatched with their recipients possess high-frequency NK clones that are alloreactive against recipients' target cells [153–155]. Thus, high-resolution HLA-C typing is a good predictor of NK cell alloreactivity (Tables 44.4 and 45.5). Since 3% of individuals do not possess the KIR2DL1 gene [155,105,114], the combination of a KIR2DL1$^-$ donor and a recipient without HLA-C group 2 alleles could result in a 1.5% incidence of false positivity. KIR2DL1 gene typing of the donor may be necessary to assess the NK-alloreactive potential of this combination.

In HLA-Bw4 mismatches, even when the KIR3DL1 gene is present in the donor (~90% of individuals) [145,153,159], NK repertoire studies show that alloreactive NK clones are non-detectable in around one third of individuals [90]. In some, allelic variants in the HLA-Bw4 inhibitory NK receptor gene *KIR3DL1* may not allow full receptor expression at the cell membrane and affect NK cell inhibition by the HLA-Bw4 ligand [160,161]; others apparently express alloreactive NK clones in very low frequencies. Thus, for HLA-Bw4 mismatches, functional assessment of the donor NK repertoire appears necessary.

As approximately half of unrelated donor transplants are mismatched for one or more HLA class I alleles, donor versus recipient NK cell alloreactivity may also occur in this setting. However, some retrospective studies show no advantage in transplantation from KIR ligand-mismatched donors [162–166]. Unrelated donor transplant protocols are heterogeneous in conditioning regimens, patient populations, and underlying diseases. They most commonly use T-cell-replete bone marrow harvests or, nowadays more frequently, peripheral blood progenitors that contain approximately 4 log more T cells than haploidentical grafts. Relatively few transplanted HSCs, combined with the high T-cell graft content and post-transplant immune suppression, have been associated with poor reconstitution of potentially alloreactive, KIR-bearing NK cells [167,168]. Other studies have observed an increased graft-versus-leukemia effect of KIR ligand-mismatched transplants [169–174]. A marked survival advantage was reported in patients who received ATGs pre-transplant to produce in vivo T-cell depletion, and a graft containing two- to threefold more nucleated cells than usual in unrelated donor transplantation for a population containing a high proportion of children [169]. Prospective studies are needed to determine whether strategies such as high doses of HSCs, T-cell depletion, and no post-transplant immune suppression, which harness donor-versus-recipient NK cell alloreactivity in haploidentical transplantation, can be implemented to improve outcome in more closely HLA-matched unrelated donor transplants.

Since the original report that NK cell alloreactivity in haploidentical transplantation rests upon KIR ligand mismatching and donor NK-cell recognition of "missing self" on recipient targets [154], the "missing ligand" model has been proposed as a powerful algorithm for predicting favorable transplant outcomes not only in haploidentical transplants [156,157], but also in matched sibling [175] and in unrelated donor transplants [176]. Under the perturbed conditions that exist after an HCT, it is hypothesized that NK allogeneic reactions occur when KIR ligand-matched donors possess an "extra" KIR for which neither donor nor recipient has an HLA ligand. These donors may carry KIR-bearing NK cells in an anergic/regulated state that, upon transfer into the recipient, are hypothesized to become activated and exert a GVLE.

However, even though self-tolerant NK cells that do not express inhibitory receptors for self-MHC have been described in mice [177–180] and humans [181], no studies have as yet determined whether tolerant NK cells acquire/resume cytotoxic effector function after transplant. When an adult series of AML patients transplanted from HLA-incompatible donors was analyzed according to the "missing ligand" algorithm, the "missing ligand" transplant recipients disappointingly had a worse prognosis than patients transplanted from NK-alloreactive (KIR ligand-mismatched) donors [155]. While differences in diseases, age of patients, and transplantation protocols, such as ATG [155] versus no ATG in the conditioning regimen [173–181], or peripheral blood CD34$^+$ cells [155] versus bone marrow [180,181] as a source of hematopoietic cells, may account for these conflicting results, the analysis shows that donor NK cell recognition of "missing self" on recipient targets is essential for triggering powerful NK cell allogeneic reactions that are beneficial for transplant outcomes. Indeed, a recent study demonstrated that NK cells that did not express receptors for self-HLA remained hypofunctional throughout NK cell reconstitution after HLA-matched transplantation, thus precluding any clinical benefits through breaking of NK cell tolerance [182].

Conclusion

Transplant results have demonstrated that an increasing degree of donor HLA incompatibility is associated with a proportionally increased risk of graft failure, GVHD, and transplant-related mortality. Initial studies utilizing T-cell-replete marrow transplants found that donor incompatibility for two or three HLA antigens represented a formidable barrier to success, did not allow sufficient control of GVHD, and led to unacceptably low patient survival. Great effort has been invested to investigate innovative approaches to overcome the HLA barrier. Hematopoietic growth factor-mobilized PBPCs have increased the probability of engraftment. Pre-transplant ATG plus intensified postgrafting immunosuppression or high-dose CY administered after transplantation have been developed to more effectively prevent GVHD. These new approaches have gained popularity because clinical results are promising and because they do not need dedicated laboratory facilities or specialized staff for cell manipulation. Clinical research on T-cell-depleted HCT from HLA haploidentical donors for the treatment of acute leukemia has been proceeding for over 20 years in centers worldwide. The use of T-cell-depleted marrow grafts has decreased acute and chronic GVHD, but at the expense of increased graft failure and leukemia relapse, and impaired immune reconstitution, leading to no advantage in survival. The use of T-cell-depleted PBPC has led to high probability of engraftment and GVHD prevention, it has proved crucial for the development of NK cell alloreactivity, which is important for the control of AML, and continues to offer opportunities for innovative immunotherapeutic strategies.

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CHAPTER 45

Hematopoietic Cell Transplantation from Unrelated Donors

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Introduction

Unrelated donor hematopoietic cell transplantation (HCT) is the fastest growing modality of allogeneic transplantation worldwide, with more than one third of patients older than 50 years of age (www.cibmtr.org; [1–3]). The increased safety, efficacy and availability of the procedure are the result of the development of less toxic conditioning regimens and improved graft-versus-host disease (GVHD) prevention and treatment strategies, recognition of the importance of comprehensive and precise donor human leukocyte antigen (HLA) matching, the availability of molecular technologies for the early detection of recurrent malignancy, the development of immunotherapeutic strategies for the treatment of recurrent disease, and improved supportive care of patients. Collectively, these advances have led to improved survival rates comparable to those after HLA genotypically identical sibling transplantation particularly for good-risk patients [4–8].

There has been a greater appreciation of the many factors that influence the success of unrelated donor HCT. Some factors that impact transplant outcome can be modified and/or choices are available, and thus provide the patient and physician with a means to plan the transplant at a time when these factors are optimal. Perhaps the best example of a modifiable factor is the HLA-match status of the donor. When a suitably matched donor is not available, a search can be continued until a better matched donor is identified, provided the patient's clinical status remains stable and the disease is well controlled. Other factors such as patient age cannot be changed, and yet other variables, such as comorbid medical conditions, may be difficult to modify. It has long been recognized that the success of allogeneic transplantation from related or unrelated donors is strongly influenced by the underlying malignancy and the burden of disease at the time of transplantation. For the individual patient, when to time the transplant, how the transplant should be performed, and from which donor, can be a complex decision that requires careful assessment of the risks and benefits.

Central to the success of an unrelated donor transplant is the ability to surmount the "transplantation barrier." The concept that GVHD is a complex disease of polygeneic etiology shaped by "environmental" factors, such as the conditioning and immunosuppressive regimens, provides a framework for understanding the immunogenetics of the transplantation barrier. The known genetic determinants involved in GVHD and the graft-versus-tumor (GVT) effect are genes of the HLA and the natural killer (NK) families, cytokine, and immune response genes. Identification of single genes of importance in transplant outcome is but the first step in understanding the basis of the transplantation barrier. The role of gene–gene interactions is increasingly recognized, and the inflammatory environment in which these interactions occur provides a basis for understanding individual patient susceptibility to GVHD and the strength of GVT. In turn, the impact of genetic effects varies with different conditioning regimens (high dose and reduced intensity) and immunosuppressive therapy (including manipulation of the graft, T-replete versus T-deplete grafts, and different methods for depletion), and with different cell sources [bone marrow (BM), peripheral blood progenitor cells (PBPCs) or cord blood]. Indeed, elucidation of the many genetic and environmental factors that influence outcome will continue to require methodical analysis of very large well-characterized transplant populations with complete clinical data.

The recent availability of a sequence of the human genome (www.hapmap.org) provides the much-needed map to identify the genes that constitute the transplantation barrier. With the technical ability to detect single-nucleotide polymorphisms (SNPs) in gene sequences between recipients and donors, however, our understanding of the extent and nature of human diversity is far ahead of what is known about the functional consequences of that genetic variation. Ultimately, how best to integrate genetic information into day-to-day clinical practice to benefit the individual patient will require continued clinical research on both the genetics front as well as in the development of novel prevention and treatment strategies. In this chapter, the major advances that have contributed to the increased safety, efficacy, and availability of unrelated donor transplantation are described. The reader is directed to Chapters 10, 29 and 37 for topics related to unrelated donor HCT and to Sections 5 and 6 for in-depth discussion of specific disease indications.

Patient factors

One of the most challenging aspects of planning transplantation for patients who lack a related donor is determining *who* can benefit from an unrelated HCT, *when* the transplant should be performed, *how* the transplant should be carried out, and *which* donor should be chosen. The development of reduced-intensity and non-myeloablative conditioning regimens now no longer restricts the use of unrelated donor HCT to only younger patients. In general, good candidates for unrelated donor HCT include patients who otherwise would be

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considered for a matched sibling donor transplant had a matched sibling been available [9]. Individualized risk assessment is a critical step early in the process of planning an unrelated donor transplant and is facilitated by robust, validated risk factor indexes [10,11]. One such instrument is the European Group for Blood and Marrow Transplantation (EBMT) Risk Score which was originally developed to aid decision making for the timing of transplantation for CML [10]. Its parameters include patient age, disease stage, time from diagnosis to transplant, whether the stem cell donor is an HLA-matched sibling or other allogeneic stem cell donor, and patient–donor gender. An important feature of the risk score is the concept that the effects conferred by each of the five individual features are informative for additive negative effects.

Patients derive the greatest benefit from unrelated HCT when transplantation is performed with the lowest leukemic burden, using HLA-matched donors, and timed when the patient can best tolerate the transplant procedure. The dramatic impact of disease stage is best seen among recipients of HLA-A, -B, -C, -DRB1, and -DQB1 allele-matched unrelated donor HCT. The strong influence of disease diagnosis and stage at time of transplantation among allele-matched transplant recipients was validated in a National Marrow Donor Program (NMDP) and Center for International Blood and Marrow Transplant Research (CIBMTR) study wherein 5-year survival for good-, intermediate-, and high-risk leukemia was 63%, 48%, and 31%, respectively [12]. Patient survival was not affected by donor age, donor parity, donor cytomegalovirus status, or donor gender. Similar results were found in a single-center study, where survival at 10 years after transplantation for good-, intermediate-, and high-risk disease was 78%, 45%, and 35%, respectively [13] (Figure 45.1).

The three major indications for unrelated donor HCT are AML, ALL, and MDS (www.cibmtr.org; [1]). The reader is referred to Sections 5 and 6 of this volume for in-depth discussion of specific disease indications.

Acute myeloid leukemia

Transplantation from HLA-matched unrelated donors is associated with similar survival to that from HLA genotypically matched siblings for patients with AML [6,14,15]. If a patient is a good candidate for an allogeneic transplant but lacks a sibling donor, then an unrelated donor should be considered. As discussed in Chapter 50, historically cytogenetic markers have served as a foundation for classification schemes to define patients at low, intermediate, and high risk of disease recurrence after conventional and transplant therapy [16–18]. Low-risk de novo AML patients include those with the t(8;21) and inv(16) karyotypes, and the t(15;17)(q22;q12–21)$^+$ acute promyelocytic leukemia subtype. These patients enjoy a high response rate to conventional chemotherapy and a low (35%) probability of disease recurrence after first complete remission (CR1) is attained. For low-risk patients who lack an HLA-identical sibling donor, unrelated HCT in CR1 is associated with a high transplant-related mortality (TRM) that outweighs the benefits of the GVT effect compared with chemotherapy [19]. When a patient with good-risk features relapses, however, HCT in second complete remission (CR2) is indicated. Unrelated donor HCT should also be considered when a patient is unable to complete induction/consolidation therapy as planned.

High-risk AML karyotypes include -5, -7, inv(3) or complex karyotypes, defined as containing at least three chromosomal aberrations. The probability of disease recurrence among high-risk patients is 80% after CR1, and the probability of achieving CR2 is very low. For these patients, allogeneic HCT in CR1 remains the best option to prevent relapse [20,21]. Survival, disease-free survival (DFS), and relapse after unrelated HCT for high-risk AML in remission may be comparable to those obtained after related donor HCT, especially when the transplant can be performed in remission (www.cibmtr.org) [16]. A second group of very high-risk patients includes those who fail primary induction chemotherapy. Application of unrelated HCT for salvage in these patients requires individualized risk assessment. Patients requiring fewer than three courses of induction chemotherapy prior to transplantation, a low percent of marrow blasts and CMV seropositivity were favorable risk factors [22]. These results support the immediate initiation of an unrelated donor search for patients who fail to achieve a complete remission after the first attempt.

Historically, patients with normal cytogenetics were classified as "intermediate" risk, with a 50% probability of disease recurrence after CR1, and low chances that CR2 can be attained. In a retrospective CIBMTR analysis of unrelated HCT for AML in CR1 and CR2, the 5-year DFS for CR1 patients with favorable-, intermediate-, and poor-risk cytogenetics was 29%, 27%, and 30%, respectively; OS at 5 years for the same three groups was 30%, 29%, and 30%, respectively [23]. When unrelated HCT was performed in CR2, 5-year DFS for patients with favorable-, intermediate-, and high-risk cytogenetics was 42%, 38%, and 37%, respectively; OS at 5 years was 35%, 45%, and 36%, respectively. Patients with high-risk cytogenetics had a high risk of relapse after HCT, but the overall outcome after transplantation was superior to that with chemotherapy. Relapse after HCT was higher for patients with unfavorable cytogenetics whether

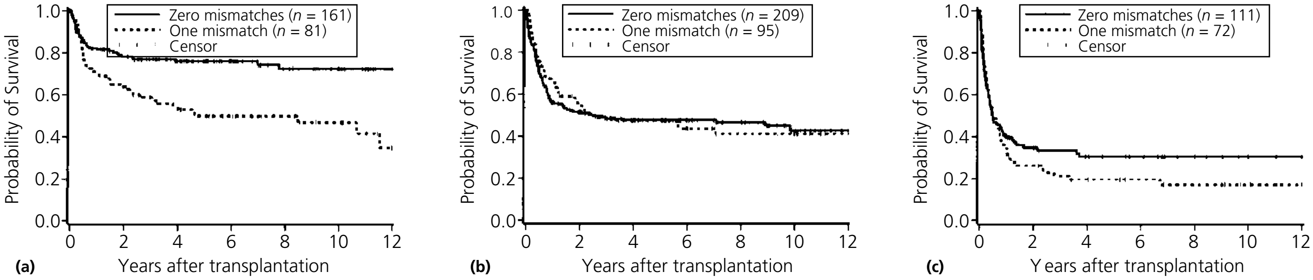

Figure 45.1 Impact of disease stage and human leukocyte antigen allele matching at the time of unrelated donor transplantation. (a) Low-risk chronic myeloid leukemia [chronic phase (CML-CP) and transplantation within 2 years of diagnosis]. (b) Intermediate-risk hematologic malignancies [CML-CP and transplantation more than 2 years from diagnosis; CML acute phase and CML blast phase/remission (CML-BP/rem), and transplantation within 3 years of diagnosis; acute leukemia in remission; myelodysplastic syndrome-refractory anemia]. (c) High-risk hematologic malignancies (CML-BP; CML acute phase and CML-BP/rem, and transplantation more than 3 years from diagnosis; acute leukemia in relapse; myelodysplastic syndrome beyond refractory anemia). Source: Petersdorf et al. 2004 [13]. Reproduced with permission of the American Society of Hematology.

the transplant was performed in CR1 or CR2, and there was evidence that there was less of a GVT effect with transplantation for high-risk AML in CR1 or CR2. More recently, molecular mutation data suggest that there are subgroups of better-risk and poorer-risk patients within the intermediate-risk group, based on nucleophosmin (NPMI), fms-related tyrosine kinase-3 (Flt3), and CCAAT/enhancer-binding protein α (CEBPA) markers [24]. NPMI mutations are generally associated with improved survival, in contrast to Flt3 mutations which have high potential for disease recurrence [25]. CEBPA is generally associated with a favorable prognosis [26]. In related donor transplantation, patients with the Flt3 mutation benefited significantly from transplantation, whereas patients without Flt3, regardless of their NPMI or CEBPA marker status, derived no benefit [26]. Hence, newer classification schemes incorporate molecular markers to refine risk strata within the intermediate-risk group of patients which have prognostic significance (Chapter 50) [27].

The clinical challenges for treating older patients with AML rest on the toxicities associated with both conventional chemotherapy as well as with the transplant conditioning regimen. A prospective single-center study designed to evaluate the applicability of reduced-intensity conditioning for related or unrelated donor transplantation found a 57% DFS among patients who underwent transplantation, an outcome that was superior to that of patients who did not have a transplant due to no donor [28]. These results support the application of reduced-intensity conditioning for older patients who, despite their chronological years, are excellent candidates for allogeneic transplantation.

Acute lymphoid leukemia

Unrelated donor HTC for acute lymphoid leukemia (ALL) is potentially curative [29]. Survival after unrelated HCT for ALL in CR1 is 42% compared to 48% after sibling donor HCT [30,31] (www.cibmtr.org). The prognostic indicators for high-risk adult ALL can be used to plan allogeneic transplantation. Factors associated with a high risk of disease recurrence include older patient age at diagnosis (>35 years), total white blood cell count >30,000/μL at diagnosis, failure to attain a first CR with conventional chemotherapy, and presence of the t(4;11) pro-B cell karyotype, t(9;22) translocation, hypodiploidy, and the rare t(1;19) karyotype.

The findings of a recent evidence-based review support unrelated donor transplantation for high-risk ALL in CR1, but not for standard-risk ALL [32]. For patients in CR2, transplantation from related or unrelated donors yields comparable survival, both superior to chemotherapy alone and to autologous HCT. Some very high-risk patients who relapse after chemotherapy may be salvaged with unrelated donor HCT [33]. The design of effective and less toxic conditioning regimens hold promise for controlling disease long term especially for older patients with comorbidities [34,35].

Myelodysplastic syndrome

Allogeneic transplantation is the only known cure for MDS [36]. The major limitation of allogeneic transplantation is disease recurrence among patients with advanced MDS and/or high-risk karyotype [37,38]. Since this disease is generally a disorder of older patients, high TRM has been observed with traditional conditioning regimens, and has led to the application of non-ablative conditioning approaches. A careful assessment of individual risk factors that may increase TRM is critical. The disease morphology, blast count, neutrophil count, disease duration, patient age, and cytomegalovirus serostatus all influence transplant outcome for MDS. The International Prognostic Scoring System stage at the time of transplantation strongly correlates with the risk of post-transplant disease recurrence, and can aid in the identification of patients who might benefit from HCT early after diagnosis.

Unrelated donor HCT for early MDS shows a low probability of disease recurrence. Data from the CIBMTR find that overall survival rates for adults are within 10–20% of those after HLA genotypically matched sibling transplantation [39]. When the disease has advanced with excess blasts, and when cytogenetics indicates higher risk disease (-5 or -7, or complex cytogenetic abnormalities), initiation of the unrelated donor search and careful monitoring of marrow function are critical. When MDS has advanced to AML, high relapse rates are seen after transplantation. The integration of hypomethylating agents both pre- and post-transplant into the treatment strategy remain important goals for the future [40,41].

Transplant factors

The effects of HLA mismatching after reduced-intensity conditioning, particularly at HLA-C, are similar to those observed after ablative conditioning, namely increased risk of clinically severe acute GVHD, increased non-relapse mortality, and lower overall survival [42,43]. Hence, the principles of HLA matching, which include limiting the total number of HLA mismatches and avoidance of HLA-C mismatching, hold true for both ablative and non-myeloablative approaches. A larger non-myeloablative transplant experience will be necessary to fully evaluate the relative risks associated with specific HLA locus mismatches and with allele versus antigen mismatches. The negative effects of HLA mismatching after non-myeloablative conditioning may be abrogated with the use of promising novel GVHD regimens [18].

Product factors

Over 60% of all allogeneic transplants performed worldwide today use PBPCs [2,3]. The source of hematopoietic cells influences transplant outcome. The BMT Clinical Trials Network completed a phase III multicenter randomized study of unrelated donor BM versus PBSC transplantation [44]. The overall 2-year survival rates in the PBSC and BM groups were 51% and 46%, respectively ($p = 0.29$); however, major differences in graft failure (3% vs. 9%; $p = 0.002$) and chronic GVHD (53% vs. 41%; $p = 0.010$) were observed. Therefore, PBSC transplantation was associated with higher rates of engraftment and chronic GVHD, whereas BM was associated with lower rates of engraftment and chronic GVHD. These findings open new strategies for the individualized use of PBSCs in clinical settings where engraftment is a major concern.

Donor factors

One of the most important aspects of planning an unrelated donor transplant is to avoid, whenever possible, the “urgent” donor search situation. As detailed in Chapter 10, the availability of high-throughput HLA typing laboratory support has decreased the time required to identify well-matched donors. Depending on the patient’s genotype, it is currently feasible to offer transplantation for high-risk patients within 3 months. For patients with high-risk features at diagnosis, prompt initiation of an unrelated donor search provides sufficient time for a suitable donor to be identified and for the transplant procedure to be performed when the disease is in remission. For all patients, but particularly high-risk patients, the identification of back-up donors provides options and avoids delays when the primary donor is unable to donate, or when the patient's condition changes [45].

The roles of donor age [5,46], donor sex [47], and CMV serostatus [46], have been extensively investigated as predictive factors in unrelated donor HCT, although recent registry data suggest only donor HLA [12] is a factor for survival. After HLA criteria are used to select among potential unrelated donors, further selection based on CMV negativity for CMV-negative recipients and avoidance of multiparous female donors for male recipients are often used by transplant centers when several otherwise equivalent donors are available (Figure 45.2).

Increased awareness of donor health and safety has prompted international efforts to standardize donor recruitment, management, and informed consent through two registries, the Serious Events and Adverse Effects Registry (SEAR) and the Serious Product Events and Adverse Effects Registry (SPEAR) [48]. These safety nets are designed for the reporting of donor events leading to life-threatening disease, death, in-patient hospitalization, or disability (SEAR). The stem cell product, the quality of the graft, labeling of the product, infusion reactions, unpredictable risk of transmissible infection, serious transportation problems, or damage to the cells during transport, are surveyed by SPEAR.

The unrelated donor search process

HLA studies are performed on the patient, parents, and full siblings to determine the availability of an HLA genotypically identical sibling donor (Figure 45.2). The resolution of HLA typing usually entails intermediate-high resolution of HLA-A, -C, -B, -DRB1, and -DQB1. When maternal and paternal haplotypes share antigens, high-resolution typing is required to define heterozygosity for two different alleles. A preliminary unrelated donor search is undertaken through Bone Marrow Donors Worldwide (BMDW; www.bmdw.org), a comprehensive datafile of unrelated donors and cord blood units currently on file in registries and banks worldwide. As of May, 2015, there are over 25 million registered unrelated donors. The likelihood of identifying HLA 8/8 or 10/10 matched donors ranges from 20% to 80% and depends on the recipient's genotypes and haplotypes, and the size and composition of the donor registries (www.worldmarrow.org; www.nmdp.org). A preliminary search of the BMDW provides clinicians with an excellent overview of the likelihood that suitably matched unrelated donors can be identified. The BMDW preliminary search does not offer direct access to donors or cord blood units for actual work-up, but provides essential information that can guide the search team and clinicians on the potential difficulties in identifying suitable donors, and hence an anticipated timeline for coordinating the transplant.

For potentially matched donors that lack sufficient HLA typing on all loci of interest or incomplete high-resolution typing, a strategy

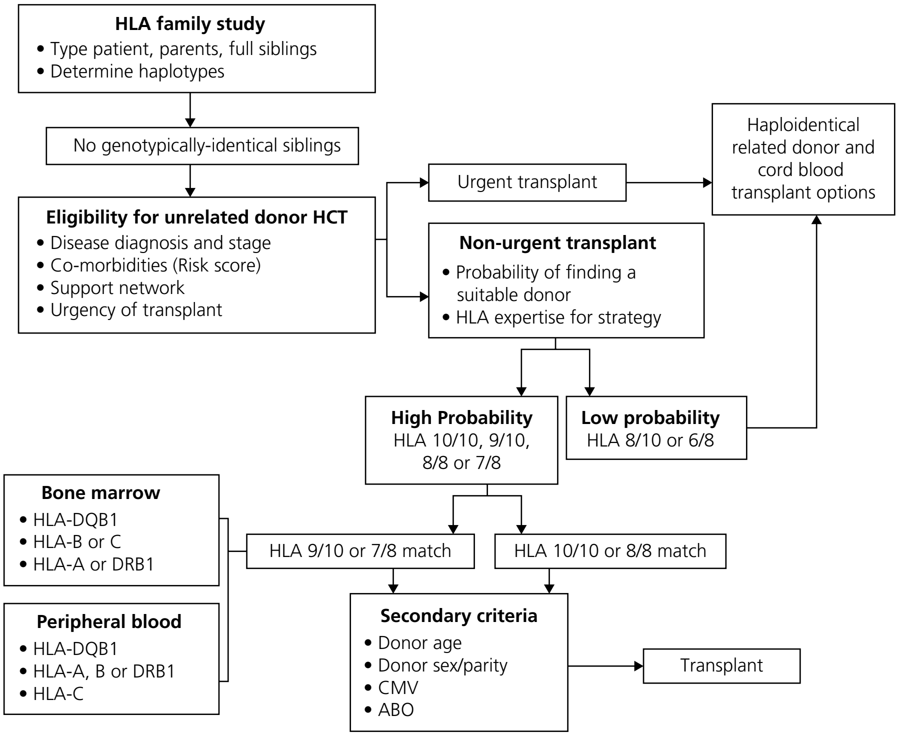
HLA family study
• Type patient, parents, full siblings
• Determine haplotypes
No genotypically-identical siblings
Eligibility for unrelated donor HCT
• Disease diagnosis and stage
• Co-morbidities (Risk score)
• Support network
• Urgency of transplant
Urgent transplant
Haploidentical related donor and cord blood transplant options
Non-urgent transplant
• Probability of finding a suitable donor
• HLA expertise for strategy
High Probability
HLA 10/10, 9/10, 8/8 or 7/8
Low probability
HLA 8/10 or 6/8
Bone marrow
• HLA-DQB1
• HLA-B or C
• HLA-A or DRB1
Peripheral blood
• HLA-DQB1
• HLA-A, B or DRB1
• HLA-C
HLA 9/10 or 7/8 match
HLA 10/10 or 8/8 match
Secondary criteria
• Donor age
• Donor sex/parity
• CMV
• ABO
Transplant

Figure 45.2 The unrelated donor search process and selection of donors. The search process begins with complete family typing where the patient's tissue type is confirmed through segregation of the maternal and paternal HLA haplotypes. Eligibility for unrelated donor HCT is an important early step where the urgency of the transplant is determined. For very urgent clinical situations, consideration of a haploidentical related donor or cord blood transplant is appropriate. For non-urgent cases, input from HLA experts may greatly facilitate the search and selection process. Patients whose searches yield only highly mismatched donors may be candidates for haploidentical related donor or cord blood transplantation. Searches that yield one or more suitable donors proceed to typing of donor samples for confirmatory typing. When several donors with a single mismatch (9/10 or 7/8) are under consideration, the preferred mismatched HLA locus may depend on the source of stem cells. For bone marrow, high risks are observed with HLA-A and -DRB1 mismatches, and low risk for -DQB1; for PBPC, high risks are observed with HLA-C mismatches, and low risk for -DQB1 [12,58]. Secondary criteria may be applied to select the donor for the transplant, and potential back-up donors.

for typing donor samples to maximize the yield of identifying at least one acceptable donor and back-up donors is important. When the BMDW report shows only potential HLA-mismatched donors, guidance from HLA experts at this early stage of the search is highly desirable. Several strategies based on known allele, antigen, and haplotype frequencies in world populations can be used to direct the donor search towards mismatching for one specific HLA determinant in an attempt to match at all remaining loci, thus limiting the total number of HLA mismatches. Allele and haplotype frequencies are available from several sites including http://allelefrequencies.net; http://hla.alleles.org; http://www.ebi.ac.uk/ipd/imgt/hla/; http://bioinformatics.nmdp.org; http://hlexplorer.net; and http://pypop.org (websites accessed February 2, 2015).

Once a formal search is initiated, transplant centers begin the typing process to confirm the HLA match status of donors of interest. Since donors may not have complete typing on HLA-A, -C, -B, -DRB1, the NMDP has developed a program, HapLogic™, to estimate the likelihood that donors are potentially HLA matched with the patient (http://www.ebi.ac.uk/ipd/imgt/hla/searchdet_nmdp.html; accessed February 2, 2105). The concept is that even though an HLA locus has not been typed, information at the other typed loci can permit probability estimates that the sample encodes a specific HLA determinant. Allele codes used by the National Marrow Donor Program (NMDP) provide information on the possible four-digit allele that could be encoded in a donor sample. This information is helpful for prioritizing the HLA typing of donors to maximize the chances that a high-resolution matched donor can be identified [49]; http://bioinformatics.nmdp.org (accessed February 2, 2015).

Donors are volunteers. Different circumstances may arise long after the donor has registered that preclude donation such as medial deferral or loss of contact; donor issues have also included unavailability for the desired dates and wrong tissue typing. The importance of back-up donors is critical to the coordination of an unrelated donor transplant [45,50]. When the clinical status of the patient is ultra-urgent, then consideration of haploidentical related donors and cord blood units is appropriate.

Donor selection

GVHD is commonly observed after unrelated HCT, and is a complex phenotype shaped by both genetic and "environmental" factors. As a complex phenotype, GVHD has a polygenic etiology. The genes known to be involved in the pathogenesis of GVHD are the HLA, killer immunoglobulin-like receptor (KIR), and cytokine genes. Although the recipient's genotypes are not modifiable, the potential to select a donor with the "optimal" genetic make-up to reduce GVHD and TRM is feasible. The environmental factors that influence a given patient's risk of GVHD are contributed by the chemotherapy- and radiation-containing regimens used to prepare the patient, the immunosuppressive agents used to prevent and treat GVHD, and the source of donor stem cells, to name but three important factors. To a great extent, these conditions can be modified to fit each patient's need, and there has been tremendous progress in the development of novel approaches such as reduced-intensity conditioning regimens.

Recognition that a complex phenotype such as GVHD arises from the effects of more than one gene is an important platform for future research in immunogenetics. Gene–gene interactions may vary under widely different transplant procedures and with different approaches for GVHD prevention and treatment, and might explain the heterogeneity of results reported from different studies. As more information becomes available on the extent of HLA, NK, and immune response gene diversity in human populations, it is appreciated that the race of the recipient and donor plays a major role in defining the genetics of a given transplant. A full understanding of the genetic basis of host-versus-graft (HVG) and graft-versus-host (GVH) alloreactivity will come from the continued evaluation of racially and ethnically diverse populations.

This section reviews the sentinel advances in the identification of the immunogenetic risk factors in unrelated donor HCT: major histocompatibility (MHC) region genes and haplotypes, HLA–NK interactions, and cytokine and immune response genetics. Based on extensive worldwide experience, the current criteria for the selection of unrelated donors are (Figure 45.2; Table 45.1):

1. Type for 10 alleles (high resolution) at HLA-A, -B, -C, -DRB1, and -DQB1.
2. Match for a minimum of 8/8 high-resolution defined HLA-A, -B, -C, and -DRB1 determinants [12,13,51–55].
3. When several 10/10 donors are available, some centers type and match for HLA-DPB1 ("12/12" donors) and consider permissible HLA-DPB1 mismatches [56,57].
4. When there are no 10/10 matched donors, use of a donor with an isolated HLA-DQB1 mismatch may be acceptable [12].
5. When donors with mismatching at HLA-A, -B, -C, or -DRB1 must be considered, the selection of mismatched donors may depend on the graft source. *Marrow* grafts from HLA-A and -DRB1-mismatched donors are the most risky, followed by HLA-B and -C [12]. *PBPC* grafts from HLA-C antigen mismatched donors are the most risky, followed by HLA-A and -B.
6. When a mismatched donor is considered, allele mismatches are as risky as antigen mismatches at HLA-A, -B, -DRB1. At the HLA-C locus, antigen mismatches are more risky than allele mismatches [12,58].

The data that support these criteria are derived from retrospective research studies that employed high-resolution typing at all loci, had sufficient power to detect statistically significant differences when they exist, and used multivariate analysis to control for non-HLA variables that affect clinical outcome [59,60] (Table 45.1) and are summarized as follows:

Which HLA loci should be typed? A "matched" donor may be defined on the basis of high-resolution allele definition at four loci – HLA-A, -B, -C, -DRB1 ("8/8") – or five loci HLA-A, -B, -C, -DRB1, -DQB1 ("10/10") – or 6 loci HLA-A, -B, -C, -DRB1, -DQB1, -DPB1 ("12/12"). HLA 10/10 matched transplants have superior DFS compared with any mismatch [12,13,52,53]. The impact of donor HLA matching is especially dramatic for a subset of good-risk patients with AML, MDS, ALL, and CML; transplantation using a high-resolution matched unrelated donor can yield survival rates that are very similar to those seen after HLA-identical sibling transplantation (www.marrow.org) [12,13,61–66] (Figure 45.1).

More recently, a minimal level of matching has been defined among HLA-A, -B, -C, -DRB1 genes [12]. In this analysis of 3860 patients transplanted from unrelated donors following myeloablative conditioning, high-resolution matching at HLA-A, -B, -C, and -DRB1 was associated with highest survival rates. Outcomes following a 10/10 matched HCT were comparable to those after 9/10 HCT when the single mismatch was at HLA-DQB1, indicating that the 8/8 high-resolution matched unrelated donor can be used as a predictor for patient survival. Based on these findings, the NMDP recently recommended the use of 8/8 matching criteria for all retrospective research studies employing HLA data [59]. For prospective donor search and selection, information on HLA-DQB1 becomes

Table 45.1 Role of HLA in unrelated donor hematopoietic cell transplantation

Model	Locus	Graft failure	Graft-versus-host disease	Graft-versus-leukemia effect	Survival	Comments
Single locus mismatch	A	[51,52]	[12,52–54]		[12,52–54]	Antigen mismatches are associated with higher risks than allele mismatches [53]. TRM is increased with any mismatch [12]. HLA-A mismatch is high risk in BM HCT [12] and intermediate risk in PBSC HCT [58].
	B	[51,52]	[12,54]		[13,53,54]	Antigen mismatches are associated with higher risk than allele mismatches [53]. TRM is increased with any mismatch [12]. HLA-B mismatch is intermediate risk in both BM and PBSC HCT [12,58].
	C	[51,55]	[52,54]	[52]	[13,53]	Single allele or antigen mismatches are detrimental, notably HLA-C in low-risk patients [13]. Antigen mismatches are associated with higher risk than allele mismatches [12,53]. TRM is increased with any mismatch [12]. HLA-C mismatch is intermediate risk in BM, and high risk in PBSC [12,58].
	DRB1		[13,51,54]		[12,13,53]	Antigen mismatches are associated with higher risk than allele mismatches [53]. There is a trend for lower survival [12]. HLA-DRB1 mismatch is high risk in BM HCT [12].
	DQB1		[13]		[13]	HLA-DQB1 mismatch is low risk in BM and PBPC HCT [12,58].
	DPB1		[72]	[57,67]		HLA-DP is a classical transplantation antigen.
Epitopes	HLA-A, -B, -C, -DRB1		[77,80]	[81]	[77,80,81]	Residue 116 substitutions in class I are detrimental [77]. 15 high-risk allele mismatches are identified; 6 class I residues are identified as non-permissive [80]. Residues for acute GVHD may be different to those for relapse.
	HLA-DP		[56,71]		[71]	HLA-DPB1 HVG vector mismatches increase graft failure in thalassemia [71]. TCE permissive model provides approach for selecting lower risk donors who are DPB1-mismatched [56].
Haplotype	HLA-A, -B, -DRB1		[98]	[98]		Among HLA 10/10 matched transplants, haplotype matching was associated with higher severe acute GVHD, lower relapse, and no difference in overall survival compared to haplotype-mismatched transplants.
	Extended MHC		[69]			The presence of specific haplotypes in Japanese patients was associated with higher or lower GVHD risk.
	Common haplotypes		[99]			Presence of 1 or 2 common Caucasian haplotypes correlated with lower risk of grades II–IV acute GVHD.

Summary of studies that meet the criteria for an interpretable study: large populations typed at high resolution for a minimum of five loci (HLA-A, -B, -C, -DRB1, and -DQB1) and use of multivariable biostatistical methodology.
HVG, host-versus-graft; TRM, transplant-related mortality.

important when available donors have a known HLA-A, -B, -C or -DRB1 mismatch, because multilocus mismatching increases morbidity and mortality [12].

At which HLA locus is it acceptable to use a mismatched donor? Data on the relative risks of HLA mismatching have been largely drawn from patients receiving conventional GVHD prophylaxis (e.g., an antimetabolite and a calcineurin inhibitor). When a 10/10 matched donor cannot be identified, current data support the selection of donors with an isolated HLA-DQB1 mismatch (i.e., an 8/8 match at HLA-A, -B, -C, and -DRB1). However, when donors are mismatched HLA-A, -B, -C, or -DRB1, an HLA-DQB1 mismatch may increase morbidity and mortality [12,13]. CIBMTR data show that survival after 8/8, 7/8, and 6/8 transplantation was 52%, 43%, and 33%, respectively, a 9–10% survival decrement with each additional HLA mismatch [12]. When donors with mismatching at HLA-A, -B, -C, or -DRB1 must be considered, the selection of mismatched donors may depend on the graft source. Marrow grafts from HLA-A and -DRB1-mismatched donors are the most risky, followed by HLA-B and -C [12]. PBPC grafts from HLA-C antigen mismatched donors are the most risky, followed by HLA-A and -B [58]. Whether these same principles of HLA mismatching apply in the setting of T-cell depletion or use of Cytoxan after HCT as GVHD prophylaxis, remain to be determined.

The negative effects of HLA disparity on risk of graft failure [51,54,55], GVHD [12,51–54], and mortality are offset by the beneficial effects on GVL [52,54,67]. One clinical setting where multiple mismatches may be tolerable has recently been identified in Japanese patients transplanted for severe aplastic anemia, where any single or multiple HLA-C/DRB1/DQB1 mismatch did not lower survival or increase acute GVHD [68] and may be due to the more conserved HLA haplotypes in this population [69,70].

When there is a choice of donors, is there a difference between allele mismatches and antigen mismatches? When allele-matched donors are not available, selection among donors with allele or antigen mismatches is not always straightforward. Early data suggested that antigen mismatches conferred greater risks than single-allele mismatches (also previously referred to as "micro" mismatches) [53,55], implicating the immunogenicity of the number and nature of nucleotide substitutions in the peptide binding groove and TCR contact residues. Registry data indicate that allele mismatches are as risky as antigen mismatches at all HLA loci with the exception of HLA-C where antigen mismatches are more risky than allele mismatches [12,58]. These data are consistent with the graft failure study [55] where there was a predominance of HLA-C mismatches, and allele disparities did not contribute to the increased risk.

When should HLA-DPB1 be considered? HLA-DP is a classical transplantation antigen [57,67,71,72]. Six-locus 12/12 matching is associated with optimal outcome particularly for patients with low-risk disease [57]. Higher rates of GVHD are accompanied by lower rates of relapse, and suggest that HLA-DP can participate in the

GVT effect [56,66]. The negative effects of HLA-DPB1 mismatching are additive to haplotype-linked polymorphisms in the class III region of the MHC within the tumor necrosis factor (TNF) locus [73].

Because most HLA-A, -B, -C, -DRB1, -DQB1-matched donors are DPB1-mismatched, it has not been practically possible to identify DPB1-matched donors without significant typing resources and only in non-urgent clinical settings. Recently, a novel concept has been applied to identify permissible DPB1 mismatches [56] in which the specific immunogenetic amino acid residues of the polymorphic exon 2 were identified through cytotoxic T-cell assays, referred to as T-cell epitope (TCE) [71]. In an analysis of 8539 unrelated donor transplants, patients receiving a 12/12 matched donor had significantly higher risk of relapse and lower risk of grades III–IV acute GVHD compared to patients transplanted from a donor with a TCE-permissive DP mismatch. HLA 10/10 with a non-permissive DP mismatch, however, had significantly increased mortality, non-relapse mortality and grades III–IV acute GVHD. An HLA-A, -B, -C, -DRB1, or -DQB1 mismatch together with an HLA-DP TCE non-permissive mismatch was associated with the poorest survival compared to 12/12, 10/10 TCE-permissive or non-permissive, and 9/10 TCE-permissive donors. Patients transplanted from 9/10 TCE-permissive donors did not have significantly different risks of mortality compared to patient transplanted from 10/10 TCE-non-permissive donors. The beneficial effect has been confirmed [74]. For transplant centers wishing to use DPB1 TCE to select among HLA 10/10 matched donors, an interactive web-based tool has recently been developed for automated scoring of permissive or non-permissive DPB1 mismatch combinations (http://www.ebi.ac.uk/imgt/hla/dpb.html; accessed February 2, 2015).

Are there ancillary tools for predicting permissible HLA mismatches? "Permissible" HLA mismatches may be defined by polymorphisms that alter the peptide repertoire or that directly contact the T-cell receptor (TCR). Several approaches have been used to identify the sequence variation that governs permissivity, including functional assay of cytotoxic T-lymphocyte precursor (CTLp) cells [75] and direct examination of sequence motifs [76]. Early studies identified substitutions at residue 116 of class I molecules as immunogenic epitopes [77]. More recently, models for exploring permissible HLA mismatches in North American [78] and Japanese [79,80] patients have been described. In a large retrospective study of 4866 unrelated donor transplants facilitated by the JMDP [80], Tyr9–Phe9 amino acid mismatch of HLA-A and the Tyr9–Ser9, Asn77–Ser77, Lys80–Asn80, Tyr99–Phe99, Leu116–Ser116, and Arg156–Leu156 mismatches of HLA-C were each associated with a significantly increased risk of severe acute GVHD. In a follow-up analysis of relapse, four HLA-C and six HLA-DPB1 mismatch combinations were identified with lower risk for relapse [81]. Bioinformatic strategies have thus far not been successful in predicting permissible mismatches for clinical practice [82–84].

When should a cord blood transplant or a haploidentical related donor transplant be considered? In the absence of prospective studies, side-by-side evaluation of retrospective studies of HLA-matched and -mismatched unrelated donor, cord blood, and haploidentical related donor HCT permits an algorithm for the selection of stem cell source (Figure 45.2). HLA-matched unrelated donor HCT has, on the whole, lower toxicity and better if not equivalent outcomes to cord blood transplantation (CBT) after ablative conditioning with respect to survival, acute GVHD and TRM [85–88]. Single CBT is associated with higher TRM but similar DFS and survival as HLA-matched unrelated donor HCT [87]; HLA-mismatched unrelated donor HCT has lower TRM but increased relapse when compared to double CBT [88]. Hence, when an unrelated donor search fails to identify an HLA 9/10 or 7/8 unrelated donor (i.e., only 8/10 or 6/8 donors are available), then consideration of cord blood or haploidentical related donor is indicated. Cord blood and haploidentical related donors are also indicated when there is clinical urgency (Figure 45.2).

New frontiers: the clinical importance non-HLA gene polymorphisms

Ethnicity and race

Ethnicity and race affect the probability of identifying 8/8 matched donors [89] and the success of transplantation [90–92] that cannot be ascribed to socioeconomic differences [91]. Low socioeconomic status regardless of race was also identified to have a negative impact on outcomes for all patients. One unifying hypothesis is the role of undetected genetic variation that could have different alleles or variants in one population but not others, described in the following section on HLA haplotypes [69,70,93].

HLA haplotypes

The trends for lower relapse after unrelated compared with matched sibling HCT suggest a more intense GVT effect that arises from undetected genetic disparity among unrelated individuals. Because of its gene density, the MHC is an attractive candidate region for novel genetic variation that may affect transplant outcome even in the HLA-matched setting. Several recent lines of evidence support the role of untyped MHC-resident genes in transplant outcome. An increased risk for bacterial infections and corresponding TRM at day 180 after transplant was found in recipients transplanted from HLA-E*01:01,01:01 homozygous unrelated donors [94]. Mapping studies conducted in Japanese and Caucasian transplant populations uncovered microsatellite-linked risks [95,96] pointing to the existence of non-HLA MHC-linked variation that is clinically relevant.

One successful approach for mapping the MHC has used information from HLA haplotypes to define regions that merit fine mapping. The concepts are based on the strong positive linkage disequilibrium (LD) between HLA alleles and non-HLA variants. Because HLA allele-matched unrelated donors and recipients are not related to one another, the physical linkage of donor HLA alleles to one another may be different from that of the recipient's haplotypes (Figure 45.3). Hence, 10/10 matched unrelated donors and recipients who have the same haplotype linkage of HLA alleles may be more similar to each other with respect to undetected MHC variation than are 10/10 allele-matched pairs whose HLA haplotypes differ. A long-range phasing method was developed to specifically test these hypotheses [97], and when applied to 10/10 allele-matched unrelated donor–recipient pairs, uncovered a 20% frequency of haplotype mismatching [98]. Haplotype mismatching was associated with a significantly increased risk of grade III–IV acute GVHD. The increased risk of GVHD was offset by lower relapse, leading to similar survival (Figure 45.4). In a recent study by the Europdonor Foundation in the Netherlands, the unrelated donors' haplotypes were estimated using frequency data [99]. The presence of one or two frequent haplotypes was associated with a statistically significantly lower risk of grade II acute GVHD (HR 0.49). These observations provide evidence that haplotype-linked non-HLA variation may significantly affect transplant outcome, and that the HLA haplotype can be used as a surrogate marker for GVHD risk.

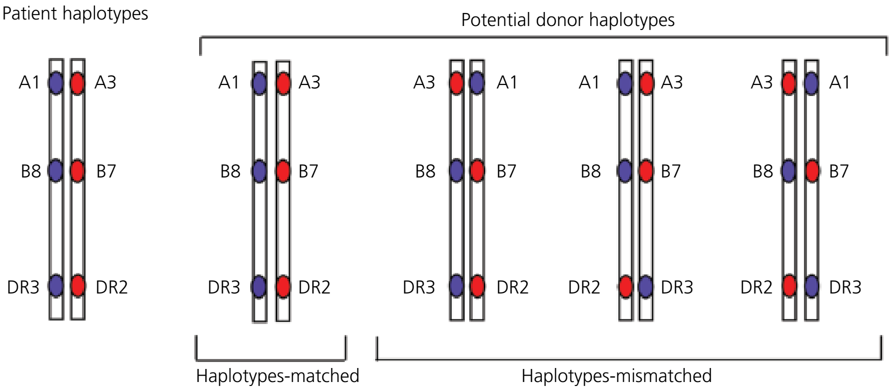

Figure 45.3 Human leukocyte antigen matched unrelated donors and recipients may be haplotype matched or haplotype mismatched [98].

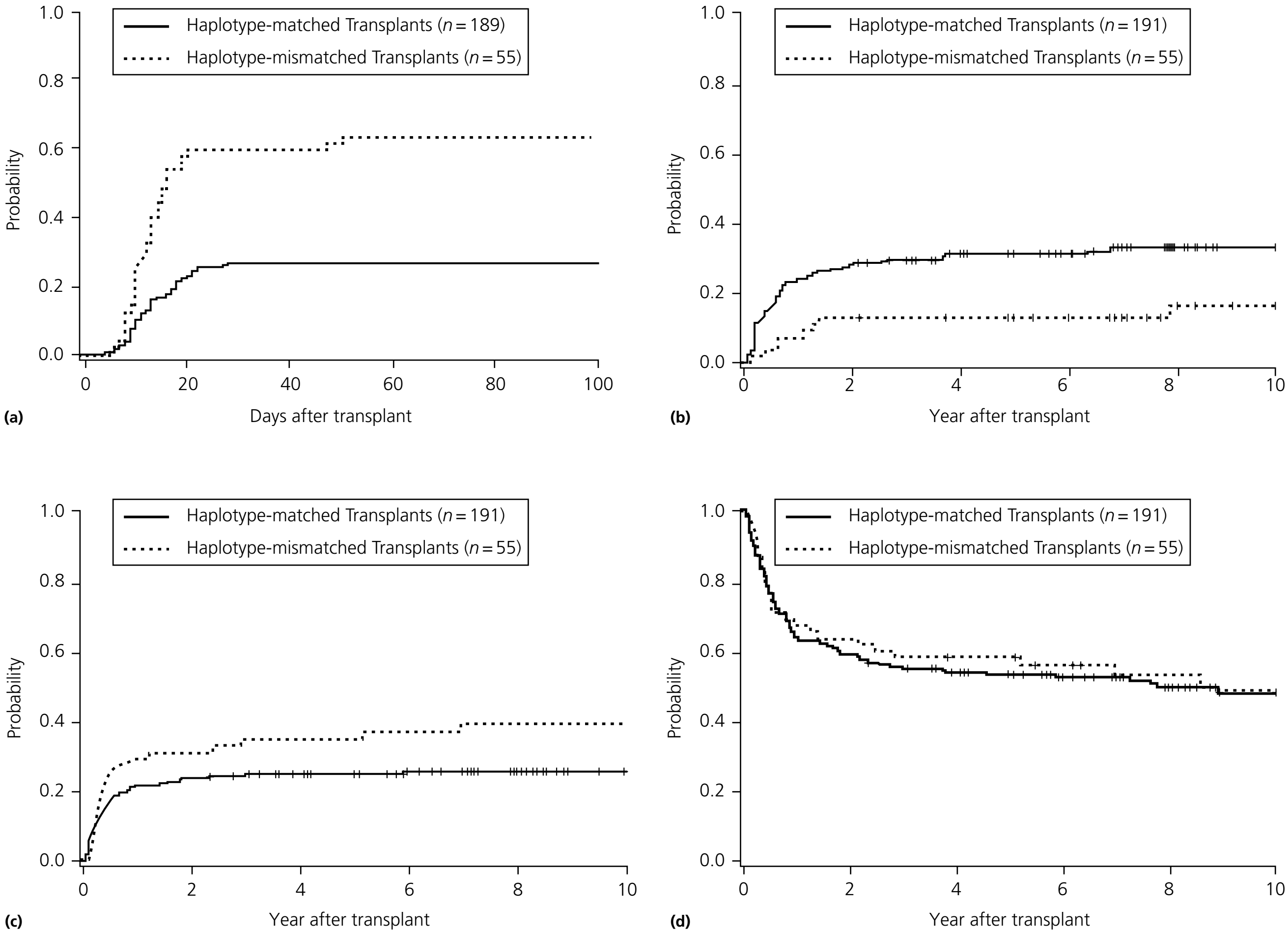

Figure 45.4 Impact of haplotype matching after human leukocyte antigen (HLA)-A, -B, -C, -DRB1, -DQB1 allele-matched unrelated donor hematopoietic cell transplantation. (a) Probability of grade III–IV acute graft-versus-host disease. (b) Probability of relapse. (c) Probability of transplant-related mortality. (d) Probability of survival. Source: Petersdorf et al. 2007 [98]. Reproduced under Creative Commons Attribution.

Undetected variation within the MHC may produce effects through patient–donor mismatching and/or the presence of specific variation. The JMDP surveyed a 9 Mb region of the MHC with SNPs to identify the degree of sequence conservation of three common Japanese haplotypes in a large cohort of unrelated donor–recipient pairs [69]. The presence of certain HLA haplotypes was associated with higher risks than others. A follow-up analysis of risks associated with specific SNPs has recently been carried out and is described later [70]. In the North American experience, two SNP markers were validated in 10/10 matched unrelated donor transplants [100]. A class I SNP residing close to the POU5F1 genetic locus was a marker for survival, whereas a second SNP telomeric to the HLA-DPB1 3′ untranslated region was a marker for acute GVHD. SNP genotyping of patients with two or more 10/10 matched unrelated donors revealed that most patients have a choice of donors with favorable SNPs at these two positions. These data suggest that prospective donor evaluation for MHC SNPs may help to lower risks to patients in the future.

KIR

The role of NK cells in the immunobiology of transplantation provides new avenues for optimizing allogeneic transplantation [101]. The majority of the data for NK in clinical transplantation are derived from the haploidentical related transplant experience (see Chapter 44). More recently, data on the role of HLA ligand–KIR interactions in unrelated donor HCT have demonstrated lower post-transplant disease recurrence in selected populations of mismatched transplants. These discoveries open the potential for integrating recipient ligand and donor KIR information into donor selection to fully maximize NK-mediated antitumor effects. That the HLA and KIR genetic systems segregate independently by classical Mendelian genetics has implications for HLA-matched and HLA-mismatched, related and unrelated donor HCT. The reader is referred to a comprehensive review of the NK–KIR models and typing methods (Chapter 10), basic immunobiology of NK cells (Chapter 11), and of the role of NK cell alloreactivity in haploidentical related donor transplantation (Chapter 44). The following section focuses on the recent clinical observations of HLA ligand–KIR interactions in unrelated HCT.

KIR ligands

The *ligand mismatch mechanism* of NK-cell alloreactivity is operational when there is an HLA class I disparity between the transplant recipient and donor. In unrelated donor HCT, the impact of ligand mismatching depends on disease diagnosis (lymphoid vs. myeloid), the conditioning regimen, the use of pretransplant antithymocyte globulin or ex vivo T-cell depletion methods [67,102–112] (Table 45.2). A recent study measured risks after T-cell-replete HCT for ALL, AML, MDS, and CML in three groups of donor–recipient pairs: HLA class I antigen matched (KIR ligand matched); class I antigen mismatched but KIR ligand matched, and HLA class I and inhibitory KIR ligand mismatched [111]. The 1-year survival for these three groups was 59%, 49%, and 30%, respectively. The mismatched groups had lower survival and event-free survival compared with the matched group; further, the group with both HLA and KIR mismatching had higher relapse and TRM than the other groups. A detrimental effect was observed for patients who lacked inhibitory KIR receptors; among HLA-matched cases, those who lacked the C1 or C2 ligand had lower survival compared with patients with either present. Patients who lacked C1 or C2 had higher TRM compared with patients who had all ligands present. These data suggest that inhibitory KIR ligand mismatching and missing inhibitory KIR ligands confer higher risk after T-replete unrelated donor HCT.

Table 45.2 The role of KIR in unrelated donor hematopoietic cell transplantation

Model and mechanism		Clinical endpoint	References
Mismatched ligand	Due to the donor–recipient HLA class I mismatch, the recipient's target cells lack the class I allotype presentin the donor	↓ Relapse	[67,103–109,111]
Missing ligand	Despite HLA identity, host cells are missing the class I ligand to provide the inhibitory signal	↓ Relapse	[109–112]
KIR haplotypes	Number of donor B haplotypes	↓ Relapse ↓ Mortality	[123]
	Number of activating KIR genes	↓ Relapse ↑ DFS	[125]
	Number of donor KIR3DS1 genes in B haplotypes	↓ II–IV acute GVHD ↓ TRM ↓ Mortality	[124]
	Donor homozygosity for A haplotype	↑ II–IV acute GVHD	[154]
KIR genes	Presence of KIR2DS1	↓ Relapse	[126]
HLA-E	Ligand for NKG2A	↓ Acute GVHD (E*01:03)	[117,118]
		↓ TRM ↓ Mortality	[119,120,127]
		No effect	[121]
MICA	Ligand for NKG2D		
	Patient–donor mismatching	↑ II–IV acute GVHD (GI)	[115]
		No effect	[116]

KIR, natural killer immunoglobulin-like receptor; DFS, disease-free survival; GVHD, graft-versus-host disease; TRM, transplant-related mortality; GI, gastrointestinal.

KIR-ligand mismatching had adverse effects on acute GVHD and rejection and no survival benefit for patients undergoing T-replete unrelated HCT in an analysis of 1790 transplants by the JMDP [67]. This study demonstrates an important role for HLA-C, -DPB1, KIR ligand mismatching on post-transplant relapse. Namely, donor–recipient HLA-C disparity was associated with reduced relapse among patients with ALL. HLA-DPB1 mismatches were associated with lower relapse among CML transplants. Mismatching for the KIR2DL ligand was associated with an increased risk of relapse for ALL. An increased risk of rejection was observed for KIR2DL ligand mismatches in the host-versus-graft vector. The risk of acute GVHD was increased with disparity for HLA-A, -B, -C, -DPB1, and KIR ligand mismatching in the GVHD vector; mismatching for HLA-A, -B, -DQB1, and KIR ligand in the GVH vector increased mortality.

When a recipient and donor are HLA matched, the recipient may lack the appropriate ligand for donor KIR receptor. This *missing recipient ligand* mechanism is also present between HLA-mismatched individuals, and therefore the lack of ligand is independent of donor–recipient HLA match status (see Chapter 10; Table 45.2). Missing ligand in the recipient has been associated with improved outcome after unrelated donor HCT [109,112]. Risks associated with missing recipient ligand and donor–recipient mismatched ligand were evaluated in patients who received T-replete BM or PBPCs after high-dose conditioning from either HLA-matched or HLA-mismatched unrelated donors [109]. Among HLA-mismatched recipients, the presence of homozygosity for HLA-Bw6 and C1 or C2 (missing ligand) was associated with a decreased hazard of relapse. Most of the protective effect was contributed by the subset of patients who were missing C2 or Bw4. The impact of missing ligand was most pronounced in patients with AML, CML, and ALL, and the effect was observed among HLA-mismatched and not HLA-matched transplants. Among HLA-B- and/or HLA-C-mismatched pairs, there was no difference between ligand-mismatched and ligand-matched cases. There was a trend for lower relapse among patients who were missing ligand compared with patients with ligands present. Taken together, these data indicate that missing ligand in the recipient is a useful indicator for lowered risk of post-transplant relapse.

The missing ligand model was tested in a large dataset of unrelated donor transplants for CML, AML, MDS, and ALL facilitated by the NMDP [112]. When compared with patients with all ligands present, patients who were missing one or more ligands had lower post-transplant disease relapse. Among the subset of good-risk CML patients, missing recipient ligand was an independent risk factor for clinically severe grade III–IV acute GVHD, indicating that NK-cell alloreactivity may play an indirect role in the pathogenesis of GVHD.

Recent analysis of C2 homozygosity in patients reveals different survival outcomes depending on whether the stem cell source was PBPCs (lower progress-free survival) or BM (improved survival) [113].

The highly polymorphic MICA locus in the class I region has been the subject of several new analyses concerning its role as a ligand for the activating NKG2D receptor [114–116]. Patient homozygosity for a valine at MICA residue 129 correlated with chronic GVHD risk in sibling donor HCT [114] but has thus far not been confirmed in unrelated donor HCT. Patient–donor mismatching at MICA was associated with an increased risk of grades II–IV acute GVHD particularly of the gastrointestinal tract after HLA matched and mismatched unrelated donor HCT [115]. The negative effect of MICA mismatching was independent of HLA-B or -C mismatching, two loci that display strong positive LD with MICA. The most commonly observed MICA allele, 008, did not impact transplant outcome. Different observations were made in an independent study of MICA after 12/12 matched unrelated donor HCT [116]. Among MICA*008-positive patients, there was a trend for less acute GVHD. Among 8/8 patient–donor transplants, GVHD risk did not depend on the specific HLA-B alleles present in the patient. A role for MICA in unrelated donor HCT remains to be elucidated.

In addition to MICA, the non-classical class I locus HLA-E may play a role in HCT. HLA-E is a ligand for the inhibitory NK cell receptor NKG2A. A protective effect of E*01:03 on acute GVHD [117,118] and lower TRM with improved survival [119,120] are reported in some studies, but not others [121]. Patient–donor mismatching for the HLA-E rs1264457 arginine/glycine residue 128 lowers survival [70].

KIR haplotypes and receptors

The specific genes that comprise KIR haplotypes content and their alleles may affect transplant outcome [110,122]. A higher number of donor B haplotypes is associated with lower relapse and improved survival in patients with AML but not ALL [123]. The protective effects were observed most strikingly with donors having two or more B haplotype-associated genes, and did not depend on coexisting HLA mismatching. The specific content of B haplotypes is also informative for outcome [124]. The higher the number of donor KIR3DS1 genes, the lower the risk of grades II–IV acute GVHD, TRM, and mortality after HCT from T-replete HLA-matched or -mismatched unrelated donors. These gene dose-related effects stemmed mainly from KIR3DS1, rather than B haplotype-associated polymorphisms, as GVHD risk was lower in B haplotype-positive recipients the effect of which was weaker than that from KIR3DS1.

The number of activating KIR genes has also been shown to influence risks of relapse and disease-free survival [125]. Transplantation from donors with group A KIR haplotypes or donors with lower numbers of activating KIR genes was associated with reduced relapse and improved DFS among AML/MDS recipients and to lesser extent among CML patients. There were no haplotype effects observed in patients with ALL. These data suggest that not only is the HLA ligand an important factor, but also the composition of donor KIR haplotypes may influence the risk of relapse and DFS. Use of donors with fewer activating KIR genes or with group A haplotypes, is associated with improved outcomes with respect to lower relapse and higher DFS for transplantation for AML or MDS.

Relapse after unrelated donor HCT for AML is lower when the unrelated donor carries KIR2DS1 and is not C2 homozygous [126], suggesting that the NK effects on leukemic relapse and possibly GVHD, are affected by the patient's and donor's KIR ligands, their KIR haplotypes, and specific KIR genes. Knowledge of both KIR ligands and receptors may be important in predicting suitable donors for transplantation; C1C2-positive patients receiving transplants from KIR2DS2-positive donors had lower risk of II–IV acute GVHD [127]. Finally, the level of KIR gene expression, in particular KIR2DS4, may also provide new information [128].

Cytokine and immune response gene variation

GVHD arises as a consequence of donor T-cell-mediated recognition of recipient HLAs not shared by the donor, and/or recognition of minor histocompatibility determinants. Additionally, the lack of donor inhibition of NK-cell KIRs may lead to lower GVHD risk, either because the donor and recipient differ for class I or because the recipient is missing the HLA ligand for which the donor has an inhibitory receptor. The activation of T-cell and NK-cell pathways that lead to GVHD is mediated by cytokines [129]. The activation of NK cells by immature dendritic cells in the periphery is facilitated by interleukin (IL)-2, IL-12, IL-15, and IL-18, and NK-cell cytotoxicity can be induced by TNF produced by dendritic cells. In lymphoid tissues, the IL-2- and IL-12-induced activation of NK cells leads to granulocyte–macrophage colony-stimulating factor and interferon-gamma (IFN-γ) production; these events induce T helper (Th) cell differentiation, notably polarization of Th1 cells. The intensity of tissue injury and inflammation of GVHD may be modulated through the effects of allelic diversity within the coding and promoter regions of cytokine and immune response genes [130]. Polymorphisms located in the 5′ and 3′ regulatory sequences influence transcription and cytokine serum levels, and may play a role in the release of cytokines during the three classical phases of the GVHD pathway. Recipient genetic variation for cytokine genes is hypothesized to exert its effect during the peritransplant period, when direct tissue injury from chemotherapy and radiation therapy initiates the activation of the cytokine storm; both recipient and donor genetic variation is hypothesized to modulate the severity of inflammation in the GVHD process.

The events that lead to clinical GVHD are classically described in three stages [129]. The inciting injury begins as early as the conditioning regimen when irradiation and chemotherapy directly damage host tissues and cause the release of the proinflammatory cytokine TNF-α. TNF-α serum levels peak during the conditioning regimen and correlate with increased risk of death (see later). The inflammatory environment promotes increased expression of HLA and minor histocompatibility antigens, recognition of which by donor T cells leads to the expansion of donor T-cell clones. This response is amplified by the production of cytokines by $CD4^+$ and $CD8^+$ T cells. Further augmentation of injury of host tissues from donor T cells ensues. The cytokines implicated in this phase include IL-1, IL-2, IL-8, and IL-10 [129]. The severity of GVHD may be further increased or diminished through the effects of INF-γ, and Th1 and Th2 cytokines, in particular IL-1, IL-2, IL-8, and IL-10. The final phase is characterized by cytotoxic T-cell-mediated tissue destruction.

Most of the published literature on cytokine and immune response genes has examined the effects of polymorphism of single genes after HLA-matched sibling donor HCT, with TNF-α and IL-10 being the two best studied genes. Data in unrelated donor HCT continue to emerge [131–133]. Since the distribution of cytokine gene and immune response gene variation differs worldwide, the racial and ethnic background of the study population is an important feature. This section will review the current literature that supports the hypothesis that genetic variation of selected cytokine and immune response genes modulates risks of GVHD and mortality after unrelated HCT (Table 45.3). The literature on

Table 45.3 Summary of studies of cytokine and immune response gene polymorphisms in unrelated donor HCT

Gene	Polymorphism	Model	Endpoint	Reference
CTLA4	CTLA4-CT60 (rs3087243 A/G)	Donor GG genotype	↓ III–IV aGVHD	[70]
		Patient genotype GG	↑ III–IV aGVHD	[140]
HPSE (Heparanase gene)	Rs4364254 C/T	Donor dominant model	↑ II–IV aGVHD	[140]
	Rs4693608 and rs4364254	Patient genotype	↑ II–IV, III–IV aGVHD	[144]
HSP70hom (Heat shock protein A1L gene)	2763G/A	Patient AA genotype	↑ II–IV aGVHD	[145]
IL1A	Rs1800587 (-889C/T)	Donor or patient genotype	n.s.	[137]
			↑ Survival	[155]
		Mismatch	n.s.	[70]
IL1B	Rs16944 (IL1B-511T/C)	Donor and recipient T genotype	AA ↓ aGVHD GG ↑ aGVHD ↑ Survival ↓ TRM	[155]
IL2	Rs2069762 (IL2-330T/G)	Donor genotype dominant model	↑ III–IV aGVHD	[140]
		Patient genotype G	↑ II–IV aGVHD	[138,155]
			↑ cGVHD (GT)	[70]
IL6	Rs1800795 C/G	Donor allele, recessive	↑ II–IV aGVHD	[140]
	-1082, -1064			[134]
IL10	-1082/-819/-592 Haplotype	R2 long allele G-C-C Donor	↓ TRM	[133]
		C-C-G/A Donor	n.s.	[70]
	Rs1800871 (-819); Rs1800896 (-1082); Rs1800872 (-592)		n.s.	[70]
	Levels			[139]
IL17	Rs2275913 (-197A/G)	Donor A genotype	↑ aGVHD	[146]
IL18	-137/-607/-656 haplotype	Patient haplotype (GCG)	↓ TRM ↑ OS	[142]
	Levels			[143]
IL23R	A1142A/G (Gln381Arg)	Donor genotype G (Arg)	↓ II–IV aGVHD ↑ III–IV aGVHD	[147]
			↓ aGVHD	[148]
	Rs6687620	Donor genotype	↓ II–IV aGVHD	[149]
			n.s.	[70]
	Rs11209026		n.s.	[140]
MTHFR (Methylenetetrahydrofolate reductase)	C677T	Patient T genotype	↓ aGVHD	[150]
		Donor genotype	n.s.	[151]
			n.s.	[70]
	A1298C	Patient C genotype	↑ aGVHD	[150]
NOD2	“SNP13”	Donor genotype	↑ III–IV aGVHD ↓ OS	[152,153]
	Rs17313265 Rs1077862 Rs1861757 Rs1861759 Rs6500328 Rs2111234 Rs211235 Rs7203344 Rs17313265		n.s.	[70]
			n.s.	[148,149]
TNF	Rs1799964 (-1031)	Mismatch	↑ IV aGVHD	[70]
		Patient C genotype	↑ III–IV aGVHD	[136]
		C genotype	↑ TRM	[133]
			n.s.	[140]
	Rs1800630 (-863) d4/d4	Patient genotype	↑ III–IV aGVHD	[136]
	D4/d5 microsatellite	Donor alleles	↑ Mortality	[73]
	-1031/-863/-857 (rs1799724) alleles	Patient and donor U02/U03 alleles	GVHD Relapse	[132]
				[131–135]
TNFR2	Rs1061622 (TNFRSF1B; residue Met196Arg)	Donor arginine (Arg) genotype	↑ III–IV aGVHD	[132]
			n.s.	[140]

aGVHD, acute graft-versus-host disease; cGVHD, chronic GVHD; n.s., not significant; TRM, transplant-related mortality.

cytokine gene and immune response gene polymorphisms in related donor transplantation is vast, and the reader is referred to outstanding comprehensive reviews on this topic [129,130].

Phase I: injury of host tissues and the initiation of the cytokine storm

In this phase, the skin, liver, and gastrointestinal tract of the recipient are the target organs of injury. The proinflammatory cytokines TNF-α and IL-1, together with the anti-inflammatory cytokine IL-10, are among the first to participate in the cytokine storm. TNF-α (chromosome 6p21.3) plays a multifaceted role in the pathogenesis of GVHD. TNF-α is produced by macrophages, monocytes, NK cells, and T cells, and functions as a Th1 cytokine by inducing apoptosis in target tissues and increased expression of HLA alloantigens, as well as by promoting further production of the IL-1, IL-6, and IL-10 cytokines [129]. The TNF-α and lymphotoxin-α genes are encoded within the HLA class III region, and are in strong positive LD with HLA [73,130]. The TNF promoter has extensive SNP diversity at positions -1031, -863, -857, -376, -308, -238, the most extensively studied polymorphisms of which are -308G/A and -863C/A. TNF-α variation is associated GVHD, TRM, and survival [131–134]. A role for TNF-α levels with grade III–IV acute GVHD [135] as well as the presence of arginine or methionine at residue 196 in the TNFRII receptor (chromosome 1p36) have been described [132]. More recently, SNP genotyping in discovery/validation cohorts have clarified the role of TNF haplotypes [70,73,136].

The IL-1 gene family (chromosome 2q14) consists of IL-1A, IL-1B, and the IL-1 receptor agonist. IL-1A is an intracellular regulator, and the IL-1A promoter has a C/T SNP at -899 which alters gene transcription and expression. The IL-1A -889T SNP in both unrelated donors and patients was associated with improved 1-year survival in one study [137]. The IL-1B gene encodes an extracellular protein. The IL-1B promoter has a T/C SNP at -511, and the T allele is associated with increased binding of transcription initiation factors. In a single-center study of unrelated transplant recipients, the presence of the -511T allele in the recipient and/or donor was associated with a significantly reduced risk of mortality and TRM that was independent of GVHD [138].

IL-10 (chromosome 1q31–q32) is a Th2 cytokine and a member of the IFN receptor superfamily. IL10 is anti-inflammatory, reduces HLA expression, lowers CTL recognition of HLA, and strongly inhibits TNF-α, IL-1A, IL-1B, IL-6, IL-12, and IFN-γ. The IL-10 promoter has several polymorphic positions (-3575, -2763, -1082, -1064, -819, and -592), of which the -1082A allele is associated with lower in vitro IL-10 production. In this way, IL-10 influences risks of acute and chronic GVHD. Most studies of IL-10 variation in allogeneic HCT have defined an inverse relationship between IL-10 levels and risk of acute GVHD; high IL-10 levels, or the presence of genetic variants associated with high production of IL-10, correlate with lower incidence of GVHD and mortality, consistent with anti-inflammatory protective effects. Recipient homozygosity for the -592AA promoter genotype was associated with decreased risk of severe acute GVHD and death after unrelated HCT [139], and may be a result of increased IL-10 production associated with the -592A/C and A/A genotypes. These data suggest that the antigen-presenting cells of recipients homozygous for AA are high IL-10 producers capable of inducing tolerance in donor T cells. In unrelated HCT, GVHD and mortality risk have been reported with -1082 and -1064 [134], confirming the role of IL-10 originally observed in sibling transplantation [140].

Phase II: activation of donor T cells

In this phase, donor T-cell recognition of host MHCs and minor antigens leads to T-cell proliferation and differentiation. Several pathways contribute to the activation of T cells in this phase. Th1 responses amplify the effects of the cytokine storm and involve INF-γ, IL-2, IL-12, and IL-18. Th2 responses are mediated by IL-10, IL-4, and IL-18. Data in unrelated donor HCT are currently available on the impact of genetic variation of INF-γ, IL-2, and IL-18 on GVHD.

IL-2 is a proinflammatory cytokine on 4q26. The promoter -330T/G SNP is associated with early and sustained production. Recipients encoding the IL-2 -330G allele had an increased risk of acute GVHD, and the presence of at least one G allele was associated with a twofold increase in risk of acute GVHD [138]. IL-18 (11q22) is produced by macrophages, T cells, dendritic cells, and keratinocytes and regulates both Th1 and Th2 responses and induces the secretion of growth factors in murine models [129]. As a Th1-inducing cytokine, it reduces the severity of acute GVHD when given after high-dose conditioning. When administered to the donor, IL-18 reduces the severity of acute GVHD as a Th2-inducing cytokine. IL-18 can preserve GVT effects after HCT [141]. The promoter -137G/ -607C/-656G haplotype was associated with lower TRM at day 100 and 1 year post-transplant, and with improved survival [142]. Patients with acute GVHD after unrelated HCT have high levels of IL-18 [143], and serum levels are reduced after successful GVHD therapy.

Phase III: inflammatory effectors

In this phase, cellular and inflammatory effectors produce the end-organ target damage that comprises the clinical GVHD phenotype. The two major cytokines that participate in this phase are TNF and IL-1. Injury of the gastrointestinal tract and skin caused by direct toxicity from the conditioning regimen and from GVHD causes leakage of bacterial toxins and byproducts. Mononuclear phagocytes are stimulated and secrete TNF and IL-1.

Novel genes and pathways with clinical significance in unrelated donor HCT

In addition to the genes and pathways involved in the three phases of acute GVHD, there have been new substantial data on CTLA4 [70,140], HSPE [140,144], HSP70hom [145], IL6 [140], IL17 [146], IL23R [147–149], MTHFR [150,151], NOD2 [152,153], Tim-3 [156], elafin [157] and REG3α [158] in unrelated donor HCT. These polymorphisms are summarized in Table 45.3.

Conclusion

The success of unrelated HCT as a curative therapy has come through innovative research in transplantation genetics and biology, the development of safer conditioning regimens, more effective GVHD preventive and therapeutic strategies, advances in the monitoring and detection of early disease recurrence, and improved supportive care of patients. The ultimate goal of individualizing treatment strategies will require a more complete understanding of the correlation between genotype and phenotype, and the impact of different transplant procedures on transplant-related toxicities and GVT effects. In the future, the optimal transplant approach for a given patient may include combined modalities of immunogenetic measures for the prevention of GVH and promotion of GVT effects, together with the use of post-transplant immunotherapy to lower risk of disease recurrence.

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CHAPTER 46

Hematopoietic Cell Transplantation for Aplastic Anemia

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Epidemiology

Aplastic anemia (AA) is a rare disease with an incidence of about 2–3 cases per million per year in the United States and Western Europe and about 4–7.4 cases per million per year in East Asia [1–3]. The gender ratio is close to 1:1, and there are two peaks of incidence in young adults (age 15–24 years) and above age 65 years. When treated with supportive care, including blood transfusions and antibiotics, only about 28% of patients are alive 2 years after diagnosis [4]. More than 50% of patients with severe aplastic anemia (SAA) may die within 6 months of diagnosis if they do not receive definitive therapy [5].

Effective therapies for AA consist of either immunosuppressive therapy (IST) with agents such as antithymocyte globulin (ATG) or allogeneic hematopoietic cell transplantation (HCT).

Clinical description

The disease is characterized by pancytopenia and marrow parenchyma that is lacking hematopoietic elements. Typically, the marrow cavities are filled with fat, and the aspirates show mainly lymphocytes, plasma cells, and fibroblasts. The differential diagnosis of a marrow aspirate with this appearance includes myelodysplastic syndrome (MDS), clonal T-cell disorders and AA associated with paroxysmal nocturnal hemoglobinuria (PNH). One of the most difficult entities to distinguish from AA is hypocellular myelodysplasia (see Chapter 54), but the difference is important as the two disease entities are treated differently. The conditioning regimen for HCT for MDS often contains busulfan (BU) or total body irradiation (TBI) as for acute leukemias, whereas conditioning regimens without radiation are preferred for patients with AA (see later discussion). Cytogenetic analysis of marrow is sometimes useful in distinguishing between hypocellular MDS and AA. Marrow cytogenetics are normal in AA, in contrast to MDS, and, in contrast to hypocellular myelodysplasia, developing blood cells are usually normal in appearance. Occasionally, there may be dysplastic erythroid cells in AA [6]. However, the presence of dyshematopoiesis in non-erythroid cell lines is more consistent with MDS [7].

SAA is defined as marrow cellularity less than 25% with at least two of the following:

1 absolute neutrophil count (ANC) less than 0.5×10^9/L;
2 platelet count less than 20×10^9/L; and
3 absolute reticulocyte count less than 40×10^9/L [8].

Very severe aplastic anemia (VSAA) is defined by a neutrophil count less than 0.2×10^9/L and at least one other peripheral blood criterion of SAA and marrow features consistent with SAA [9].

Etiology

Aplastic anemia may be the result of a variety of causes, including ionizing radiation, benzene, and chemotherapeutic agents [7,10,11]. Drugs causing idiosyncratic marrow injury include chloramphenicol, phenylbutazone, sulfonamides, gold, and anticonvulsants such as felbamate (reviewed in [6,7]). Rarely, AA is associated with viral diseases such as non-A, non-B, non-C hepatitis (in 5% of AA) [1,12], parvovirus (pure red cell aplasia), or Epstein–Barr virus (EBV), and autoimmune disorders such as eosinophilic fasciitis [6,7]. Some cases of AA result from congenital or hereditary disorders, including Diamond–Blackfan anemia and Fanconi anemia. A diagnosis of dyskeratosis congenita may be apparent when typical clinical features of nail dystrophy, reticulate skin pigmentation, and leukoplakia are present. Mutations in telomerase complex component (TERC and TERT) can be found in about 5% of adult patients with apparent acquired AA who lack the above clinical abnormalities and who typically show a lack or poor response to IST ("cryptic dyskeratosis congenita") [7,13,14]. In most patients, the disease etiology is unknown (idiopathic or primary AA).

Molecular and clinical biology

Possible pathophysiologic mechanisms of AA include quantitative and/or qualitative deficiencies of hematopoietic stem cells (HSCs), a defective marrow microenvironment, impairment of cellular interactions needed to sustain hematopoiesis, and immunologic suppression of marrow function. The result is a reduction in morphologically recognizable precursor cells in the marrow aspirate [15,16].

The variable response of SAA to therapy and the diverse evolution of the disease with time are perhaps a reflection of the multiple pathophysiologic mechanisms. The observation that the infusion of syngeneic marrow without prior immunosuppressive conditioning results in cure of the disease in approximately 50% of patients

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suggests that a defect in hematopoietic stem cells is a likely cause in these patients [17–19]. Although most patients with AA have normally functioning stromal cells [17,18], there are a few cases where defects in the marrow microenvironment have been thought to have a role in the pathogenesis of their disease [20]. Recent evidence suggests that many cases of AA may be due to immune-mediated destruction of marrow. The immune attack leads to bone marrow failure. Furthermore, defective immune homeostasis between regulatory T cells and effector T cells plays an important role in AA. Regulatory T cells in AA appear to be intrinsically impaired, with decreased frequency, reduced cell migration capacity, and defective immunosuppression of effector cells [21]. T helper type 17 cells are increased particularly in patients with non-severe AA [22]. The responsiveness of AA to IST is evidence for the immune pathophysiology [15].

Hematopoietic cell transplantation

This chapter summarizes the results of marrow HCT for SAA over the past 40 years. Early reports of marrow HCT for SAA identified three major transplant related problems:

1 graft rejection;
2 acute graft-versus-host disease (GVHD); and
3 chronic GVHD [23–25].

The incidence of graft rejection has decreased in part because of the use of more effective immunosuppressive conditioning regimens and in part because of changes in transfusion practices (see below). The incidence of acute GVHD has also decreased owing to improved GVHD prevention protocols. The incidence and mortality of chronic GVHD may also have declined slightly. These changes, in turn, have resulted in significant improvement in survival. With longer follow-up, late sequelae, including impaired growth and development among children [26,27] and secondary malignancies [28], have become better understood.

Syngeneic grafts

Patients with syngeneic donors were among the first to receive marrow HCTs. Six of 12 patients in Seattle showed complete and sustained marrow recovery after HCT without preceding immunosuppression. In the other six patients, second marrow infusions were performed after conditioning with 200 mg/kg cyclophosphamide (CY). Ten of 12 patients were alive with an overall survival of 83% and follow-up ranging from 1.5 to 30 years [29]. The Center for International Blood and Marrow Transplant Research (CIBMTR) reviewed data on 40 syngeneic transplants worldwide. Twenty-three patients did not receive an initial conditioning regimen, and eight of the 23 had full hematologic recovery, whereas 15 required second transplants that were preceded by conditioning regimens. Twelve of 17 patients whose initial HCT was preceded by conditioning had full hematologic recovery. Four of these 17 patients died within 20 days of transplantation from pulmonary complications: fungal pneumonia, acute respiratory distress syndrome, and diffuse alveolar hemorrhage. One patient required a second transplant with conditioning for a full hematologic recovery. Although initial sustained engraftment was higher in patients who had transplants with conditioning, their 10-year survival was lower (70%) than that of patients who did not receive conditioning initially (87%) [30].

These observations suggest that syngeneic HCT can be attempted without conditioning and, if the graft fails, a second marrow infusion with a conditioning regimen is usually successful without compromising overall survival. The results of syngeneic HCTs are consistent with the concept that some cases of AA are caused by a defect in HSCs that can be corrected by simple intravenous infusion of syngeneic marrow. However, there are other cases in which the infused twin marrow HCT either fails to engraft or is rejected following a period of transient recovery of hematopoietic function. The etiology of the aplasia in these cases may be T cell mediated or be the result of unknown factors whereby the dysfunction of marrow can be overcome by conditioning with CY and a second marrow infusion [15,31].

HLA-identical related bone marrow transplantation (BMT)

The largest number of transplants for SAA performed to date have been from human leukocyte antigen (HLA)-matched sibling donors. In preparation for allogeneic HLA-identical HCT, recipients have been treated by intensive immunosuppression to prevent rejection of the grafts. Immunosuppressive agents have included CY given either alone at a dosage of 50 mg/kg/day intravenously on each of four successive days or combined with horse (or rabbit) ATG. In other patients who received earlier, less effective regimens, CY at a dosage of 60 mg/kg/day for 2 days was combined with TBI or limited field radiation (LFR), such as total lymphoid irradiation (TLI) or thoracoabdominal irradiation (TAI). Recently, some trials have decreased the CY dose and added fludarabine (FLU) with the goal of decreasing side effects related to the conditioning regimen [32]. However, these attempts at reducing the CY dose have not achieved definitively superior outcomes [33,34].

Table 46.1 summarizes recent reports on transplant outcome. The median age of patients reported in studies was approximately 19 years or less with a range of 0–69 years. The conditioning regimens and agents used for GVHD prophylaxis varied widely, even within studies. Most transplanted patients were previously transfused, a variable that has been shown to affect transplant outcome adversely by increasing the risk of graft rejection. The survival rates have improved with time in part because of decreased incidences of graft rejection, the result of changes in transfusion policies, and improved conditioning programs [29], and in part because of a decreased incidence of acute GVHD owing to the introduction of the combination of cyclosporine (CSP) and methotrexate (MTX) as GVHD prophylaxis [35,36].

Graft rejection

Graft rejection occurs because of genetic disparity between donor and recipient. Graft rejection may also be due to persistent host T cells that mediated the bone marrow failure in the first place. Two forms of graft rejection may be seen. Primary rejection is defined by the absence of any sign of hematologic function of the graft, and late rejection is defined as graft loss after initial graft function. Patients with primary or late graft rejection can often be rescued with a second HCT [37].

Graft rejection was formerly a frequent problem among patients who received CY alone for conditioning. Graft rejection at the Fred Hutchinson Cancer Research Center (FHCRC), Seattle, was seen in over 35% of patients transplanted in the early 1970s [38]. A report from the European Group for Blood and Marrow Transplantation (EBMT) showed a rejection incidence of 32% among patients transplanted before 1980 [39]. However, as described in the following section, significant progress has been made with respect to decreasing the risk of graft rejection. With most modern conditioning regimens, graft rejection has become the exception, but it is important to review the interventions necessary to prevent graft rejection.

Table 46.1 Selected recent reports of HLA-identical HCT for SAA

Transplant team[a]	Year of report	Year of trans plant	No. of patients[b]	Age range in years (median)	Conditioning program[c]	Hematopoietic source[d]	Prevention of GVHD[d]	Graft rejection/ failure (%)	GVHD (%)		Survival (%)	Range of follow-up in years (median)
									Acute	Chronic		
Seattle, Kahl et al. [59]	2005	1988–2004	81	2–63 (25)	CY + ATG	BM	CSP + MTX	4	24	26	88	0.5–16.4 (9.2)
CIBMTR, Champlin et al. [62]	2007	1994–2001	130	1–51 (24)	CY ($n = 60$)	BM	CSP + MTX	11	18	21	74	0.4–10.2 (6.3)
					CY + ATG ($n = 70$)			11	11	32	80	
GITMO, Locatelli et al. [60]	2000	1991–1998	71	4–46 (19)	CY	BM	CSP *vs.*		38	44	78	0.6–7.8 (4.0)
							CSP + MTX		30	30	94	
EBMT, Locasciulli et al. [107]	2007	1991–2002	1275	1–67 (18.7)	CY + ATG, CY ± TBI ± varied	–	CSP + MTX CSP or –	–	–	–	(1991–1996) 74 (1997–2002) 80	2.0–13.2 (3.6)
EBMT, Maury et al. [34]	2009	1998–2007	30	31–66 (46)	Flu + CY + ATG	BM ($n = 20$) PBPC ($n = 10$)	CSP + MTX or other	3	10	13	77	1.1–6.8 (4.1)
Sangiolo et al. [116]	2010	1998–2009	23	40–68 (49)	CY + ATG	BM	CSP + MTX	4	35	26	65	0.9–19.2 (9.1)
CIBMTR, Chu et al. [115]	2011	1997–2003	547	(18)	CY + ATG (57%), and various other regimens	BM (547)	CSP + MTX (83%), or other	9 Δ	13	16	80	0.2–10.4 (5.2)
			78	(25)		G-BM (78)		10 Δ	14	10	72	0.2–10.2 (3.7)
			134	(24)		PBPC (134)		7 Δ	28	43	76	0.2–9.9 (4.2)
Marsh et al. [32]	2011	1999–2009	21 MSD 29 UD	8–62 (35)	FLU + CY + Alem	BM ($n = 24$) Other ($n = 26$)	CSP	12	14	4	88	0.2–9.8 (1.5)
Atta et al. [70]	2012	1995–2011	20	4–48 (21)	CY + ATG horse	BM	CSP + MTX	5	35	34	65	0.02–14 (4.5)
			20		CY + ATG rabbit			5	0	0	63	0.03–4.8 (0.7)
Kim et al. [33]	2012	2003–2010	50 MSD	15–60 (35)	CY + ATG vs. FLU + CY + ATG	BM ($n = 59$)	CSP + MTX	13	15	17	78	0.1–7.1 (1.5)
			33 AD			PBPC ($n = 23$)		17	23	16	85	
Burroughs et al. [111]	2012	1971–1984	98	1.8–19 (12.8)	CY	BM	MTX	22	21	21	66	11–37.2 (31.1)
		1981–1988	19		CY		CSP + MTX	32	11	21	95	15.2–27.1 (23)
		1989–2010	31		CY + ATG		CSP + MTX	7	39	10	100	0.3–21.5 (6.1)
EBMTR, Bacigalupo et al. [53]	2012	1999–2009	1886	1–68 (18)	CY + ATG (41%), and various	BM ($n = 1163$)	CSP + MTX (41%), or other	9	11	11	age ≤20: 90 age >20:74	(2.1)
				1–69 (24)		PBPC ($n = 723$)		10	17	22	age ≤20: 76 age >20:64	(2.0)

In all series, the majority of patients were previously transfused. – = Data not reported; Δ = failure to achieve platelet engraftment ($>20 \times 10^9$/L) by day + 60 after transplant.

[a] EBMT, European Group for Blood and Marrow Transplantation; GITMO, Gruppo Italiano Trapianti di Midollo Osseo; CIBMTR, Center for International Blood and Marrow Transplant Research.

[b] For studies that included both HLA-identical sibling and alternative donors: MSD = HLA-identical sibling donor, UD = unrelated donor, AD = alternative donors.

[c] CY = cyclophosphamide (200 mg/kg, unless otherwise indicated); ATG = antithymocyte globulin; TBI = total body irradiation; FLU = fludarabine; Alem = alemtuzumab.
Flu + CY + ATG (Maury et al. [34]) = FLU 30 mg/m^2/day × 4 days, CY 300 mg/m^2/day × 4 days, rabbit ATG (Thymoglobulin) 3.75mg/kg/day × 4 days.
Flu + CY + ATG (Kim et al. [33]) = FLU 30 mg/m^2/day × 5 days, CY 50 mg/kg/day × 2 days, rabbit ATG (Thymoglobulin) 3 mg/kg/day × 3 days.
Flu + CY + Alem (Marsh et al. [32]) = FLU 30 mg/m^2/day × 4 days, CY 300 mg/m^2/day × 4 days, alemtuzumab total dose 40–100 mg (median, 60 mg) infused over 5 days.

[d] MTX = methotrexate; CSP = cyclosporine; PBPC = granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood progenitor cells; BM = bone marrow.

A major factor associated with graft rejection has been sensitization to histocompatibility antigens through previous blood product transfusions, as shown by extensive animal studies and confirmed in clinical trials [40–44]. Early studies reported that previously transfused patients had a significantly lower overall survival compared with untransfused patients [43], mainly because of complications of graft rejection.

Data from animal studies showed that dendritic cells in the transfusion product are important in sensitizing recipients to disparate minor histocompatibility antigens of the transfusion (and marrow) donor [45]. Treating blood products with 20 Gy of gamma irradiation *in vitro* before transfusion almost eliminated sensitization to minor histocompatibility antigens and prevented rejection of dog leukocyte antigen (DLA) identical marrow grafts [46,47]. Other methods to reduce graft rejection in the canine model included the use of platelet and red blood cell transfusions that were leukocyte depleted [42]. These data strongly suggest that all human patients with AA who are potential candidates for HCT should be given blood products that are both irradiated and leukocyte-poor [48].

In historical studies, patients with AA who proceeded to BMT without prior blood transfusion had a reduced incidence of graft rejection. However, fewer than 15% of patients were untransfused prior to transplant [43]. Other methods were therefore developed to overcome marrow graft rejection. In the 1970s, an inverse relationship was observed between the number of donor marrow cells infused and the risk of graft rejection in patients conditioned with CY [38]. A low incidence of graft rejection was seen with a high ($>3 \times 10^8$ cells/kg) marrow cell dose and vice versa. On the basis of that observation, an attempt was made to increase the number of hematopoietic cells transplanted by infusing unirradiated donor buffy coat cells in addition to the marrow [49]. Although effective in reducing the incidence of graft rejection and improving survival compared with patients given marrow alone, the infusion of donor buffy coat cells was subsequently abandoned because of the increased the risk of de novo chronic GVHD [50,51].

Historically, another approach to overcome graft rejection that was subsequently abandoned included intensifying the conditioning regimen, for example, using CY combined with TBI or LFR. Although effective in reducing the incidence of rejection, radiation-based regimens were associated with worse long-term survival due to a higher rate of transplant-related mortality (TRM) and an increased risk of secondary cancer, in addition to problems with growth, development, and fertility (see later discussion and Chapters 102, 103 and 104).

In the past decade, there was an interest in the use of granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood hematopoietic cells (G-PBHCs) as the source of HSCs for allogeneic transplantation. G-PBHCs contain an increased number of $CD34^+$ hematopoietic progenitor cells and an approximately 10-fold increased numbers of T cells compared with marrow [52]. With the infusion of a larger number of donor cells, G-PBHCs could potentially decrease the risk of graft rejection in patients with SAA. EBMT registry data indicated that approximately 38.8% of HLA-identical sibling transplants for SAA in the period 2008–2009 received G-PBHCs as the HSC source [53,54]. The use of G-PBHCs resulted in a significantly increased risk of chronic GVHD, which also significantly decreased overall survival. For patients with AA there is no compensatory benefit of a graft-versus-leukemia effect provided by PBHCs, therefore G-PBHCs should be avoided.

Animal studies showed synergistic immunosuppressive effects between ATG and alkylating agents such as procarbazine and CY as assessed by the criteria of skin graft prolongation and overcoming marrow graft rejection [55,56]. On the basis of these experimental animal data, the regimen of CY (200 mg/kg) and horse ATG was developed and was initially used to rescue patients in whom their first graft had been rejected. Fifteen of 19 previously transplanted patients who were reconditioned with CY and ATG had successful second marrow grafts, and 50% became long-term survivors [57]. More recently, survival of patients after second HCT increased to 83% at FHCRC, a result not significantly different from survival after the first transplant [37].

Because of the success of CY (200 mg/kg) and ATG in second transplants, the combination was chosen as a conditioning regimen for first transplants from HLA-identical family members beginning in 1988. This led to the initial report of significantly improved outcome of HCT compared with the historical regimen of CY alone with buffy coat infusions [57].

Changes in transfusion policies (consistent use of irradiated, leukodepleted blood products), and improved conditioning regimens (combined CY + ATG) before transplant, have led to significant decreases in graft rejection [39]. In addition, post-grafting immunosuppression aimed at controlling GVHD may also have a role in controlling host-versus-graft (HVG) reactions. A retrospective analysis by the EBMT suggested that the use of CSP as GVHD prophylaxis decreased the rejection rate compared with MTX alone [39], but a randomized prospective study in Seattle failed to show differences in rejection when long-term MTX was compared with a short course of MTX combined with CSP [29,36]. Studies in animals showed MTX and CSP to be superior to CSP alone, not only in controlling GVHD but also in suppressing HVG reactions, thereby enhancing engraftment [58]. Hence post-grafting immunosuppression used for GVHD prevention also has to be considered when evaluating new conditioning regimens for their efficacy in decreasing the risk of graft rejection.

Updated results from the Seattle study of CY and ATG for patients with SAA confirmed that graft rejection had become a minor problem (Figure 46.1a). Of the 81 consecutive patients enrolled, 76 had received multiple transfusions and 44 had failed prior immunosuppressive therapy. The ages of the patients ranged from 2 to 63 years. After transplantation, 81 patients received MTX and CSP for GVHD prophylaxis. There was a 3.7% incidence of graft rejection between 1 and 7 months after transplantation (Figure 46.1a). Of the three patients with graft rejection, two were alive following successful second HSC transplantation. With a median follow-up of 9.2 years (range 0.5–16.4 years), the overall survival rate was 88% (Figure 46.1e) [59].

Some transplant centers have continued to use CY without ATG as the conditioning regimen for younger patients receiving marrow HCT from HLA-identical siblings. Results from a prospective randomized multicenter trial sponsored by the Gruppo Italiano Trapianti di Midollo Osseo (GITMO) and the EBMT that compared CSP alone with MTX and CSP for GVHD prophylaxis in 71 patients with SAA showed that the overall incidence of graft rejection was 8% [60]. Several factors may explain why the GITMO/EBMT results with CY conditioning alone were superior to the historical data from the Seattle group in the 1980s. Since the initial Seattle report, there has been increased awareness in the medical community of the benefit of leukodepleted and irradiated blood products for patients with SAA. Seventy of 71 patients in the GITMO/EBMT study had been transfused with a median of 6 units of blood products prior to transplantation. Although not specifically addressed in the GITMO/EBMT study, the widespread use of

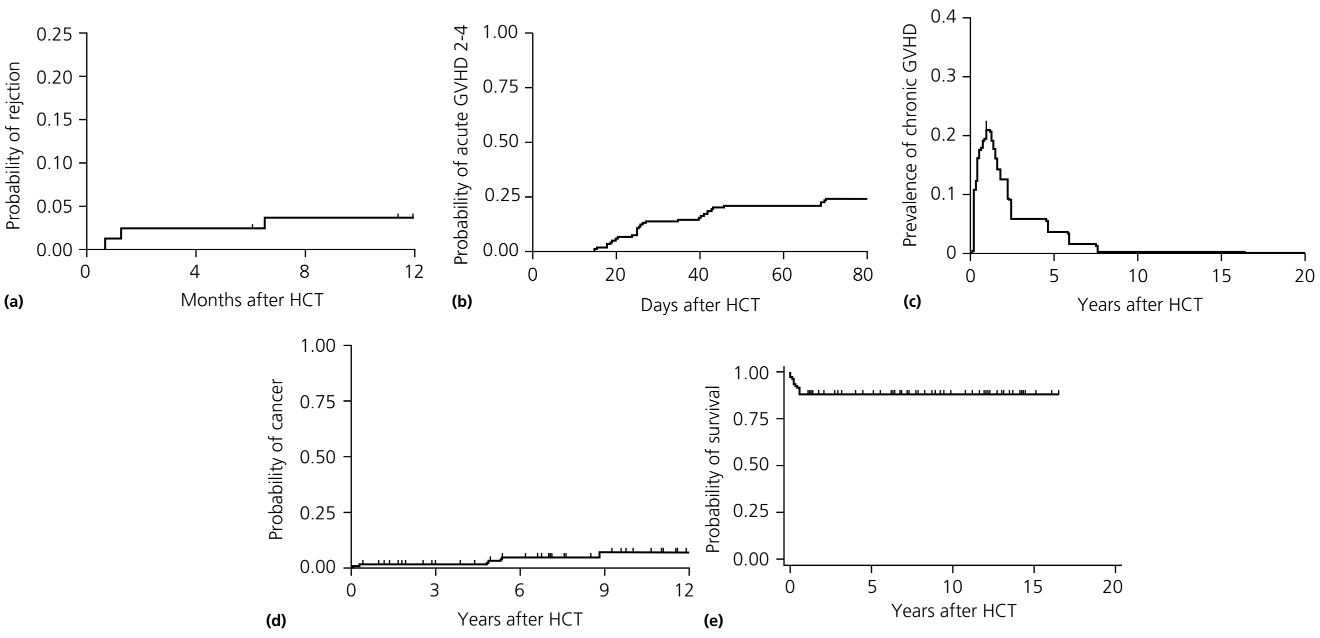


Figure 46.1 Cumulative incidences of (a) graft rejection and (b) developing acute grade II–IV graft-versus-host disease (GVHD) in 81 consecutive patients with aplastic anemia given human leukocyte antigen (HLA)-identical marrow grafts following cyclophosphamide (CY) and antithymocyte globulin (ATG) conditioning and GVHD prophylaxis with methotrexate (MTX) and cyclosporine (CSP). (c) Prevalence of chronic GVHD. (d) Probability of malignancy after HCT. (e) Overall survival. Source: Kahl et al. 2005 [59]. Reproduced with permission of John Wiley & Sons, Ltd.

irradiated and leukodepleted blood products for transfusion support prior to transplantation for patients with AA may have contributed to the lower incidence of graft rejection seen since the 1980s. The GITMO/EBMT study included predominantly younger patients, most of whom were not treated with a trial of immunosuppression prior to proceeding to allogeneic HSC transplant. In contrast, the updated multicenter trial of CY and ATG included older, heavily transfused patients whose prior treatment with immunosuppressive therapy had failed [59,61].

In a multicenter prospective trial conducted over 7 years, 134 patients were randomly assigned to receive CY alone or in combination with horse ATG. The majority of patients had not received prior IST. Patients received T-cell-replete (unmodified) bone marrow from an HLA-identical sibling and GVHD prophylaxis consisted of CSP and MTX. The 5-year probabilities of survival were 74 and 80% in the CY and the CY–ATG groups, respectively. Graft failure rates were similar at 18 and 16%, respectively. Fifteen of 22 patients with graft failure underwent a second HCT from their initial donor, but three patients in the CY alone group required more than two transplantations. At the last follow-up, 12 of the 22 patients with graft failure were alive. Unfortunately, this randomized study was underpowered to detect a statistically significant difference in survival, so the data are inconclusive as to whether the combination of CY plus ATG leads to superior outcome after BMT [62].

Perhaps for younger patients (under 20 years of age) who have not received prior IST for AA and who received only a very limited number of blood product transfusions, all which were irradiated and leukodepleted, the risk of graft rejection following HLA-identical sibling BMT is low and the addition of ATG to the CY conditioning regimen may not be necessary. However, some data indicate that transplantation of a high cell dose of donor marrow (corrected for donor peripheral blood cell content) results in significantly increased risk of chronic GVHD [59]. Therefore, to avoid possible graft rejection when the marrow cell dose is restricted, ATG should be administered with CY.

The addition of FLU to a reduced dose of CY with ATG has been evaluated as an alternative approach to overcoming graft rejection. However, studies of FLU–CY–ATG were confounded by the use of G-PBHC instead of bone marrow to help overcome the risk of graft rejection. The incidence of graft rejection was relatively low, but the incidence of severe acute GVHD was high, and the overall outcome was not superior to the accepted standard CY (200 mg/kg)–ATG regimen [34,63–65]. A recently completed randomized study compared FLU (120 mg/m^2), CY (100 mg/kg), and rabbit ATG (9 mg/kg) with CY (200 mg/kg) and rabbit ATG [33]. There was no significant difference in combined primary and secondary graft rejection in the two arms (13.4 vs. 16.8%, respectively), or the incidence of acute or chronic GVHD and overall survival. However, there was a lower incidence of pulmonary toxicity among recipients of HLA-matched sibling grafts in the FLU–CY–ATG arm. This study was confounded by relatively small number of patients, inclusion of patients with the diagnosis of hypoplastic myelodysplastic syndrome, use of alternative donors, and use of G-PBHC. Furthermore, pulmonary toxicity was not observed in the Seattle series of patients given the CY(200 mg/kg)–ATG regimen [59]. Nonetheless, the data suggest that FLU 120 mg/m^2 may be potential substitute for up to 100mg/kg of the CY dose. Since FLU has some non-overlapping toxicity with CY, the FLU–CY combination may be beneficial particularly in older patients who may have an increased risk of cardiac or pulmonary toxicity from high-dose CY. However, to date, there is no definitive evidence of a survival benefit in patients receiving the combined reduced-intensity FLU–CY–ATG regimen compared with the standard-dose CY–ATG. Although reduction of the CY dose with addition of FLU may be a reasonable option for HCT in patients older than 40 years of age, additional studies are needed to confirm this recommendation.

Other centers have evaluated the combination of FLU (120 mg/m^2) and CY (1200 mg/m^2) with alemtuzumab (40–100 mg) as an alternative conditioning regimen [32]. The report of 50 patients with AA treated with the FLU–CY–alemtuzumab regimen was limited owing to several factors such as: inclusion of patients with non-severe aplastic anemia, wide variation in the dose of alemtuzumab, less than half of the patients received unmodified bone marrow as the hematopoietic cell source, and the inclusion of alternative donors. The incidence of graft rejection was relatively high at 12%, and there were infectious complications after transplant, including cytomegalovirus (CMV), adenovirus, and EBV [32]. Altemtuzumab-containing regimens may be considered as an alternative to ATG for patients who are not able to tolerate ATG due to prior exposure to ATG treatment or who have anti-ATG antibodies detected [66]. The combined FLU plus reduced-dose CY (with either ATG or alemtuzumab) appears to be an alternative conditioning regimen suitable for older patients, but owing to the apparent increased risk of graft rejection the reduced-dose alternative regimens are not recommended for patients who can tolerate the standard CY (200 mg/m^2)–ATG Seattle regimen.

In summary, results from clinical trials to date indicate that combined CY and ATG conditioning followed by MTX and CSP as post-grafting immunosuppression is the most effective and reliable regimen to prevent graft rejection and GVHD following HLA-identical BMT for SAA. Similar survival might be obtained without ATG in younger, relatively untransfused patients. The use of the least toxic transplant regimen associated with the lowest incidence of graft rejection avoids the need for having to deal with this potentially life-threatening complication after transplant. For first transplants, the use of G-CSF mobilized PBHC is not appropriate given the increased risk of chronic GVHD. Treatment guidelines for SAA include uniform adherence to the following:

1. Use irradiated and leukodepleted blood products before transplant.
2. For patients with an HLA-identical sibling donor, proceed to BMT as first-line therapy.
3. Use the well-established and well-tolerated conditioning regimen of CY (200 mg/kg) and ATG.
4. Infusion of 2.5×10^8 nucleated donor marrow cells (corrected for donor peripheral blood cell count)/kg.
5. Do not use G-CSF-mobilized peripheral blood progenitor cells.
6. Use combined MTX and CSP post-grafting immunosuppression.
7. Despite the potential increased risk of graft rejection, reduced-intensity conditioning regimens such as FLU–CY with either rabbit ATG or alemtuzumab may be considered for older patients (age >40 years) unable to tolerate CY 200 mg/kg.

Mixed donor–host hematopoietic chimerism

Transient mixed chimerism is common in patients after marrow allografts for AA. In one study, almost 60% of patients had mixed chimerism in peripheral blood or bone marrow after HCT, and two-thirds of these eventually converted to complete donor-type hematopoiesis while the remainder rejected their grafts [67]. In a larger study of 116 patients with AA transplanted from gender-mismatched HLA-identical siblings, 54% were mixed chimeras detected in either blood or marrow. Although patients with mixed chimerism appeared to have a higher incidence of graft rejection (14%) than those who were complete chimeras (9%), this difference was not statistically significant [68]. A study of 45 patients with AA who received HLA-identical sibling BMT and serial monitoring with short tandem repeat (STR) polymerase chain reaction (PCR) chimerism analysis reported that 72% developed complete donor chimerism, 11% had stable mixed chimerism, and 17% had an increase in host chimerism after transplantation. Patients with increased host chimerism after transplantation had a 50% incidence of graft failure and an overall survival of 38%. Patients with complete donor or stable mixed chimerism had superior survival. None of the patients with stable mixed chimerism developed chronic GVHD [69]. The inclusion of more intensive in vivo T-cell-depleting antibody therapy in the conditioning regimen results in a higher incidence of mixed chimerism after transplant. A recent study of FLU–CY–alemtuzumab conditioning reported that all evaluable patients had long-term, sustained mixed T-cell chimerism after HCT [32]. Among patients receiving rabbit ATG (8 mg/kg) plus CY 200 mg/kg conditioning, 80% had stable mixed hematopoietic chimerism [70]. These results suggest that monitoring chimerism after allogeneic HCT is useful for predicting the subsequent transplant outcome. T-cell chimerism is now routinely determined after HCT. Chimerism analysis is an indispensable tool for monitoring the kinetics of engraftment and the ultimate fate of the graft; it also appears to be useful in predicting impending graft rejection.

Acute graft-versus-host disease (see Chapter 83)

Once engraftment has been accomplished, acute GVHD may develop, usually within the first 6 weeks after HCT. Grade II–IV acute GVHD has an adverse effect on survival in patients transplanted for SAA. Early Seattle data showed an actuarial probability of survival at 11 years of 45% for SAA patients with preceding grade II–IV acute GVHD compared with 80% survival among patients with grade 0 and I acute GVHD [71]. Similarly, subsequent CIBMTR data showed a 31% 5-year actuarial probability of survival for SAA patients with grade II–IV acute GVHD compared with 80% among patients with no or mild acute GVHD [72]. During the 1970s, MTX was the most commonly used agent given after BMT to prevent acute GVHD, and 35% of MTX-treated SAA patients developed grade II–IV acute GVHD [71].

Because of the high mortality associated with acute GVHD, a number of studies were carried out in the 1980s to investigate whether the incidence of this complication could be reduced and survival improved. CSP was initially studied as a single agent to prevent acute GVHD. Retrospective analyses showed that CSP favorably influenced the survival of more recently transplanted SAA patients compared with historical control patients given MTX alone [73–76].

A number of transplant centers combined prednisone (PSE) and CSP in hope of improving control of acute GVHD. A randomized prospective study of patients with high-risk lymphohematopoietic malignancies showed that the combination of CSP and PSE was only marginally better than CSP alone in preventing acute GVHD, but this came at the expense of a significant increase in chronic GVHD [77,78]. Encouraging data in experimental animals led, in 1981, to the introduction in the clinic of a combination of MTX (15 mg/m^2 given at 24 hours after completion of bone marrow infusion and 10 mg/m^2 given on days 3, 6, and 11 after BMT) combined with CSP. One randomized trial from Seattle showed a significant reduction in incidence and severity of acute GVHD in 22 SAA patients given the combination versus 24 patients receiving MTX alone [36], and overall survival was higher in patients who received the combination of drugs [36,79]. Similar findings were obtained in patients with leukemia where the combination of MTX and CSP was found to be superior to CSP alone in preventing acute GVHD [80]. In a later non-randomized retrospective analysis of CIBMTR data from 595 patients with SAA who had received BMTs from matched siblings, patients who received either CSP alone or CSP with MTX compared with MTX alone had a statistically significant improved survival rate of 69% versus 56%, respectively [72]. The reduction in acute GVHD resulted in improved survival, a finding that was especially impressive among pediatric patients with SAA [73]. One retrospective analysis suggested that optimal doses of both MTX and CSP were important in controlling acute GVHD [81]. Because of the success of the combination of MTX and CSP, a randomized study was carried out to compare MTX + CSP with CSP + MTX + PSE for patients with hematologic malignancies and SAA. Among patients with HLA-identical donors, the addition of PSE increased the risk of acute and chronic GVHD unless the PSE was administered after the completion of a short course of MTX [82]. A subsequent randomized trial of MTX and CSP versus MTX, CSP and "late PSE" was completed in patients with leukemia and suggested improved survival in the MTX and CSP arm ($p = 0.1$) [83].

The prospective randomized study by GITMO/EBMT confirmed the initial Seattle report and showed that the combination of MTX and CSP resulted in superior 5-year estimated survival compared with the use of CSP alone for SAA patients transplanted with an HLA-identical sibling marrow graft. The incidence of acute GVHD in the MTX and CSP group versus CSP alone was 30 and 38%, respectively [60].

Updated results of the study with the Seattle regimen consisting of CY and ATG, HLA-identical marrow followed by combined MTX and CSP immunosuppression in 81 consecutive patients showed that the cumulative incidence of grade II–IV acute GVHD was 24% (Figure 46.1b) [59]. The severity of GVHD observed was grade II (21.6%), grade III (0%), and grade IV (2.4%). Thus, despite the greater age of patients in the Seattle regimen study compared with the GITMO/EBMT study, the overall incidence of acute GVHD was lower in the Seattle study. Similarly, the randomized CY versus CY + ATG multicenter study with MTX + CSP GVHD prophylaxis reported an acute GVHD incidence of 18 and 11%, respectively [62]. These findings suggest that the incorporation of ATG in the conditioning regimen may be effective in preventing acute GVHD, particularly in older patient age groups. ATG may have an effect on depletion of the infused donor T cells.

Several smaller studies have evaluated potential alternatives to the standard CY–horse ATG regimen. As an alternative to horse ATG, the use of rabbit ATG (total dose 8 mg/kg) in the conditioning regimen with CY 200 mg/kg and infusion of HLA-identical sibling bone marrow and post-grafting CSP + MTX in 20 SAA patients was associated with a 0% incidence of acute GVHD. However, the rabbit ATG recipients experienced a high incidence of invasive fungal infection and delayed immune recovery and overall survival was not improved compared with recipients of horse ATG [70]. In addition, there is significant variability in the methods for assessing the incidence of acute GVHD among transplant centers, which can lead to significant under-reporting of the incidence of acute GVHD in retrospective single-center studies [84]. A reduced-intensity regimen of FLU (120 mg/m^2), CY (100 mg/kg), and rabbit ATG (9 mg/kg) included post-transplant CSP and abbreviated MTX. The incidence of acute GVHD was 23%, although 70% of the patients received bone marrow and 37% received alternative donor grafts [33]. In a limited study of 30 SAA patients treated with the reduced-intensity FLU (120 mg/m^2)–CY (1200 mg/m^2)–rabbit ATG (15 mg/kg) conditioning regimen, 20 patients were transplanted with bone marrow from HLA-identical siblings and most received post-grafting CSP + MTX: there was a 10% incidence of acute GVHD [34]. A study incorporating the FLU–CY–alemtuzumab regimen used CSP alone as post-grafting immunosuppression and reported a 16% cumulative incidence of acute GVHD [32]. In summary, the use of rabbit ATG or alemtuzumab given during the conditioning regimen has the potential to decrease the risk of acute GVHD after allogeneic HCT. However, rabbit ATG and alemtuzumab have been associated with a significant risk of increased opportunistic infections such as potentially fatal invasive fungal and viral infections, hence overall survival has not been significantly improved.

Chronic graft-versus-host disease (see Chapter 84)

Whereas significant progress has been made in reducing the incidences of graft rejection and acute GVHD, chronic GVHD continues to be a major complication of marrow grafting for AA. Unlike the graft-versus-leukemia effect associated with chronic GVHD that can improve the survival of patients with leukemia, there is no benefit of chronic GVHD in patients transplanted with AA. Historically, the incidence of chronic GVHD varied depending on the patient groups studied. Studies of a few small series of children transplanted from HLA-identical siblings reported incidences of 0–25% [85–87], whereas the incidence was as high as 40–60% among other series that included adult patients and those who received supplemental buffy coat infusions [88,89]. In most current series of matched sibling transplants that included adults, the incidence of chronic GVHD was 10–30% [54,59,60,62,65,90,91] (see Table 46.1).

Early studies identified a history of acute GVHD as the most important risk factor for chronic GVHD [50,92]. Older patients also experienced more chronic GVHD. De novo chronic GVHD occurs without preceding acute GVHD. In addition to donor buffy coat infusions, other risk factors, including increased patient age and preceding corticosteroid therapy, have been associated with de novo chronic GVHD development [50,93]. More recently, infusion of high corrected marrow cell dose (greater than 3.4×10^8 cells/kg) was associated with a significantly increased incidence of chronic extensive GVHD [hazard ratio (HR) 7.7] and lower overall survival [59].

G-CSF--mobilized PBHC increases the risk of chronic GVHD, which is more protracted and less responsive to treatment compared with bone marrow [94]. Because of the excellent overall survival of SAA patients after HLA-identical BMT given combined CY + ATG conditioning and CSP + MTX GVHD prophylaxis, it is very unlikely that future studies will demonstrate any significant benefit of utilizing T-cell-depleted or CD34-enriched PBHC over HLA-identical bone marrow as stem-cell source. Therefore, for patients with SAA, HLA-identical bone marrow with a target-corrected cell dose of 2.5×10^8 nucleated cells per kilogram body weight is the optimal source of hematopoietic stem cells.

Chronic GVHD is associated with significant morbidity and requires prolonged IST, although ultimately immunosuppression can be discontinued in most patients as chronic GVHD resolves [59,95]. In the past, up to one-third of affected patients died, often from infections. PSE, azathioprine, CY, procarbazine, CSP, thalidomide, mycophenolate mofetil (MMF), tacrolimus (FK-506), and extracorporeal photopheresis given either alone or in combination have all been used for treatment of chronic GVHD (see Chapter 84) [95–100].

Recent evidence suggests that persistent chronic GVHD is a risk factor for the development of solid tumors late after transplantation. In addition, in the Seattle survey of long-term survivors after allogeneic transplantation for AA, chronic GVHD was a risk factor for development of cataracts, lung disease, bone and joint disease, and depression [101]. In the Seattle experience, since the introduction of the CY and ATG conditioning regimen and the discontinuation of added buffy coat cell infusions, the prevalence of chronic GVHD has significantly decreased. Chronic GVHD was not only less frequent but also appeared to be more responsive to therapy in patients conditioned with CY and horse ATG and given marrow alone compared with historical control patients treated with CY and marrow plus buffy coat. In the updated Seattle transplant regimen report with HLA-identical sibling marrow grafts consisting of CY and horse ATG followed with MTX and CSP, the cumulative incidence of chronic GVHD observed was 26% (Figure 46.1c). In all surviving patients, chronic GVHD responded to therapy with complete responses. Of the patients with chronic GVHD, 83% survived long term. The peak incidence of chronic GVHD was at 1 year after transplantation. By 7 years, no patients required IST for chronic GVHD [59].

The incidences of chronic GVHD in the GITMO/EBMT trial with CY conditioning and post-grafting immunosuppression with either CSP alone versus MTX + CSP were 30% and 44%, respectively. The majority of patients with chronic GVHD had limited disease of the skin; the overall incidence of extensive chronic GVHD was 9% [60]. Most of the patients in the GITMO/EBMT study were children, who had a lower incidence of chronic GVHD than adults.

Prompt diagnosis of chronic GVHD combined with aggressive initiation of IST with the combination of CSP and PSE has resulted in an apparent decrease in the time needed for inducing remission of chronic GVHD, and the overall response to treatment has improved over time. However, this improvement has not been associated with a significant increase in survival for patients with SAA who develop chronic GVHD [97,102]. Hence mortality associated with chronic GVHD remains a significant problem for patients following allogeneic HCT.

These observations suggest that improvements in the prevention of chronic GVHD must be sought to improve patient survival further. Perhaps prompt diagnosis and treatment of chronic GVHD may help improve response to treatment and overall survival. However, in a randomized prospective study comparing CSP continued at "therapeutic levels" for 24 months with 6 months after HCT for patients at highest risk of developing chronic GVHD, for example, those with preceding acute GVHD, corticosteroid therapy, and older patients, there was no difference in overall survival between the two groups [98]. In the setting of HLA-identical sibling HCT, CY + ATG conditioning and GVHD prophylaxis with MTX + CSP, the most effective means of preventing chronic GVHD may be achieved by limiting the infused donor marrow to a target cell dose of 2.5×10^8 total nucleated cells/kg (corrected for donor peripheral blood cell count). Taken together, current evidence indicates that the Seattle regimen of CY and ATG conditioning followed by HLA-identical marrow transplantation and combined MTX and CSP post-grafting immunosuppression appears to provide one of the lowest risks of graft rejection and acute and chronic GVHD compared with other transplantation regimens.

Some recent studies suggest that inclusion of rabbit ATG or alemtuzumab in the conditioning regimen may decrease the incidence of chronic GVHD. In one report, the substitution of rabbit ATG (total dose 8 mg/kg) in place of horse ATG (90 mg/kg) as part of the conditioning regimen with CY 200 mg/kg prior to HLA-identical sibling BMT was associated with a decreased incidence of chronic GVHD, 0% versus 34%, respectively. However, there was increased risk of invasive fungal disease after transplantation, earlier CMV reactivation, and delayed lymphocyte recovery in rabbit ATG recipients. Despite the decreased incidence of chronic GVHD, there was no difference in overall survival after HCT between recipients of rabbit or horse ATG [70]. Following the reduced-intensity FLU–CY–alemtuzumab conditioning regimen and CSP post-grafting immunosuppression, the cumulative incidence of chronic GVHD was 4%, despite only 48% of patients receiving bone marrow as the source of hematopoietic stem cells [32]. In both studies, nearly all evaluable patients had sustained mixed T-cell chimerism after HCT. Profound in vivo T-cell depletion with rabbit ATG or alemtuzumab has the potential to decrease the incidence of chronic GVHD. Additional studies are needed to determine if these alternative regimens can consistently reduce the incidence of chronic GVHD and also result in improved overall survival.

Interstitial pneumonia

The incidence of interstitial pneumonia occurring after transplantation in patients with SAA has decreased over time. In 1989, Weiner et al. [103] reviewed CIBMTR data from 547 patients with AA receiving HLA-identical marrow grafts for the risk of interstitial pneumonia. They found an overall incidence of interstitial pneumonia of 17%. CMV pneumonia affected 37% of patients, 22% had pneumonia from other organisms, and in 41% of cases no organisms were identified. The case fatality rate was 64%. At that time, four factors predicted the development of interstitial pneumonia:

1 the use of MTX rather than CSP after HCT;
2 moderate to severe acute GVHD;
3 TBI compared with CY in the preparative regimen; and
4 increased patient age.

Results from 329 patients treated for SAA in Seattle from 1974 to 1990 showed that the overall incidence of interstitial pneumonia was 16%. Some 44% of affected patients had CMV pneumonia, 37% had pneumonia resulting from a variety of other infectious organisms, and 19% had pneumonia of unknown etiology. The overall mortality was 7%. TBI and acute GVHD were significant risk factors for the development of interstitial pneumonia, but there was no adverse influence of patient age or of MTX prophylaxis on the development of interstitial pneumonia [29]. More recent studies confirmed that grade II–IV acute GVHD was the major risk factor for the development of interstitial pneumonitis [104]. A study of idiopathic lung disease after transplant found that patients with AA conditioned with CY had a significantly lower incidence (less than 5%) than those transplanted for malignant diseases who were conditioned with TBI or BU-containing regimens [105]. The case mortality rate for these studies and more recent Seattle data, all of which contained large numbers of patients with malignant diseases, was 71–75% [104–106]. Over time, avoidance of TBI, improved prophylaxis for acute GVHD with MTX and CSP, and improved supportive care, including the prompt diagnosis and treatment of infectious agents such as CMV (by DNA detection methods), have all contributed to the decreased incidence of idiopathic interstitial pneumonitis. In the subsequent update of the patients with SAA treated with the Seattle regimen, only one of 81 patients died of idiopathic interstitial pneumonia [59].

Survival

Overall survival following HCT for SAA has increased steadily over the last 40 years. During the 1970s, survival rates of 40–60% were commonly seen, whereas the survival rates now range from 74 to 100% [54,59,60,62,64,65,90,91,107–111] (see Table 46.1). In Seattle, we observed improved 5-year overall survival among patients transplanted after July 1988 (88%) compared with patients transplanted before 1977 (41%) and compared with patients transplanted between 1977 and 1988 (61%). All patients transplanted since 1985 received MTX and CSP for GVHD prophylaxis, and since 1988 all were conditioned with CY and ATG rather than CY alone. Updated results employing the Seattle transplant regimen (CY and ATG conditioning, HLA-identical marrow HCT with combined MTX and CSP post-grafting immunosuppression) confirmed that with a median follow-up of 9.2 years (range 0.5–16.4 years), the survival rate was 88% [59]. In this study of 81 consecutive SAA patients, the median age was 25 years (range 2–63 years) (Figure 46.1e).

The Seattle group recently reported updated results for 148 consecutive children (median age 12.8 years) with SAA who received HLA-matched related bone marrow grafts between 1971 and 2010 (Figure 46.2) [111]. Patients were divided into three groups reflecting changes in the conditioning and GVHD prophylaxis regimens that occurred over time. Patients in Group 1 (Figure 46.2a) were conditioned with CY 200 mg/kg followed by "long" (day 102) MTX. Patients in Groups 2 and 3 received CY alone (Figure 46.2b) or CY with horse ATG (Figure 46.2c), followed by "short" (days 1, 3, 6, 11) MTX and CSP (until day 180). With a median follow-up of 25 years, the 5-year survivals were 66, 95, and 100% for Groups 1, 2, and 3, respectively (overall $p < 0.0001$). The 3-year estimates of graft rejection were 22, 32 and 7, respectively. The probabilities of grades III and IV acute and 2-year chronic GVHD were 15, 0, and 3% and 21, 21, and 10%, respectively. In summary, as outlined in the sections above, advances in the preparative and GVHD prophylaxis regimens and supportive care in the past 40 years have led to improved outcomes for children with SAA. The results confirm the use of allogeneic marrow transplantation as first-line therapy for children who have HLA-matched related donors [111].

The randomized GITMO/EBMT trial comparing CSP alone with combined MTX and CSP for patients conditioned with CY alone showed estimated 5-year survival of 94% for the MTX and CSP group and 78% for the CSP alone group [60]. The difference in survival between the two groups was statistically significant. This study confirmed the initial Seattle results demonstrating the superiority of combined MTX and CSP post-grafting immunosuppression. The multicenter randomized study of CY versus CY + ATG showed that the 5-year survival was 74% for the CY alone group and 80% for the CY + ATG group [62].

Analysis of transplant registry data has been helpful in identifying important trends influencing survival over time. The reported results are in general less favorable than the optimal conditioning regimens reported by some transplant centers because multiple conditioning and GVHD prophylaxis regimens are used by registry members, some of which were suboptimal. A retrospective multicenter multiregimen CIBMTR study reported that 5-year survival after HLA-identical sibling transplants had increased from 48% in 1976–1980 to 66% in 1988–1992 [89]. Because of the introduction of CSP for GVHD prophylaxis, the risk of GVHD decreased. Improved long-term survival was because of decreased mortality in the first 3 months post-transplant. The incidence of reported graft failure did not change significantly, ranging from 20% in 1976–1980 to 16% in 1988–1992. However, only 9% of reported transplants in 1988–1992 included CY and ATG conditioning.

CIBMTR data for 1699 patients receiving HLA-identical sibling transplants for SAA between 1991 and 1997 showed a 5-year probability of survival (± 95% confidence interval) of 75 ± 3% for 874 patients less than 20 years of age, 68 ± 4% for 696 who were 21–39 years old, and 35 ± 18% for 129 who were 40 years or older [112]. Survival was highest in untransfused patients transplanted early in the course of their disease and who were without active infection at the time of transplant. Only a minority of patients reported to the registry between 1991 and 1997 received CY and ATG conditioning with combined MTX and CSP post-grafting immunosuppression. More recently, since children with HLA-identical siblings were increasingly referred for transplantation as first-line therapy, and

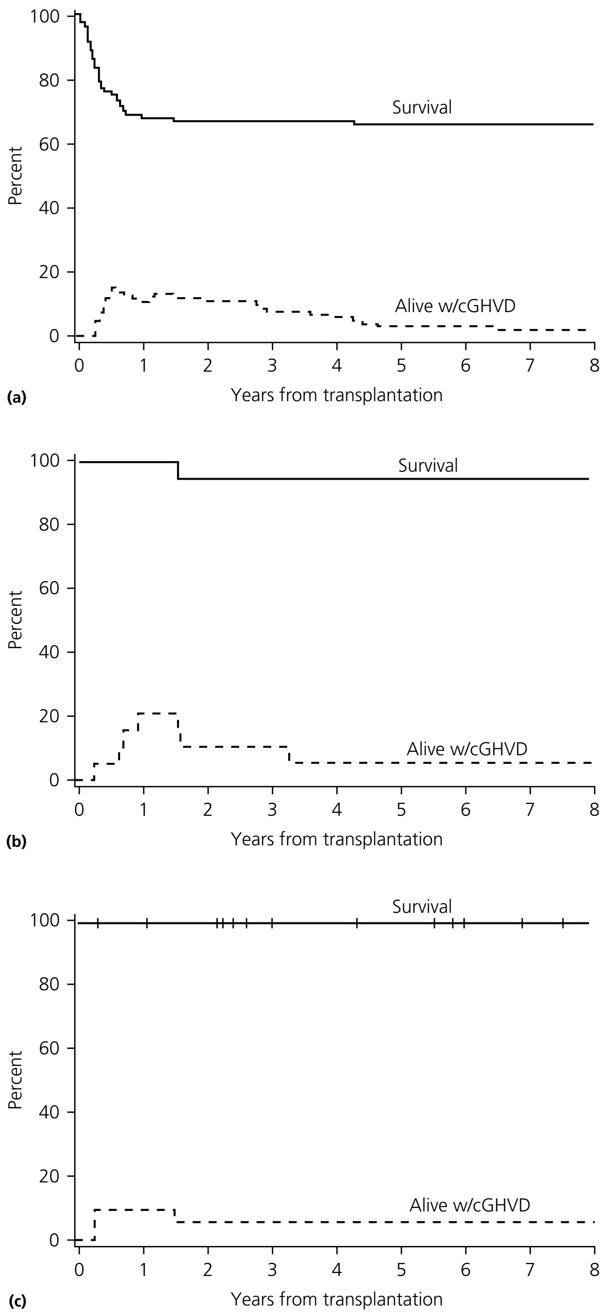


Figure 46.2 Survival (upper curves) and prevalence of chronic graft-versus-host disease (cGVHD, lower curves) among pediatric patients in Seattle with severe aplastic anemia that received an allogeneic bone marrow transplant (BMT) from an HLA-identical sibling over three time periods, (a) Group 1, BMT between 1971 and 1984: cyclophosphamide (CY) 200 mg/kg conditioning and post-transplant GVHD prophylaxis with methotrexate (MTX) only ($n = 98$). (b) Group 2, BMT between 1981 and 1988: CY conditioning with MTX + cyclosporine (CSP) ($n = 19$). (c) Group 3, BMT between 1989 and 2010: CY + anti-thymocyte globulin (horse ATG) conditioning, MTX + CSP GVHD prophylaxis ($n = 31$). Source: Burroughs et al. 2012 [111]. Reproduced with permission of John Wiley & Sons, Ltd.

because the majority of transplant centers now administered CY + ATG conditioning with MTX + CSP for GVHD prophylaxis, the survival trends have continued to improve. Between 1995 and 2003, 305 patients aged 20 years and under who received bone marrow as the HSC source had a 5-year probability of survival of 85%. The 253 patients more than 20 years old had a 5-year survival of 64%. Survival was significantly worse for the 134 patients who received G-PBHC as the graft source owing to the increased incidence and severity of chronic GVHD [54].

EBMT data for 1275 patients receiving HLA-identical sibling transplantation between 1991 and 2002 confirmed and extended the CIBMTR results. The year of the transplant, the age of the patient, and the interval from diagnosis to transplant predicted outcome after HCT. Comparing patients transplanted in the periods 1991–1996 and 1997–2002, there was an improvement in 5-year survival rate: 74% versus 80%, respectively. In the period 1997–2002, actuarial survival was 91% for children (<16 years old) and 74% for adults. ATG was added to the conditioning regimen in 20% of patients and their overall survival was 81% compared with 73% among patients not receiving ATG [107].

Several other studies found a survival of more than 85% for patients conditioned with the regimen of CY (200 mg/kg) and ATG [61,86,108–110]. In contrast, reports of patients conditioned with CY and irradiation had survival rates ranging from 57 to 78% [109,113,114], although the follow-up on some of these reported studies was short. Pediatric patients (younger than 19 years) generally fared better than adult patients [29,73,85,86,111], with survival rates of 94–100%.

A striking difference in transplant outcome for AA between radiation- and non-radiation-based conditioning regimens was reported from a single center by Ades et al. [109]. From 1978 to 1992, 100 patients received A CY/TAI conditioning regimen and had an overall survival at 15 years of 58%. From 1995 onwards, 33 patients (26 under the age of 30 years) received CY + ATG conditioning with MTX + CSP GVHD prophylaxis and had a 94% overall survival.

Radiation-containing regimens were widely used in the late 1970s and 1980s because of their better immunosuppressive properties resulting in a decreased incidence of graft rejection. However, the use of radiation has been associated with risks of interstitial pneumonia, growth impairment, development of secondary solid tumors (see later discussion), and lower overall survival compared with CY and ATG. Radiation is no longer recommended as an initial preparative component for patients with HLA-matched sibling donors.

The steady improvement over the past four decades in overall survival after allogeneic HCT for SAA was recently threatened by the widespread use of G-PBHC and/or G-CSF primed bone marrow (G-BM) in place of unmodified bone marrow (BM). Two large registry studies have clearly demonstrated worse overall survival when either G-PBHC or G-BM was used instead of unmodified BM. A CIBMTR study compared outcomes in patients with SAA transplanted between 1997 and 2003, infused with BM (n = 547), G-BM (n = 78), or G-PBHC (n = 134) from HLA-identical siblings. There was no difference in the likelihood of achieving neutrophil recovery at 30 days after HCT or platelet recovery at 60 days after HCT among the three groups. However, there was a significantly increased risk of acute GVHD for G-PBHC recipients and an increased risk of developing chronic GVHD among G-BM and G-PBHC recipients compared with BM. The 3-year adjusted overall survival was 80, 72, and 76% for recipients of BM, G-BM, and G-PBHC, respectively. The difference in overall survival for G-BM recipients was significantly worse compared with unmodified BM [115]. An EBMT study with increased numbers of SAA patients showed a more striking difference in the overall survival in favor of unmodified BM. Among patients receiving HLA-identical sibling grafts between 1999 and 2009, 1163 received BM and 723 received G-PBHC and actuarial 10-year overall survival was 84 and 68%, respectively. Multivariate analysis identified the following negative predictors for overall survival: older age, longer interval between diagnosis and transplantation, no ATG in the conditioning regimen, a regimen other than CY 200 mg/kg, and G-PBHC as the stem-cell source. For patients age 20 years or less, the 10-year overall survival was 90 and 76% for BM and G-PBHC, respectively. For patients over 20 years of age, the 10-year overall survival was 74 and 64% for BM (n = 507) and G-PBHC (n = 489), respectively. When stratified for all negative predictors of survival, BM was consistently superior to G-PBHC in overall survival after HCT (Figure 46.3) [53]. In conclusion, there is no indication for the use of G-PBHC or G-BM as the stem-cell source for patients with SAA.

Transplantation for older patients

An analysis of 23 consecutive patients with SAA who were above the age of 40 years (range 40–68 years) and underwent BMT from HLA-identical siblings in Seattle with CY (200mg/kg) + ATG conditioning and MTX + CSP GVHD prophylaxis between July 1988 and October 2008 showed an overall survival of 65% with a median follow-up of 9.1 years. Five patients (22%) died from pre-existing infections prior to engraftment. Three patients died from infections at 3, 6, and 7 months after BMT related to acute or chronic GVHD. Six patients (26%) developed fluid overload, requiring specific medical treatment, either during the conditioning regimen or within the week after HCT; this complication was observed in the setting of concurrent systemic infection. Graft rejection was observed in 4%. The overall incidence of acute and chronic GVHD was 35 and 26%, respectively; grade IV GVHD was not observed. The median time to discontinuation of immune suppression was 6 months (range 6–92 months) [116]. These data support the use of BMT for patients with SAA well above the age of 40 years who have no significant medical comorbidities.

Registry data from CIBMTR identified 84 patients above 40 years of age with SAA who received HLA-identical sibling BMT following a wide variety of conditioning regimens (48% with standard CY + ATG) and reported 53% overall survival at 5 years. Independent risk factors for worse outcome included poor performance score and delay of greater than 3 months between diagnosis and transplant [117]. An analysis of older Korean patients transplanted with various conditioning regimens and cell sources, including 53% with standard CY + ATG and 74% with BM, reported that patients between age 40 and 50 years had a 5-year overall survival of 68% [118].

The outcome with a FLU (120 mg/m^2), CY (1200 mg/m^2), and rabbit ATG (15 mg/kg) conditioning regimen was reported in 30 patients with SAA over 30 years of age. Two-thirds received unmodified BM and one-third received G-PBHC from HLA-identical siblings. Most patients received CSP and MTX GVHD prophylaxis. There was one patient with graft failure and the incidence of acute and chronic GVHD was 10 and 13%, respectively. At a median follow-up of 4 years, the overall survival for the 21 patients above 40 years of age was 71% [34]. Finally, results from the FLU–CY–alemtuzumab regimen were reported in 12 SAA patients older than 50 years of age, with 71% overall survival at 2 years [32]. Although there may be an increased risk of subsequent graft rejection, the

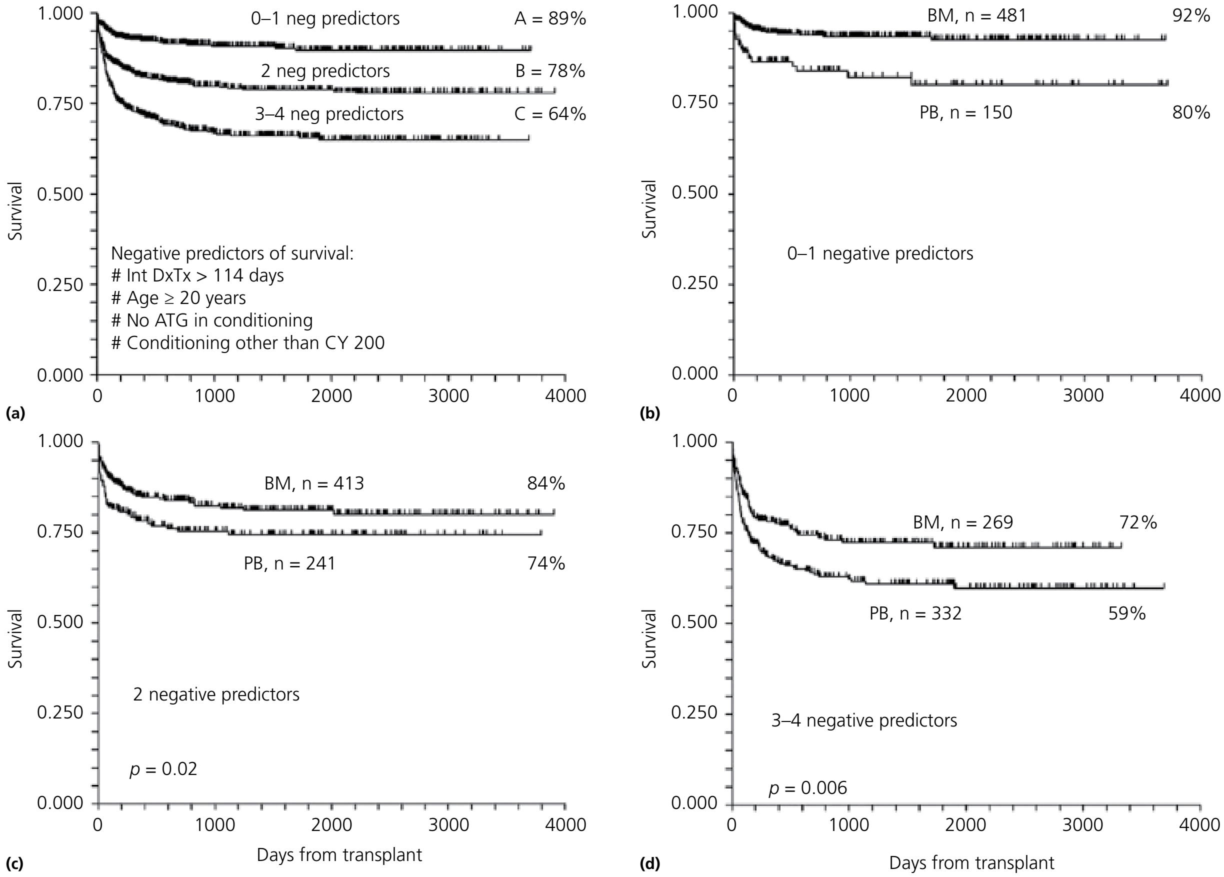


Figure 46.3 European Group for Blood and Marrow Transplantation (EBMT) registry data summarizing survival outcome in 1886 patients with acquired aplastic anemia who received a first transplant of HLA-identical sibling between 1999 and 2009 with either bone marrow (BM, $n = 1163$) or G-CSF-mobilized peripheral blood (PB, $n = 723$) as the source of stem cells. (a) Actuarial survival of all patients stratified by negative predictors of survival: interval diagnosis-transplant (Dx–Tx) ≥114 days, recipients' age ≥20 years, no antithymocyte globulin (ATG) in the conditioning, a conditioning regimen other than cyclophosphamide 200 mg/kg. Three groups were identified: low risk (survival 89%), intermediate risk (survival 78%), and high risk (survival 64%). (b) Actuarial survival for BM versus PB graft recipients in the low-risk group ($p = 0.0001$). (c) Actuarial survival for BM versus PB graft recipients in the intermediate-risk group ($p = 0.02$). (d) Actuarial survival for BM versus PB graft recipients in the high-risk group ($p = 0.006$). Source: Bacigalupo et al. 2012 [53]. Obtained from *Haematologica* website, http://www.haematologica.org. Copyright 2012 Ferrata Storti Foundation.

reduced-intensity regimen of FLU–CY–alemtuzumab [32] or FLU–CY–rabbit ATG [34] with HLA-identical sibling bone marrow as the stem-cell source is an effective alternative treatment for older patients with SAA who are deemed unable to tolerate the standard higher dose CY 200 mg/kg + ATG regimen.

Influence of etiology of aplastic anemia on the outcome of marrow grafts

HCT can cure AA resulting from causes other than an idiopathic etiology, including patients with hepatitis-associated AA, Fanconi anemia, and dyskeratosis congenita. For hepatitis-associated AA, there was concern either that liver damage caused by hepatitis might lead to increased liver damage from CY or that the damaged liver might not be able to activate CY enzymatically. Neither of these concerns proved valid [119,120]. Marrow grafts were successful, even when CY was administered at times of highly abnormal liver function tests. Also, there were no long-term hepatic sequelae related to the HCT procedure in these patients. Survival was in excess of 80% [119].

Fanconi anemia is covered in detail in Chapter 76, but is often included in reports describing patients with SAA. Fanconi anemia is an autosomal recessive disease with progressive pancytopenia, chromosomal fragility, and hypersensitivity to DNA cross-linking agents that can progress to acute leukemia. Currently, the only successful treatment for marrow failure in patients with Fanconi anemia is HCT. Conditioning regimens used in the past to prepare Fanconi anemia patients for transplantation have been associated with considerable toxicity and transplant-related mortality [121,122]. In addition, irradiation and high-dose CY are associated with a very high risk of secondary malignancies [123]. It has been recognized that unlike patients with idiopathic AA, those with Fanconi anemia can be conditioned with lower doses of CY than conventionally used because of their unusual sensitivity to toxicity from alkylating agents [124]. Results from Curitiba in Brazil and Seattle showed that patients with Fanconi anemia can be transplanted successfully without incorporation of irradiation into the conditioning regimen with substantially lower doses of CY than used in the past [125]. Subsequently, further dosage reduction of CY to 15 mg/kg/day given for four consecutive days (total dose, 60 mg/kg) has been successful in achieving durable donor engraftment of HLA-identical sibling bone marrow accompanied with decreased toxicity and excellent long-term survival of 93% with follow-up to 7.9 years [126]. Similar results with CY 60 mg/kg combined with ATG have been reported [127]. Thus, the optimal total dosage of CY that will allow sustained engraftment with minimal toxicity appears to be 60 mg/kg. For patients who do not have an HLA-identical sibling donor, regimens have been developed that incorporate FLU and reduced-dose CY with low-dose TBI and either in vivo or ex vivo T-cell depletion.

Dyskeratosis congenita is a rare inherited syndrome of ectodermal dysplasia that is associated with AA in 50% of cases [128]. The initial trials using conventional conditioning regimens were associated with significant morbidity and mortality [129,130], particularly an increased incidence of late chronic pulmonary and vascular complications. Recent reports have shown successful outcomes in patients given a reduced-intensity conditioning regimen such as the Seattle nonmyeloablative regimen of FLU 90 mg/m^2 + 2 Gy TBI [131] or, alternatively, CY (60 mg/kg) + ATG [132].

HCT has also been used to treat SAA resulting from PNH and Diamond–Blackfan anemia [133–135]. PNH is discussed in greater detail in Chapter 47. PNH may be classified into three categories based on flow cytometry characteristics, reticulocyte count, serum LDH concentration, and bone marrow analysis, namely (1) classic PNH, with florid hemolysis and erythroid hyperplasia, (2) PNH in the setting of another bone marrow failure syndrome (SAA or MDS), and (3) subclinical PNH, with no clinical or biochemical evidence of intravascular hemolysis [136]. Patients with PNH in the setting of a bone marrow failure syndrome typically have less than 10% of nucleated cells deficient in glycosylphosphatidylinositol-anchored proteins (GPI-APs). Patients with bone marrow failure syndrome should have treatment focused on the underlying bone marrow failure with either allogeneic HCT or immunosuppressive therapy. PNH-AA syndrome has been reported to respond favorably to IST, hence allogeneic BMT is generally offered to younger patients and those with HLA-identical siblings or to those who have not responded to IST. All patients with clinically significant hemolysis (typically with >10% GPI-AP-deficient cells) should receive therapy with the complement inhibitor eculizumab to prevent ongoing intravascular hemolysis. Patients with classic PNH who fail to respond adequately to eculizumab therapy and who have persistent intravascular hemolysis and thrombotic events should consider proceeding to allogeneic HCT [136]. Although allogeneic HCT offers potentially curative treatment for PNH, fewer patients with classic PNH are undergoing HCT since the introduction of eculizumab. Long-term eculizumab treatment for patients with classic PNH has favorable survival [137]. A retrospective registry analysis compared the outcomes of 211 patients with PNH who received allogeneic HCT between 1978 and 2007 with those of 405 PNH patients who did not undergo HCT between 1950 and 2005. There did not appear to be a benefit for allogeneic HCT when patients were approximately matched based on the severity of thromboembolism and PNH disease. The 5-year overall survival for 118 patients with the PNH AA syndrome who underwent transplantation was 69%. Nearly half of these patients received high-dose CY + busulfan or CY + TBI based conditioning regimens [138]. The reduced-intensity conditioning regimen of FLU 90 mg/m^2 + 2 Gy TBI has been successful for patients with PNH [139]. It is likely that reduced intensity conditioning regimens will reduce transplant related mortality and may improve the overall survival after allogeneic HCT. Of 61 patients with Diamond–Blackfan anemia who were refractory to prednisone and other therapies reported to the CIBMTR, 41 received HLA-identical sibling transplantation with 76% survival at 4 years [140].

Late effects

Long-term survival

Long-term outcome among 212 patients transplanted for SAA was initially reported by the Seattle group in 1998 [101]. The survivors included children and adults (aged 1–42 years at the time of transplant); 93% were conditioned with CY ± ATG, and 89% received HCT from HLA-identical siblings. Chronic GVHD was the dominant risk factor for late complications. The 20-year survival among patients without chronic GVHD (n = 125) was 89% compared with 69% among those with chronic GVHD (n = 86) [101].

A subsequent study of late effects was reported among 137 pediatric patients with acquired SAA who were followed for nearly four decades after allogeneic HCT [27]. Transplantation regimens were primarily CY ± ATG (77%) and 18% had a TBI-based regimen. Donors were 88% family members, 12% unrelated, and 2% syngeneic. Bone marrow was the source of stem cells in 98.5% of transplants; cord blood was used in 1.5%. The incidence of chronic GVHD was 26%. With a median follow-up of 21.8 years, survival for acquired SAA at 30 years after HCT was 82%. The 30-year survival for patients without chronic GVHD was 83% and for those with chronic GVHD it was 62%. There was an increased incidence of thyroid function test abnormalities and secondary malignancies in recipients of TBI-based conditioning. CY recipients demonstrated normal growth, with basically normal development and pregnancies with mostly normal offspring. Quality of life studies in adult survivors of HCT indicated that patients were comparable to age-matched control subjects, except for difficulty in obtaining health and life insurance in 20% of HCT patients. The data indicate that the majority of long-term survivors after transplantation for AA during childhood can have a normal productive life [27].

Gonadal function and fertility (see Chapter 102)

Gonadal function in patients conditioned only with CY + ATG often returns to normal. Among 65 women between the ages of 13 and 25 years who received CY, all had evidence of recovery of ovarian function, whereas among women aged 26–38 years, 37% developed primary ovarian failure [26,141–144]. These data suggest that older age is a risk factor for ovarian failure. Testicular function returned to normal in most men aged 14–41 years who received CY + ATG only for conditioning [26]. Many successful pregnancies were reported after conditioning regimens containing CY + ATG only [101]. Among a cohort of 63 women who underwent HCT for SAA as children (median age at transplant 10 years), there were 73 evaluable pregnancies among women who received CY-based conditioning. The median age at time of the first pregnancy was 24 years and 82% of pregnancies resulted in live births. The infants born to these women were normal, except for one with congenital aortic stenosis. Among the 74 men with SAA who received the CY-preparative regimen, their partners had 59 evaluable pregnancies, 80% of which resulted in live births. The infants were normal except for two: one had hip dysplasia and the other cleft lip [27]. In contrast, pregnancy and fathering a child after conditioning with TBI were rare events. Among the 10 patients who received a 12 Gy TBI conditioning regimen for SAA, there were two women who also became pregnant, and both delivered normal infants. One of these women was 3 years of age at transplantation and the other 13 years [27]. There is insufficient information regarding fertility after FLU-based regimens, since these regimens are predominantly used for older patients.

Growth and development (see Chapter 102)

A study of hormonal function and growth after HCT found that children conditioned with CY only had normal height and normal thyroid and adrenal function, and no growth hormone deficiency. However, in children given TBI as part of the conditioning, although prepubertal growth in the first 3 years after transplant was normal, the final height was lower than the target height [145]. Therefore, TBI-containing regimens should be avoided in children if possible. Both children and adults treated with TBI-containing regimens were also more likely to develop thyroid dysfunction after transplantation [26], and these patients should be monitored with yearly thyroid function tests.

Secondary malignancies (see Chapter 104)

Since the first patients were transplanted successfully for AA over 40 years ago, data have been accumulated on the incidence and types of secondary malignancies resulting from HCT. Deeg et al. [123] described results in 700 patients with AA who had an estimated 14% incidence of secondary malignancies at 20 years. The cancer risk among transplanted patients was ninefold higher than that among the general population. Overall, 18 of 621 patients (excluding those with Fanconi anemia) developed secondary malignancies a median of 7.5 years post-transplant. Of the tumors, five were lymphoid malignancies seen a median of 3 months after transplant, and 13 were solid tumors occurring a median of 8.2 years after transplant. Nine of the solid tumors were squamous cell carcinomas of the head and neck and most of these patients were cured following surgical resection. All of the patients with lymphoid malignancies died, whereas 46% of those with solid tumors died. Significant risk factors for the development of secondary malignancies in this series included the use of azathioprine for chronic GVHD treatment, increased age, and use of irradiation in the conditioning regimen [123]. With regimens not containing irradiation there was a cumulative incidence of secondary cancers at 10 years of 1.4% [146] compared with a 22% (standard error ±11%) incidence at 8 years [147] with radiation-containing regimens. Other studies have also implicated irradiation-based conditioning regimens as risk factors for late malignancies [28,109], so these regimens should be avoided in HLA-identical HCT candidates. The incidence of secondary malignancy for 81 patients receiving CY + ATG conditioning is shown in Figure 46.1d.

Among the pediatric cohort of 137 patients with acquired AA treated in Seattle, 18 (13%) developed a secondary malignancy between 1 and 31 years after transplantation and this was fatal for four patients. The most common malignancy was squamous cell carcinoma, which occurred in seven patients and was the cause of death for one. Five of the seven with squamous cell carcinoma also had severe skin chronic GVHD. Three patients developed a thyroid malignancy, all of whom were successfully treated. Three patients developed breast cancer between 21 and 31 years after transplantation. This was the cause of death for one with invasive breast cancer, and the other two survivors had carcinoma in situ. Two patients developed hepatocarcinoma associated with hepatitis C (due to blood transfusion given prior to the availability of donor screening). This was the cause of death for one, and the other is alive after a liver transplantation. One patient developed MDS and underwent a second allogeneic HCT. One patient developed a melanoma 2 months after receiving the transplant, which was treated with resection. A final patient died of metastatic cervical cancer. The proportion of patients who developed a secondary malignancy was greater among the patients who had TBI in the conditioning regimen [27].

Chronic GVHD is also a risk factor for the development of solid tumors after transplant for AA, with an estimated 17–30% probability at 20 years for patients with both acute and chronic GVHD [59,101]. Carcinomas of the oropharyngeal mucosa developed in patients with a history of chronic GVHD. These findings were also confirmed in a larger study of 19,229 patients who had undergone transplantation between 1964 and 1992, which showed that chronic GVHD was associated with a significantly increased risk of squamous cell carcinoma of the buccal cavity and skin [148]. The severity of chronic GVHD and duration of IST for chronic GVHD appear to be risk factors for secondary malignancy. These findings emphasize the importance of avoiding infusion of buffy coat or G-CSF-mobilized PBHC to prevent chronic GVHD in patients with AA.

Newer reduced-intensity conditioning regimens (see Chapter 21)

Considerable interest has been generated recently with the development of reduced-intensity conditioning regimens, which can decrease transplant-related mortality and permit successful allogeneic HCT of older or medically infirm patients with hematologic malignancies or inborn errors of metabolism (see Chapter 21) [149–151]. The current standard CY (200 mg/kg) + ATG regimen for SAA is non-myeloablative, although it is a more intensive conditioning regimen than the newer, less intensive regimens with FLU and reduced-dose CY. Two fairly recently reduced-intensity regimens include FLU (30 mg/m^2/day × 4 days), CY (300 mg/m^2/day × 4 days), and rabbit ATG (Thymoglobulin, 3.75 mg/kg/day × 4 days) [34] and, alternatively, FLU (30 mg/m^2/day × 4 days), CY (300 mg/m^2/day × 4 days), and alemtuzumab (12 mg/day × 5 days, although lower doses may be sufficient) [32]. In the two clinical trials for SAA reported to date, a substantial number of patients received G-PBHC; however, bone marrow is the preferred source of stem cells. CSP and MTX was primarily used as post-grafting immunosuppression for the FLU–CY–rabbit ATG trial and CSP monotherapy was given after the FLU–CY–alemtuzumab regimen. However, it is unclear if the optimal alemtuzumab dose or the optimal post-grafting therapy has been identified for the FLU–CY–alemtuzumab regimen.

Whether or not an alternative conditioning regimen such as fludarabine or reduced-dose CY could be less toxic or better tolerated and yet as effective as CY 200 mg/kg + ATG for SAA patients remains to be determined. To date there is no evidence of improvement in the survival or definitive reduction in transplant-related mortality with the intensified FLU with reduced CY-based regimens. A report of six patients with SAA who received FLU 90 mg/m^2 and 2 Gy TBI, a G-PBHC graft, and the triple-drug GVHD prophylaxis regimen of CSP + MMF + MTX showed that all six patients survived with donor engraftment, although follow-up was brief [152]. These results suggest that alternatives to the standard CY (200 mg/kg) + ATG regimen are worth considering in the context of clinical trials for patients at high risk for medical complications from the CY 200 mg/kg and ATG regimen. Given the historical experience with high rates of graft rejection for transfusion-sensitized SAA patients, the potential benefit of a slight reduction in conditioning regimen toxicity must be very carefully balanced against the increased risk of complications associated with graft rejection or acute and chronic GVHD.

Grafts from alternative marrow donors

Transplantation of marrow obtained from unrelated or HLA-mismatched related donors has been less successful than grafts from HLA-identical siblings. There are probably two major reasons for this discrepancy:

1 there is an increased risk of transplant-related complications because of the greater genetic disparity between donors and recipients; and
2 most patients are not considered candidates for HCT from alternative donors until they have failed attempts at IST.

By the time these patients come to transplantation, they are often refractory to platelet transfusions, have received multiple antibiotics because of life-threatening infections, and are often severely infected at the time of transplantation. In order to improve patient survival, transplantation from alternative donors should be considered at earlier time points before patients become severely ill.

HLA-non-identical related donors (see Chapter 44)

Data from Seattle and the EBMT group indicate that patients transplanted for aplastic anemia with phenotypically HLA-matched related donors have better overall survival, ranging from 64 to 100%, than patients with HLA-mismatched related donors, whose survival is generally 50% or less [153,154]. For patients with phenotypically HLA-matched donors, irradiation proved not to be a necessary component of the conditioning regimen, and survival of CY-conditioned patients was similar to that of patients with HLA-identical sibling donors [153].

For patients with HLA-mismatched related donors, conditioning with CY with or without ATG did not provide sufficient immunosuppression to prevent graft rejection in most patients, and none of the patients achieved long-term survival. High rates of acute and chronic GVHD occurred among those few patients who did engraft. For subsequently transplanted patients with HLA-mismatched related donors, the use of CY combined with TBI decreased the incidence of graft failure and increased survival, but pre- and peri-transplant infections and post-transplant GVHD have remained major problems [153]. The optimal dose of TBI for these patients to prevent rejection with a minimal amount of regimen-related toxicity is not known.

Increasing HLA disparity adversely affects transplant outcome [153,154], consistent with findings in patients transplanted for leukemia [155]. A CIBMTR survey showed 49% projected survival for one HLA-locus mismatched HCT, similar to Seattle data that showed 50% survival for these patients [156]. The CIBMTR series reported 35% survival for two or more HLA-loci mismatched recipients, with other studies reporting similarly poor outcomes [153]. The Beijing transplant group reported on a cohort of 19 younger patients with SAA who received HLA-haploidentical family member donor G-BM combined with G-PBHC following conditioning with busulfan (BU 6.4 mg/kg intravenous), CY (200 mg/kg), and ATG (rabbit or pig). Post-grafting immunosuppression consisted of the triple regimen of MTX, CSP, and MMF. Two patients (11%) had late graft failure, and three patients did not achieve platelet engraftment. Acute GVHD developed in 42% and chronic GVHD in 56% of evaluable patients. The 2-year overall survival was 64%. Although these early survival results are better than expected, the increased risk of GVHD following this transplant regimen is of concern, and will limit the applicability of this approach for more patients with SAA [157].

Unrelated donors (see Chapter 45)

Most patients with AA who do not have suitably HLA-matched related donors receive IST as first-line therapy. They proceed to unrelated donor BMT only if they fail to respond to IST. Historically, rates of transplant-related morbidity and mortality have been high. In a National Marrow Donor Program (NMDP) retrospective analysis of 141 patients transplanted between 1988 and 1995, the overall survival at a median of 36 months after transplant was 36% [158]. Some 86% of these patients received a radiation-containing conditioning regimen, 74% received HLA-matched marrow, and 26% received marrow mismatched for at least one HLA-A, -B or -DR antigen; 32% received T-cell-depleted marrow, and all but 13% received a CSP-containing regimen to prevent GVHD. A total of 89% achieved sustained engraftment, and 52% of patients developed grade II–IV acute GVHD.

In a pilot study, five patients received unrelated donor transplants following CY and ATG conditioning as used for recipients of HLA-identical donor marrow. Three patients experienced graft failure and only one patient survived long term [159]. Other groups reported that high-dose irradiation regimens, although effective in achieving engraftment, resulted in an increased incidence of fatal organ toxicity without increasing the probability of survival.

In the past two decades, high-resolution HLA typing and identification of an optimized conditioning regimen have resulted in improved survival after unrelated donor HCT [160]. A collaborative multicenter prospective NMDP-sponsored study was undertaken to define the minimum effective dose of TBI sufficient to achieve engraftment for patients with AA transplanted with unrelated donor marrow [161]. The starting dose of TBI was 6 Gy (three fractions of 2 Gy) given after three doses of horse ATG 30 mg/kg/day, combined with four consecutive infusions of CY 50 mg/kg/day. The TBI dose was to be escalated in increments of 2 Gy if graft failure occurred in the absence of prohibitive toxicity, and de-escalated for toxicity in the absence of graft failure.

A total of 87 patients were enrolled between 1994 and 2004: 62 patients received HLA-A, -B, -C, -DR and -DQ-allele matched marrow transplants based on molecular typing and 25 patients received marrow from donors who differed by one or two alleles or one HLA antigen. The ages of the patients ranged from 1.3 to 53.5 years with a median of 18.6 years. The time intervals from diagnosis to transplantation were 2.8–328 months with a median of 14.6 months. All patients had received multiple transfusions and a median of three courses of IST. All 20 patients treated with 6 or 4 Gy TBI, CY, ATG, and HLA-matched marrow engrafted, and survival was 50%. Of the 35 patients receiving 2 Gy TBI, CY, ATG, and HLA-matched marrow, one rejected and 23 (66%) were alive. Grade 3 or greater pulmonary toxicity occurred in eight of 30 patients conditioned with 6 or 4 Gy TBI and in one of 35 patients conditioned with 2 Gy TBI. The incidence of acute GVHD was 69%, with eight deaths attributable to acute or chronic GVHD and infection. Chronic GVHD requiring therapy developed in 52% of patients. Nine patients did not tolerate ATG and instead received CY 120 mg/kg and fractionated 12 Gy TBI; five of the nine survived [161].

Age was an important risk factor for survival. Patients who were under the age of 20 years at the time of HLA-matched unrelated BMT conditioned with 2 Gy TBI + CY + ATG had a 5-year survival of 78% compared with 50% for patients older than 20 years. Recipients of HLA-non-identical unrelated donor grafts had an overall survival of 40% [161]. CIBMTR data for unrelated donor HCT showed that among 181 patients transplanted with low- or intermediate-resolution HLA-matched unrelated donor marrow between 1988 and 1998, the 5-year survival probability was 39% [156]. As multiple suboptimal preparative regimens were used, and high-resolution HLA typing was not applied, the results are inferior to those with the new regimen identified by Deeg et al. [161] for unrelated HCT. Most unrelated transplants were performed late in the course of disease after failure to respond to one or more courses of immunosuppression. Furthermore, it is now accepted that the widespread adoption of improved molecular techniques for high-resolution HLA typing will result in better outcomes for recipients of HLA-matched unrelated donor HCT [162].

Table 46.2 summarizes the hematopoietic cell transplant results for patients who received marrow grafts from HLA-matched unrelated donors. In these studies, patients who were younger than 20 years had better survival. As with transplants using HLA-mismatched related donors, non-radiation-containing conditioning regimens provided insufficient immunosuppression to prevent rejection among patients receiving unrelated marrow grafts [159].

In summary, the NMDP-sponsored study reported by Deeg et al. [161] showed improved outcome of patients and identified a conditioning regimen of 2 Gy TBI, CY, and ATG that achieved reliable engraftment with significantly decreased organ toxicity. These results, combined with the recent advances in molecular HLA typing, now strongly suggest that patients who have unrelated donors fully HLA matched for both class I and II, including HLA-C, by high-resolution DNA-based typing may have superior long-term outcome with HCT over IST. If a high-resolution 10 of 10 HLA-allele matched donor is identified, then unrelated donor HCT should be considered early in the course of treatment of patients with SAA, even as first-line therapy, particularly for patients 20 years of age or younger. Because of the time interval involved in the donor search, unrelated donor HCT might not be feasible as first-line therapy for acquired SAA. However, patients could proceed to unrelated donor HCT within 4 months if response to IST was unsatisfactory. As with the HLA-identical sibling setting, transfusion with leukodepleted irradiated blood products prior to transplantation is critical to decrease the risk of graft rejection. Although confirmatory data are lacking, younger patients (less than 35 years of age) with 1-HLA-allele mismatched donors could also proceed to BMT if treatment with one or possibly two optimal IST regimens had failed [161].

Recently, a Blood and Marrow Transplant Clinical Trials Network (BMT CTN) multicenter phase I/II study was conducted to identify the optimal dose of CY when used in a FLU (120 mg/m^2)–CY–ATG–2 Gy TBI conditioning regimen. There were four cohorts of CY: 150, 100, 50, and 0 mg/kg. All patients received either 3 mg/kg/day × 3 doses (9 mg/kg) of rabbit ATG or 30 mg/kg/day × 3 doses (90 mg/kg) of horse ATG. Bone marrow from an HLA-matched unrelated donor was the source of hematopoietic stem cells (a single HLA-locus mismatch was acceptable). CY 150 mg/kg was associated with a high incidence of pulmonary toxicity and death (seven

Table 46.2 Selected recent reports of HCT from unrelated donors

Transplant team	Year of report	Year of HCT	No. of patients	Age range in years (median)	Conditioning regimen	GVHD prophylaxis	Graft failure (%)	GVHD (%)		Survival (%)	Range of follow-up in years (median)
								Acute Grade II–IV	Chronic		
NMDP, Deeg et al. [161]	2006	1994–2004	87	1–53 (18.6)	CY + ATG + 2 Gy TBI (n = 46); CY + 4 or 6 Gy TBI + ATG[a], (n = 41)	CSP + MTX	HLA-allele matched (n = 62):				1.2–10.2 (7)
							1	70	52	61[b]	
							HLA-allele/antigen mismatched (n = 5):				
							12	75	57	44	
EBMT, Passweg et al. [156]	2006	1988–1998	232	1–55 (16)	CY + TBI + others, varied	CSP + MTX, varied	HLA-matched (low resolution, n = 181):				0.2–11.6 (5.1)
							17	48	29	39	
							HLA-mismatched (low resolution, n = 51):				
							24	37	24	36	
EBMT, Viollier et al. [197]	2008	1990–1997	149	1–48 (17)	TBI + other (50%), varied	CSP + MTX (42%)	26	37	38	32	5 year estimated overall survival
		1997–2005	349	1–65 (19)	TBI + other (32%), varied	missing data (47%)	11	28	22	57	
JMDP, Yagasaki et al. [166]	2011	1993–2005	301	0–64 (17)	TBI + CY + ATG (42%)	CSP + MTX or FK-506 + MTX, varied	10	27	25	66	0.2–10.7 (3.7)
							HLA-allele matched 10/10 (high resolution (n = 101)				
							7	15	20	75	
CIBMTR, Eapen et al. [165]	2011	2000–2008	225 BM	2–66 (19)	TBI (2–8 Gy) + CY ± ATG ± FLU (57%), varied	CSP + MTX (51%), FK-506 (30%), varied	18	31	age ≤20: 26 age >20: 40	76	3-year probability of overall survival
			71 PBPC	1–71 (36)			7	48	age ≤20: 28 age>20: 58	61	
BMT CTN, Tolar et al. [163][c]	2012	2006–2011	61	1–65	CY = 150 or 100 or 50 or 0 + FLU + 2 Gy TBI + ATG	CSP or FK-506 + MTX	CY 0 (n = 3) 100% CY 150 (n = 14) 7%	NR	NR	CY 0, 33% CY 150, 50%	1.9–2.5 (2.0) CY 0 and CY 150 arms closed to accrual 2° excess toxicity
Samarasinghe et al. [164]	2012	2000–2010	44	3–19 (8)	CY + FLU + Alem various doses	CSP or CSP + MMF or MTX	0	38	12	95	1–6.3 (2.9)

NMDP = National Marrow Donor Program; EBMT = European Group for Blood and Marrow Transplantation; JMDP = Japan Marrow Donor Program; BMT CTN= Blood and Marrow Transplant Clinical Trials Network; BM= bone marrow; PBPC = granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood progenitor cells; HCT = hematopoietic cell transplantation; GVHD = graft-versus-host disease; CY = cyclophosphamide; ATG = antithymocyte globulin; TBI = total body irradiation; FLU = fludarabine; Alem= alemtuzumab; CSP = cyclosporine; MTX = methotrexate; FK-506 = tacrolimus; MMF= mycophenolate mofetil; HLA = human leukocyte antigen; NR= not reported.

[a] Includes nine patients who did not tolerate ATG, and received CY (120 mg/kg) + 12 Gy TBI.

[b] 5-year survival among 23 patients aged ≤20 years conditioned with 2 Gy TBI and disease duration less than 1 year was 85%.

[c] BMT CTN trial is a dose-finding study comparing conditioning regimen CY 150, 100, 50 or 0 mg/kg + FLU 120 mg/m^2 + ATG (rabbit or horse) + 2 Gy TBI. Study is continuing accrual with randomization of patients to CY 50 vs. 100 mg/kg dose.

of 14 patients died from organ failure or acute respiratory distress syndrome) and CY 0 mg/kg was associated with a high incidence of secondary graft failure (three of three patients). The study will continue to accrue patients in two arms of CY dose, 50 vs. 100 mg/kg [163]. It is not yet clear if the outcome from the CY 50 or 100 mg/kg combined with FLU 120 mg/m^2, ATG, and 2 Gy TBI is superior to the standard CY(200 mg/kg)–ATG–2 Gy TBI regimen. As has been suggested by some of the HLA-identical sibling studies, it may be that the combined FLU-reduced dose CY regimen is better suited for older patients with SAA receiving unrelated donor BMT.

United Kingdom BMT centers reported on a cohort of consecutive children (age 1–17 years) with SAA diagnosed between 2000 and 2010, who initially underwent treatment with IST consisting of rabbit ATG plus CSP, followed with unrelated donor HCT when IST failed to control SAA. Among 43 evaluable children, the efficacy of first-line rabbit ATG + CSP IST was low, with a 6-month cumulative response rate of 32.5%. Next, 44 children received unrelated donor HCT, 40 of whom had failed prior rabbit ATG and CSP. The transplant conditioning regimen varied, but consisted primarily of FLU (150 mg/m^2), CY (either 120 or 200 mg/kg), and alemtuzumab (1 mg/kg total dose). Most patients received unmanipulated BM from HLA-matched unrelated donors and post-transplant immunosuppression consisted of CSP or a combination of CSP with MMF or MTX. The median follow-up was 2.9 years, there was no graft rejection, and the cumulative incidence of acute and chronic GVHD was 38 and 11%, respectively. The 5-year estimated overall survival was 95% [164]. This study demonstrated the efficacy of a rather immunoablative FLU–CY–alemtuzumab conditioning regimen without the need for TBI in children with SAA undergoing unrelated donor HCT.

Similarly to findings from HLA-identical sibling HCT for SAA, bone marrow is the preferred source of stem cells from unrelated donors. A CIBMTR study compared outcomes for patients with SAA who received an HLA-allele matched unrelated donor (matched at HLA-A, -B, -C, and -DRB1) transplant between 2000 and 2008 after a variety of standard conditioning regimens: 225 received unmanipulated BM and 71 patients received G-PBHC. Hematopoietic recovery was similar after G-PBHC and BM transplantation. Grade II–IV acute GVHD was increased after transplantation of G-PBHC compared with BM (48% vs. 31%, Figure 46.4a). Chronic GVHD was not different after adjusting for age at transplantation (Figure 46.4b). Mortality risks, independent of age, were higher after G-PBHC compared with BM transplantation (HR 1.62, $p = 0.04$). The 3-year overall survival was 61 and 76% after G-PBHC and BM transplantation, respectively (Figure 46.4c) [165]. Although this was not a randomized study, the conclusions are strongly in favor of using only unmodified BM and completely avoiding G-PBHC from unrelated donors as the stem-cell source for transplantation of patients with SAA.

Although a fully HLA-allele matched donor is preferable, a recent analysis by the Japan Marrow Donor Program (JMDP) suggests that certain HLA-allele mismatches are acceptable for younger patients with SAA [166]. Reviewing the 380 consecutive unrelated donor bone marrow transplants performed between 1993 and 2005 in Japan, a total of 301 patient and donor pairs were available for HLA-A, -B, -C, -DRB1 and -DQB1 DNA sequence analysis. Based on DNA sequencing for HLA typing completed after the transplant, patients were categorized into five groups: Group 1, complete match at HLA-A, -B, -C, -DRB1 and -DQB1 (33% of patients); Group 2, single allele mismatch at HLA-A or -B (7%); Group 3, single allele mismatch at HLA-C, -DRB1 or -DQB1 (16%); Group 4, multiple alleles mismatched restricted to HLA-C, -DRB1, and -DQB1 (23%); Group 5, all other HLA allele mismatches (21%). The median age at BMT was 17 years, the median disease duration prior to BMT was 43 months, and all patients had failed conventional IST. Various conditioning regimens were used, including predominantly TBI- or LFR-based regimens combined with either CY ± ATG, or with FLU + CY ± ATG. The incidence of neutrophil engraftment at day 42 after BMT was 90% for the entire cohort, and was highest in Group 1 and lowest in Group 5 at 93 and 84%, respectively. The incidence of grade II–IV acute GVHD was lowest in Group 1 and highest in Group 5 at 15 and 38%, respectively. The cumulative incidence of chronic GVHD was 25% in the entire cohort and was not significantly different between the five groups. The 5-year overall survival was 66% for the entire cohort and greatest in Group 1 and lowest in Group 5 at 75 and 47%, respectively. Multivariate analysis indicated that only HLA disparity of Group 5 (multiple HLA-allele mismatches not only restricted to HLA-C, -DRB1, and -DQB1) was a significant risk factor for poor survival (relative risk 1.96, $p = 0.02$). HLA-DPB1 mismatching was not associated with any clinical outcome. The results suggest that the use of an HLA 10 of 10 allele matched donor is preferable. However, if such a donor is not available, any single- or multiple-allele (HLA-C,-DRB1,-DQB1)-mismatched donor is acceptable as an unrelated donor for patients with SAA [166].

Umbilical cord blood (see Chapter 39)

A potential advantage of using umbilical cord blood (UCB) as a source of HSC for unrelated donor transplantation in SAA is that UCB is already stored and promptly available for transplantation and HLA mismatching is better tolerated and therefore may be considered when a sufficiently HLA-matched unrelated donor marrow is not available. Published data for acquired AA UCB transplantation are limited. Furthermore, the low cell dose obtained from a UCB donation poses particular problems in SAA, where an increased HSC dose is important to increase the likelihood of sustained engraftment. Initial reports of cord blood transplantation for SAA were very discouraging because of a very high incidence of graft rejection and transplant-related death [167]. The recent introduction of two-unit UCB transplants as a means of increasing the hematopoietic progenitor cell dose for adult recipients has been successful in achieving a high engraftment rate in patients with high-risk MDS/leukemia. Typically, for each two-unit UCB transplant recipient, only one of the two infused units engrafts long term [168]. However, two-unit UCB transplantation is associated with an increased risk of acute GVHD. Despite the broader introduction of two-unit UCB transplantation, the overall low cell dose of hematopoietic progenitor cells in cryopreserved cord blood units continues to limit the more widespread application of this approach for adults with SAA. Several recent reports support the role of UCB transplant for SAA.

In the setting of UCB transplantation, conditioning regimens containing ATG were associated with worse overall survival, apparently due to delayed immune reconstitution and increased infectious complications. The Japan Cord Blood Bank Network (JCBBN) reported on the initial experience with 31 SAA patients with a median age 28 years who received single-unit UCB transplantation following diverse conditioning regimens. The overall cumulative incidence of neutrophil recovery was 55%, and the incidence of acute and chronic GVHD was 17 and 20%, respectively. Overall survival was 41% at 2 years. One conditioning regimen consisting of low-dose TBI (2–5 Gy), FLU, and CY given to five patients had an overall survival of 80% [169].

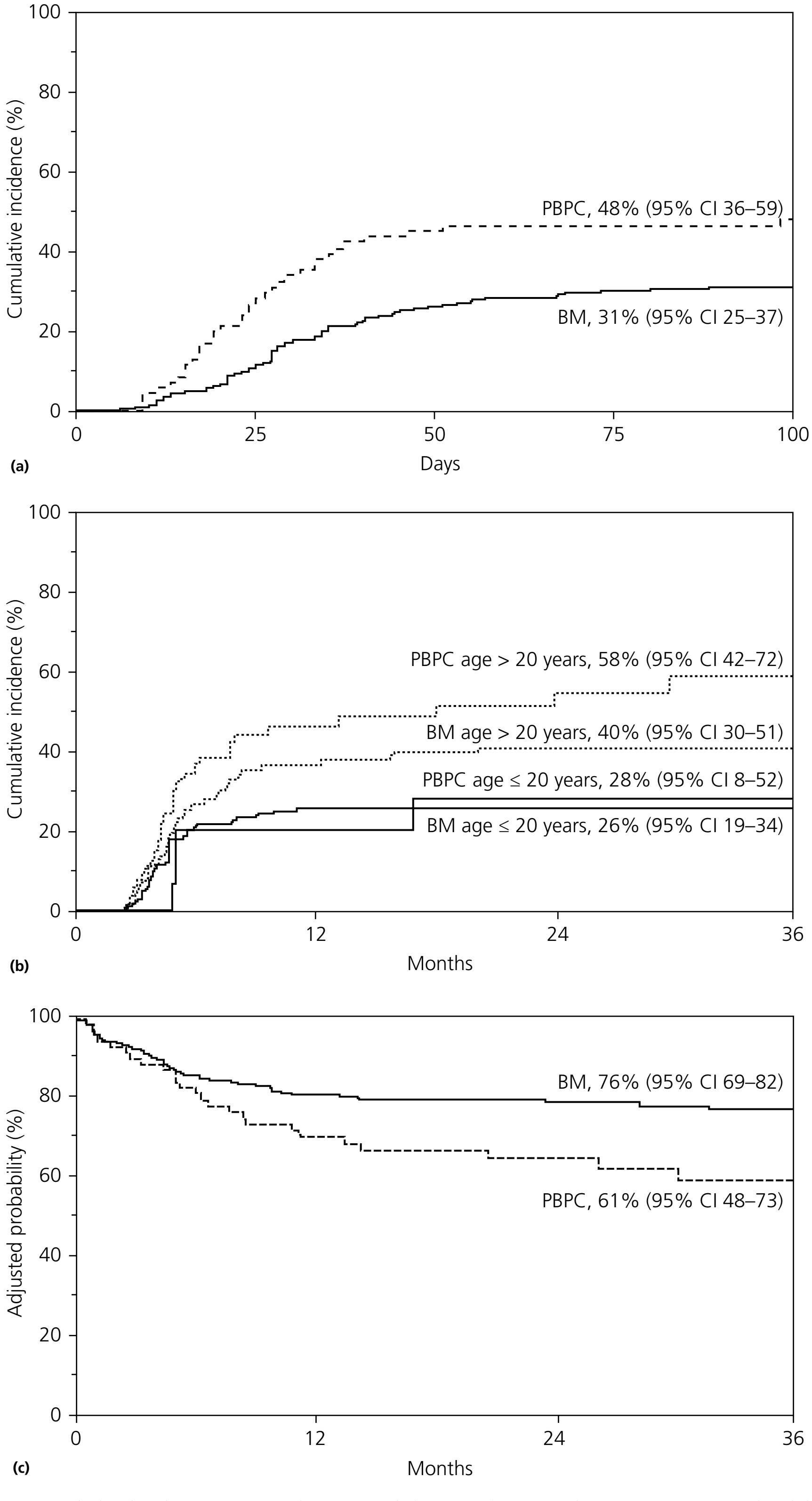


Figure 46.4 Center for International Blood and Marrow Transplant Research (CIBMTR) registry data summarizing unrelated donor hematopoietic cell transplant outcome for patients with severe aplastic anemia. Comparison of patients receiving either bone marrow (BM, *n* = 225) or peripheral blood progenitor cells (PBPC, *n* = 71) from unrelated donors matched at human leukocyte antigen-A, -B, -C, -DRB1. (a) Incidence of grade II–IV acute graft-versus host disease (GVHD). (b) 3-year cumulative incidence of chronic GVHD after PBPC and BM transplantation in patients aged <20 and ≥20 years at transplantation. (c) 3-year probability of overall survival after PBPC and BM transplantation (*p* = 0.04). Source: Eapen et al. 2011 [165]. Reproduced with permission of the American Society of Hematology.

A follow-up Japanese study of 12 SAA patients with a median age of 49 years used a reduced-intensity conditioning regimen consisting of 4 Gy TBI, FLU (125 mg/m^2), and melphalan (MEL) (80 mg/m^2) and single-unit UCB transplantation. The median infused total nucleated cell (TNC) dose was 2.5×10^7/kg. Most patients received tacrolimus/MMF post-grafting immunosuppression; 42% developed grade II acute GVHD. Ten of 12 patients had sustained engraftment; in addition, two patients died of idiopathic pneumonia syndrome. At a median of 36 months after UCB transplantation, 10 of the 12 patients were alive [170].

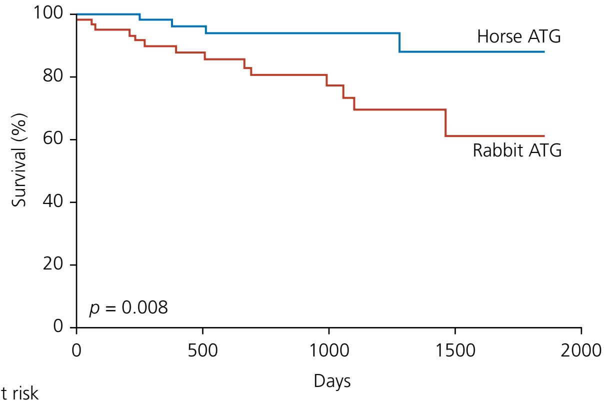
The EBMT group reported on 71 patients with SAA, including nine with PNH, transplanted with a variety of conditioning regimens and UCB between 1996 and 2009 with a median age 13 years. Either single- or double-unit UCB infusion was given to 80 and 20%, respectively, and 68% received a reduced-intensity conditioning regimen. The cumulative incidence of neutrophil recovery was 51% at day 60, with significantly improved engraftment in recipients of units with prefreezing TNC dose $>3.9 \times 10^7$/kg. The incidence of platelet engraftment was 37% at day 180, and the incidence of acute and chronic GVHD was 20 and 18%, respectively. With a median follow-up of 35 months, the 3-year overall survival was 38%. Recipients of UCB units with a prefreezing TNC dose $>3.9 \times 10^7$/kg had a 3-year overall survival of 45% compared with 18% for recipients of $<3.9 \times 10^7$ TNCs/kg. These results emphasize the importance of cell dose for both engraftment and overall survival in patients with SAA undergoing UCB transplantation [171]. In summary, although the outcome of UCB transplantation for SAA is not as favorable as with HLA-matched unrelated donor BMT, for patients who lack a suitably HLA-matched donor, UCB transplantation offers effective salvage therapy for those with refractory SAA who have failed to respond to one or two courses of IST, provided that the infused UCB cell dose is adequate (ideally $>4 \times 10^7$ TNCs/kg). Additional clinical trials are needed to confirm the efficacy of the TBI–FLU–MEL regimen [170] or other reduced-intensity regimens for UCB transplantation.

Non-transplant approaches

The value of immunosuppressive agents for the treatment of AA was first described by Mathé et al. in Paris in 1970 [172]. They observed recovery of autologous hematopoiesis in patients conditioned with antilymphocyte globulin (ALG) administered in preparation for transplants of marrow from HLA-mismatched donors. Later prospective studies showed that the mismatched marrow graft did not contribute to the responsiveness, and subsequently ALG or ATG was used alone to treat patients with AA. More recently, CSP with or without corticosteroids was added to ATG or ALG [173].

For patients who are not candidates for first-line BMT, the combination of ATG and CSP is the currently recommended immunosuppressive regimen for AA. The response rate to IST (as defined by transfusion independence) for patients with severe AA was 60–70% at 3–4 months [174,175]. Long-term follow-up from a German prospective multicenter randomized trial comparing ATG alone with ATG and CSP reported an overall survival of 54 and 58%, respectively, at 11 years. The addition of CSP significantly improved early response to ATG treatment, but because of the efficacy of salvage CSP treatment, there was no overall survival benefit. Failure-free survival, defined as survival in the absence of relapse, no response, PNH, MDS, acute myeloid leukemia (AML), or solid tumors, favored the combined ATG and CSP regimen (39% vs. 24%). Normalization of blood counts occurred in one-third of patients. Risk of relapse was 38%. Remissions were CSP dependent in 26% of patients treated with CSP, necessitating long-term maintenance of the drug. Clonal or malignant diseases developed in 25% of the patients. The actuarial probability of developing hemolytic PNH, MDS/AML, or a solid tumor after IST was 10, 8, and 11%, respectively, at 11 years [175]. The National Institutes of Health (NIH) Hematology Branch retrospectively analyzed risk factors predicting hematologic response to IST. Younger age, absolute reticulocyte count (ARC) $>25 \times 10^9$/L, and absolute lymphocyte count (ALC) $>1 \times 10^9$/L were associated with hematologic response to ATG and CSP at 6 months. Patients with higher ARC and ALC had 83% response versus 41% for patients with lower ARC and ALC [176].

In a randomized study conducted at the NIH, rabbit ATG was demonstrated to be inferior to horse ATG as first-line IST treatment for SAA. A total of 120 consecutive patients with SAA who were not considered candidates for BMT as first-line treatment, 2–77 years of age, were randomly assigned to horse ATG or rabbit ATG (60 in each group). Horse ATG (ATGAM, Pfizer) was administered at a dose of 40 mg/kg/day × 4 days and rabbit ATG (Thymoglobulin, Genzyme) at a dose of 3.5 mg/kg/day × 5 days. CSP was given twice daily from day 1 and continued for at least 6 months in both ATG groups; the dose was adjusted to maintain blood trough levels of 200–400 ng/mL. The two groups were equally matched in patient characteristics and the median follow-up was 28 months. The hematologic response rate at 6 months was 68% with horse ATG and 37% with rabbit ATG ($p < 0.001$). The majority of patients had a response by 3 months, and only four patients in the horse ATG group and two in the rabbit ATG group had a response between 3 and 6 months. The incidence of clonal evolution in all patients at 3 years was 21% in the horse ATG group and 14% in the rabbit ATG group. The overall survival at 3 years was 96% in the horse ATG group and 76% in the rabbit ATG group when data were censored at the time of BMT, and 94 and 70% in the two groups, respectively, when the data were not censored at the time of BMT (Figure 46.5) [177]. Lymphopenia was more protracted in the rabbit ATG recipients, with significantly lower CD4 T cells for a prolonged duration. The results suggest that depletion of CD8 T cells is linked to the



No. at risk				
Horse ATG	60	44	27	12
Rabbit ATG	60	41	22	6

Figure 46.5 Randomized trial conducted at the National Institutes of Health, Bethesda, MD, comparing front-line non-transplant therapy with two antithymocyte globulin (ATG) formulations, horse versus rabbit, combined with cyclosporine (CSP). The 3-year overall survival rate was 94% in the horse ATG group ($n = 60$) versus 70% in the rabbit ATG group ($n = 60$) ($p= 0.008$); the data were not censored at the time of proceeding to hematopoietic cell transplantation. Source: Scheinberg et al. 2011 [177]. Reproduced with permission of the American Society of Hematology.

success of treatment and that the profound loss of CD4 T cells may be detrimental. Although horse ATG is not available in many countries outside the United States, this agent combined with CSP appears to be the most effective first-line IST for SAA [177]. A follow-up report from the EBMT SAA working party also confirmed the finding that rabbit ATG + CSP was significantly less effective than horse ATG + CSP for initial IST for patients with transfusion-dependent, non-severe AA [178].

The question of whether there is a benefit in the addition of G-CSF to ATG and CSP for first-line IST was definitively addressed in a recently completed randomized EBMT study of 192 patients with SAA [179]. For patients randomized to treatment with G-CSF, the drug was started on day 8 of treatment and continued daily until remission or day 240. No difference in overall survival or event-free survival was observed at 6 years between the two groups. Among patients with very severe AA treated with G-CSF versus no G-CSF, there was a reduced rate of early infections, 32% versus 52%, respectively, and duration of hospitalization. Multivariate analysis showed that (1) age at time of enrollment in the study and (2) severity of AA were significantly associated with overall survival.

Furthermore, the use of high-dose G-CSF for prolonged duration with IST has been reported to be a risk factor for the development of MDS/AML with monosomy 7, particularly in children [180]. A European survey included 840 patients who received a first-line IST with (43%) or without (57%) G-CSF. The incidences of MDS/AML in patients who did or did not receive G-CSF were 10.9 and 5.8%, respectively. A significantly higher HR (1.9) of MDS/AML was associated with the use of G-CSF [181].

Alternative treatment to ATG and CSP has also been explored. In a study that extended over 9 years, 10 patients were treated with a high dose of CY 180 mg/kg without marrow rescue, and seven patients responded with increases in peripheral blood counts so that they no longer met the criteria for SAA. Six patients were alive and without relapse or clonal disorders with a follow-up of 21–31 years [182,183]. A more recent follow-up study of 44 patients with treatment-naïve SAA and 23 with refractory SAA received 50 mg/kg/day CY for four consecutive days without CSP but with G-CSF support. The estimated probability of 10-year survival was 88%, the hematologic response rate was 71%, and the event-free survival was 58% for treatment-naïve patients. Patients with refractory SAA fared less well, with 10-year survival, response, and event-free survival of 62, 48 and 27%, respectively. Among responders to CY treatment, the median time to recovery of neutrophil counts (ANC $>0.5 \times 10^9$/L) was 60 days and the median time to independence from transfusion support was 6 months. The primary cause of death was invasive fungal infections, and most infections began within the first 2 months of treatment. For the treatment-naïve and -refractory SAA patients, the incidence of severe invasive fungal infections was 21 and 49%, respectively [183]. However, a randomized trial conducted at the NIH that compared high-dose CY with ATG (both treatment groups included CSP) was terminated early because of excessive infectious mortality in the CY group [184]. In the NIH study, CY induced prolonged pancytopenia, which contributed to the increased risk of infectious deaths. It was concluded that high-dose CY without allogeneic HCT was a dangerous choice for treatment of AA. In a subsequent report of late complications, relapse of disease and clonal evolution of cytogenetic abnormalities occurred in both the ATG and the CY treatment groups [185]. Given these results, high-dose CY alone without subsequent HCT is not recommended as an alternative IST regimen for the treatment of SAA.

The addition of drugs to the standard ATG and CSP regimen has been routinely reported in the literature as novel therapy for SAA. Multiple regimens have been explored as alternatives to ATG and CSP IST without evidence of benefit in response rates or survival [186]. Alemtuzumab monotherapy has poor efficacy for either treatment-naïve or -refractory SAA, but it may have activity when used as second-line IST for treatment of relapsed SAA [187]. Eltrombopag (an oral thrombopoietin mimetic, daily dose up to 150 mg) was studied in 25 patients with SAA that was refractory to ATG and CSP IST. Eleven patients (44%) had a hematologic response in at least one lineage at 12 weeks and only 28% had a prolonged response with platelet transfusion independence and improvement of either red blood cells or neutrophils [188]. These agents have low efficacy but may be helpful as salvage therapy in patients who are not able to proceed to allogeneic HCT.

Comparison of immunosuppressive therapy and hematopoietic cell transplantation

Several reports have compared the effectiveness of conventional IST and HCT. There have been no randomized trials comparing IST with HCT. Recent and earlier studies have consistently shown that there was a clear survival benefit for patients who underwent HLA-matched HCT [9,107,111,116,189–191].

An earlier large Seattle trial compared the outcome of 168 patients treated with BMT and 227 patients receiving IST between 1978 and 1991. In this study, many of the patients treated with BMT already had failed initial IST. Overall survival in this study at 15 years was 69% for the BMT patients and 38% for patients receiving IST ($p < 0.001$) [189]. The higher overall survival with BMT compared with IST was seen in all groups of patients under 40 years of age. In a long-term study of 100 consecutive children under the age of 17 years with SAA, the Nagoya transplant team confirmed that HCT was superior to IST [190].

Similarly to the results from Seattle, an updated retrospective survival analysis was completed based on the initial treatment offered to 2479 consecutive patients with SAA reported to the EBMT registry from 1991 to 2002 [107]. A total of 1567 patients received BMT and 912 patients received IST. The actuarial 10-year survival was 73 and 68% for patients treated with BMT and IST, respectively ($p = 0.002$). Comparing the two periods 1991–1996 and 1997–2002, BMT outcome for all patients improved significantly with time, with overall survival of 69 and 77%, respectively, $p = 0.001$. Survival improved over time for both recipients of matched sibling donor (74 and 80%, $p = 0.003$) and for alternative donor recipients (38 and 65%, $p = 0.0001$) transplants. After BMT, survival in children was 79% compared with 68% in adults ($p < 0.0001$). In multivariate analysis, favorable predictors of BMT outcome were younger age, transplant after 1996, an HLA-identical sibling donor, a brief time interval from diagnosis to transplantation, and no irradiation in the conditioning regimen ($p < 0.001$). In contrast to the outcome with BMT, there was no significant improvement over time for patients receiving IST (69% survival for patients treated from 1991 to 1996 and 73% for patients treated from 1997 to 2002, $p = 0.29$). Survival after IST was significantly better in children than adults (81 and 70%, $p = 0.001$). Overall survival after IST for patients with severe AA was better in children (83%) than adults (62%) ($p = 0.0002$) [107].

One important difference between IST and HCT is that immunosuppression alone may not correct the underlying marrow abnormality. Up to 30% of IST patients develop disease relapse, that is, they have a return of their marrow aplasia, and a substantial proportion may develop clonal abnormalities including MDS and AML [31,174,181,192,193]. In a report of 50 children treated with a combination of G-CSF and CSP, 22% developed MDS or AML [194]. In another study of immunosuppression with ATG and CSP, survival was 72% at 2 years, but 10% of patients had developed PNH [195]. One study compared the incidence of secondary malignancies among patients with HCT versus IST. Forty-two malignancies were noted in 860 patients receiving IST whereas nine were reported in 748 patients who received HCTs. Overall, the 10-year cumulative incidence of cancer was 18.8% after IST and 3.1% after HCT. In this study, MDS and acute leukemia were seen exclusively in patients treated with IST whereas the incidences of solid tumors after IST versus HCT were similar [193]. The relatively high incidence of malignancies after IST was in agreement with earlier work by Tichelli et al. [196], who reported an incidence of PNH, MDS and acute leukemia as high as 57% at 8 years after treatment. Thus, long-term survival curves after IST have as yet not become stable, in contrast to those after HCT.

For some patients, the decision to proceed with BMT versus IST as first-line therapy depends on failure-free survival and quality of life issue analyses. One retrospective study compared quality of life outcomes between 52 transplanted patients and 155 patients receiving IST during 1976–1999. Overall and event-free survival were similar between the two groups. However, quality-adjusted time without symptoms and toxicity analysis showed that, compared with BMT, IST-treated patients had longer periods of time with (a) symptoms from drug toxicity, (b) transfusion dependency, (c) partial remission, and (d) secondary clonal disorders. Transplanted patients spent more time in complete remission without drugs and had longer periods free from symptoms [191].

In most registry studies of IST versus HCT, patients received IST because they lacked an HLA-identical family member donor and were generally not offered unrelated donor transplants as salvage if they failed IST, because at the time of these studies very little had been published about successful unrelated donor HCT for AA. With the recent increased success of unrelated donor HCT, the salvage rate for patients first given a trial of IST may be considerably greater than seen in the past. This result could change the relative benefits of early transplantation from an unrelated donor versus an initial trial of IST.

In summary, over the past four decades, overall survival has improved among patients with AA undergoing allogeneic HCT. There has also been an improvement in survival among patients treated with IST, although the improvement was not as significant as with allogeneic HCT. Based on a review of results to date, for patients up to age 65 years with an HLA-identical sibling, HCT with CY + ATG conditioning and MTX + CSP GVHD prophylaxis provides superior overall survival and should be recommended as first-line therapy over IST. Patients under 20 years of age with a 10/10 HLA-allele matched unrelated donor should proceed directly to HCT with the CY + ATG + 2 Gy TBI conditioning regimen followed by MTX + CSP GVHD prophylaxis. Older patients or patients who do not have an HLA-allele matched unrelated donor should receive first-line IST with CSP + ATG. If there is failure of IST after 3 months, patients should proceed to an unrelated donor HCT.

Conclusion

There are at least two reasons for the improved survival of patients with AA who were treated with HLA-identical HCT. One is the decreased incidence of graft rejection. The decline in rejection has resulted from the more judicious use of transfusions before HCT, the removal of sensitizing white blood cells from transfusion products, the irradiation of blood products before transfusion, and improvements in the immunosuppressive qualities of the conditioning programs used to prepare patients for transplantation. Irradiation-based programs have been effective but at the price of more transplant-related complications, and the CY–ATG combination is just as successful in preventing rejection with better long-term survival. With regard to transfusions before HCT, in vitro irradiation of all blood products may further reduce the risk of sensitization to minor histocompatibility antigens in the future [40,42]. The second reason for improved survival has been a decrease in the incidence and severity of acute GVHD through the introduction of better GVHD prevention regimens, such as combined MTX–CSP. The incidence of chronic GVHD may be decreasing, but mortality from it has not changed much despite prompt therapy. Therefore, better ways of preventing chronic GVHD are necessary, such as targeting the total corrected donor marrow cell dose to 2.0–2.5 × 10^8 cells/kg [59]. As more patients become long-term survivors, the problem of long-term sequelae from the initial conditioning programs and from certain post-grafting immunosuppressive agents such as azathioprine must be considered, in particular secondary cancer. In future studies, perhaps less toxic conditioning programs can be developed, particularly for older patients with medical comorbidities. Radiation-based regimens should not be used in HLA-identical recipients because of the greater likelihood of inducing secondary cancer, the deleterious effect on fertility, and the potential detrimental effects on growth and development for pediatric patients.

For patients without HLA-matched sibling donors, transplantation from HLA-mismatched related donors or unrelated donors should be considered. Prompt initiation of an unrelated donor search is needed after diagnosis of SAA in case there is failure to respond to IST within 3 months. Radiation appears to be an essential part of the currently used conditioning regimen for patients with alternative donors to prevent rejection. Recent results with unrelated donor marrow transplantation indicate that 2 Gy TBI, CY (± FLU) and ATG conditioning and combined MTX and CSP post-grafting immunosuppression were sufficient to prevent graft rejection and avoid TBI-induced organ toxicity [161,163]. The use of molecular-based methods for identifying optimally HLA-allele matched unrelated donors is critical to improve survival after transplant further. Cord blood transplantation is a salvage treatment option for patients who have failed to respond to IST and do not have a suitably HLA-matched unrelated donor, but only if there is a sufficient cell dose available (TNC >4 × 10^7/kg) [171].

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CHAPTER 47

Hematopoietic Cell Transplantation for Paroxysmal Nocturnal Hemoglobinuria

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Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal hematopoietic stem-cell disease disorder that manifests with intermittent hemolysis, thrombotic events, or pancytopenia [1–3]. It is a rare disorder that can occur at any age and across all human populations, with an estimated prevalence of 1–10 per million population [4,5]. Low-frequency PNH clones can be found in a good proportion of patients with aplastic anemia (AA) and may appear in association with other bone marrow failure syndromes, such as myelodysplasia or T-cell large granular lymphocyte (LGL) leukemia [6]. PNH may also develop following immunosuppressive therapy for AA [7,8]. All PNH patients to date have been shown to harbor *PIG-A* mutations; the product of this gene is required in an early step in the synthesis of the glycosylphosphatidylinositol (GPI) anchor. Two GPI-anchored proteins (GPI-APs) that protect red blood cells (RBCs) from complement activity, CD55 (DAF: decay-accelerating factor) and CD59 (MIRL: membrane inhibitor of reactive lysis), are lacking on the surface of PNH RBCs, thereby leaving these cells highly sensitive to complement-mediated destruction. The clinical course of PNH is highly variable. Some patients present with only mild symptoms whereas others have life-threatening complications of thrombosis, hemolysis, or marrow failure with infection from neutropenia or bleeding from thrombocytopenia. The disease often has a waxing and waning course, sometimes over 15 years or longer, and it is difficult to predict when a life-threatening complication will occur [9].

Pathogenesis of PNH

The *PIG-A* gene mutation

Numerous studies have elucidated the molecular basis for PNH. The abnormal PNH clone has a defect in the formation of GPI that is part of the anchor proteins on the cell surface. The defect is due to an acquired somatic mutation in the phosphatidylinositol glycan-A (PIG-A) gene of the hematopoietic stem cell [10–12]. The *PIG-A* gene product is required for the first step in the synthesis of GPI-APs [13]; therefore, *PIG-A* mutations block the biosynthesis of the GPI anchor, as shown in the left and central diagrams in Figure 47.1.

Since *PIG-A* is on the X chromosome and males and females have only one active X chromosome per cell (in females, one X chromosome is inactivated through lionization), a single *PIG-A* mutation can generate a PNH phenotype. All other genes involved in GPI anchor biosynthesis are found on autosomes; hence inactivating mutations in these genes would have to occur on both alleles in the same cell, which is highly unlikely. A wide range of somatic mutations throughout the entire coding region of the *PIG-A* gene have been described in PNH patients. There are no true mutational “hotspots,” although exon 2, which contains almost half of the coding region, is the exon where most mutations occur. Most *PIG-A* mutations are small insertions or deletions that result in a frameshift in the coding region and consequently a shortened, non-functional product (reviewed in [14,15]).

PNH clonal evolution

Although the identification of mutations in the *PIG-A* gene has provided a direct explanation of the PNH phenotype in red cells and in other blood cells, it does not explain why and how a *PIG-A* mutant clone expands. To explain this essential component of the pathogenesis of PNH, the following models have been proposed and are supported by experimental or observational studies:

1. *Immune evasion:* PNH cells may evade immune attack, possibly because of a missing cell surface GPI-AP that is the target of the immune attack [16]. This model is attractive as it can explain the close association between AA and PNH; however, no GPI-AP has been shown to be the immunologic target in AA or PNH. Furthermore, GPI-APs are translated in PNH cells but are degraded within the cells in the absence of the GPI anchor [17]; thus, protein epitopes can still be displayed in the context of human leukocyte antigen (HLA) and be immunogenic.
2. *Conditional growth advantage:* The *PIG-A* mutation may confer an intrinsic resistance to apoptosis [18] that becomes more apparent when in the immunologic environment. This also explains the link between AA and PNH. No clear survival advantage has been found under a variety of conditions in the mouse model of PNH [19], but several studies have found that primary GPI-AP-deficient myeloid cells from PNH patients are relatively resistant to apoptosis [18,20–23].
3. *Mutation-driven growth advantage:* A second anti-apoptotic mutation may occur in the PNH clone [24]. Support for this hypothesis is derived from a report of two patients with PNH in whom an acquired rearrangement of chromosome 12 produced ectopic expression of the *HMGA2* gene. However, as argued elsewhere, it is unlikely that a second mutation in the same HSC explains clonal expansion of GPI$^-$ cells in the majority of patients with PNH [25,26].

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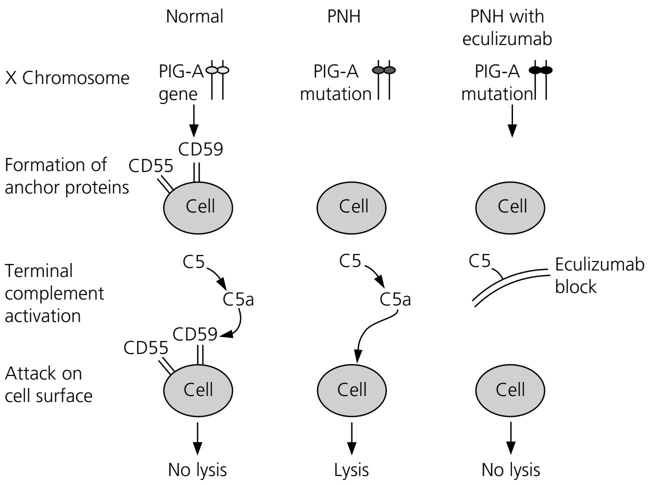

Figure 47.1 Pathway showing the formation of anchor proteins that protect cells from lysis by activated complement in normal individuals, subjects with paroxysmal nocturnal hemoglobinuria (PNH), and the effect of eculizumab to prevent activation of C5 to C5a. C5, fifth component of complement; C5a, activated fifth component of complement; CD55, CD59, anchor proteins; PIG-A, phosphatidylinositol glycan-A gene.

Clinical manifestations

Classifications

The International PNH Interest Group proposed a working classification of PNH as follows [27]:

1 *Classic PNH:* clinical evidence of intravascular hemolysis, no evidence of another defined bone marrow abnormality, a cellular marrow with erythroid hyperplasia and normal/near-normal morphology, without non-random cytogenetic abnormalities.
2 *PNH in the setting of another specific bone marrow disorder:* clinical and laboratory evidence of hemolysis, but also have concomitantly, or have had a history of, a defined underlying marrow abnormality [e.g., AA, myelodysplastic syndrome (MDS)].
3 *Subclinical PNH (PNH-sc):* no clinical or laboratory evidence of hemolysis. Small populations of GPI-AP-deficient hematopoietic cells are detected by very sensitive flow cytometric analysis. PNH-sc is observed in association with bone marrow failure syndromes (e.g., AA, MDS).

Hemolysis

The absence of CD59 (membrane inhibitor of reactive lysis) from the surface of PNH erythrocytes leads to intravascular hemolysis. CD59 blocks the aggregation of C9 into the C5b-8 complex, thereby effectively preventing the formation of the intact membrane attack complex (MAC) [28]. Hemolysis in PNH is chronic, but can be associated with episodic hemolysis (paroxysms) resulting from increases in complement activation due to infections, surgery, strenuous activity, and excessive alcohol intake. PNH patients also experience extravascular hemolysis due to absence of CD55 (DAF) on the surface of RBCs. CD55 [29] functions to accelerate the rate of destruction of membrane-bound C3 convertase. Extravascular hemolysis tends to be less evident in most PNH patients since CD59 deficiency leads more rapid intravascular RBC destruction [30]. However, treatment of PNH patients with the terminal complement inhibitor eculizumab can make the extravascular hemolysis of PNH more conspicuous [31].

Nitric oxide scavenging

Nitric oxide is synthesized in the endothelium and functions to maintain proper vascular and muscle tones and to limit platelet activation. In PNH, intravascular hemolysis generates a large amount of free hemoglobin in serum which serves as a potent scavenger of nitric oxide. The release of erythrocyte arginase into serum during intravascular hemolysis further decreases the substrate (arginine) for nitric oxide synthesis. The resulting deprivation of nitric oxide from tissues is thought to be responsible for a wide range of systemic symptoms in PNH patients, including generalized fatigue, pain, esophageal spasm, male erectile dysfunction, renal impairment, and possibly thrombosis[32].

Thrombophilia

Thrombosis in PNH patients is one of the life-threatening complications, and means to predict which patients are at high risk for thrombosis are needed. Patients with clinical symptoms of abdominal pain, hemoglobinuria, esophageal spasm, and impotence are more likely to have thrombotic events [33]. Geographic differences in the incidence of thrombosis have been observed. Latin Americans and African- Americans are at higher risk than Europeans and other groups. Ethnicity, therefore, is a risk factor for thrombosis in PNH [34]. The pathogenesis of thrombotic events associated with PNH is not well understood.

Complement-mediated hemolysis, impairment of the fibrinolytic system, platelet activation, and leukocyte-derived tissue factors are thought to be responsible [35–37]. Nitric oxide depletion has been associated with increased platelet aggregation, adhesion, and accelerated clot formation. PNH platelets undergo exocytosis of the complement attack complex to repair complement-mediated damage, which results in the formation of microvesicles with phosphatidylserine externalization, a potent in vitro procoagulant. Activation of complement cascade and subsequent generation of C5a significantly upregulates expression of tissue factor (TF), a key initiating component of coagulation cascade [38]. Fibrinolysis may also be compromised in PNH as the PNH blood cells lack the GPI-anchored urokinase receptor. In addition, tissue factor pathway inhibitor (TFPI) has been shown to require a GPI-anchored chaperone protein for trafficking to the endothelial cell surface [39].

The propensity for thrombosis is related to the size of the PNH clone. Among 49 patients with a PNH clone measured in granulocytes, thrombosis occurred in 12 of 22 patients who had more than 61% PNH granulocytes, whereas none of the remaining 27 patients with less than 61% PNH granulocytes had thromboses [33]. Such markers of increased risk of thrombosis in the blood can be of value clinically in identifying which patients are at risk and who could be candidates for anticoagulation and possibly early transplantation.

Association with AA, MDS

An expanded PNH clone is also found in up to 70% of patients with acquired AA, demonstrating a pathophysiologic link between these disorders [40–42]. In contrast to patients with classic PNH, these patients typically have a lower percentage of PNH cells. Acquired AA is an autoimmune disorder, where the target of the immune attack is primitive $CD34^+$ bone marrow progenitors [43,44]. Typically, fewer than 10% of GPI-AP-deficient granulocytes are detected in AA patients at diagnosis, but occasional patients may have larger clones [40]. DNA sequencing of the GPI-AP-deficient cells from AA patients reveals clonal *PIG-A* gene mutations [45]. Although most AA patients exhibit no signs or symptoms of PNH early in the natural history of their disease when the PNH clone size is small, many, but not all, will experience further expansion of the *PIG-A* mutant clone and progress to classic PNH. GPI-AP-deficient cells have also been reported in patients with MDS [40,45]. Patients with MDS who are reported to have small PNH populations tend to be classified as having refractory anemia and often have the following characteristics: a hypocellular marrow, HLA-DR15 positivity, normal cytogenetics, moderate to severe thrombocytopenia, and a high likelihood of response to immunosuppressive therapy [45,46].

Natural history and prognosis

The issues that confound recommendations for transplantation for PNH center both on the heterogeneous presentation and natural history and on its close association with marrow failure syndromes. In a study by Hillmen et al., a group of 80 consecutive patients with PNH in the United Kingdom was followed and it was found that the median survival after diagnosis was 10 years, with 22 patients (28%) surviving for 25 years [3]. Sixty patients have died; 28 of the 48 patients for whom the cause of death is known died from either venous thrombosis or hemorrhage. Thirty-one patients (39%) had one or more episodes of venous thrombosis during their illness. Of the 35 patients who survived for 10 years or more, 12 had a spontaneous clinical recovery. A study of 220 patients with PNH from the registry of the French Society of Hematology [47] showed a median survival of 12 years for the entire cohort, and identified risk factors associated with a worse outcome: (1) age older than 55 years; relative risk (RR) of death 4.0; (2) occurrence of thrombosis (regardless of location; RR 10.2); (3) progression to pancytopenia (RR 5.5); (4) transformation to MDS or acute leukemia (RR 19.1); and (5) thrombocytopenia at diagnosis (RR 2.2).

The French group recently analyzed an expanded cohort of 460 PNH patients diagnosed in 58 French hematologic centers from 1950 to 2005 [48], aiming to study the natural history of PNH clinical subcategories (classic PNH and AA-PNH syndrome). After a median follow-up time of 6.8 years, the median survival time was 22 [standard error (SE) 2.5] years. The study identified 113 patients with classic PNH, 224 patients with AA-PNH syndrome, and 93 (22%) intermediate patients who did not fit within these two categories. At presentation, classic PNH patients were older, with more frequent abdominal pain, and displayed higher levels of GPI-AP-deficient granulocytes. A time-dependent improved survival was observed. In classic PNH, diagnoses before 1986 [hazard ratio (HR) 3.6, $p = 0.01$] and increasing age ($p < 0.001$) were associated with worse survival prognoses, whereas use of androgens within the first year after diagnosis was protective (HR 0.17, $p = 0.01$). Transfusions before 1996 (HR 2.7, $p = 0.007$) led to lower survival rates in patients with AA-PNH syndrome, whereas immunosuppressive treatment was associated with better outcomes (HR 0.33, $p = 0.03$). Evolution to thrombosis affected survival in both subcategories (classic PNH, HR 7.8, $p < 0.001$; AA-PNH syndrome, HR 33.0, $p < 0.001$). Evolution to bicytopenia or pancytopenia for classic PNH (HR 7.3, $p < 0.001$) and malignancies for AA-PNH syndrome (HR 48.8, $p < 0.001$) were associated with worse outcomes. It was concluded that although the clinical presentation and prognosis factors are different, classic PNH and AA-PNH syndrome present roughly similar outcomes, affected mainly by complications.

Diagnosis

A diagnosis of PNH should be considered when a patient presents with non-autoimmune hemolytic anemia or AA. It should also be included in the differential diagnosis of individuals presenting with a life-threatening thrombosis or with recurrent thromboses (Table 47.1). The time-honored tests to diagnose patients in whom PNH is suspected have been the sugar water hemolysis and Ham's tests. However, these tests are positive only when abnormal cells remain in circulation after a major hemolytic episode.

Table 47.1 Clinical and laboratory characteristics of paroxysmal nocturnal hemoglobinuria (PNH), AA, and myelodysplasia

	Normal control	PNH	Aplastic anemia	Myelodysplasia
Clinical features				
Aplastic/cytopenia	–	+	+	+/–
Non-autoimmune hemolytic anemia	–	+	–	–
Thrombosis	–	+	–	–
Laboratory findings				
Ham's test on RBCs	–	+	+/–	–
Flow cytometry detection of CD55 and CD59 on RBCs and leukocytes	+	–	+/–	+
Aerolysin detection of PNH type II and III cells	–	+	+/–	–

The recognition of the abnormalities of expression of other anchor proteins including CD14 and CD55 on affected monocytes, the expression of anchor proteins CD16, CD24, CD59, and CD66b on neutrophils, and that of CD24 and CD59 on lymphocytes makes it possible to establish the diagnosis by flow cytometry of cells that remain in circulation [49,50]. In contrast to GPI-AP-deficient RBCs, the life span of PNH granulocytes is normal. Therefore, the proportion of abnormal granulocytes more accurately reflects the PNH clone size and is not affected by RBC transfusions. For initial evaluation, quantitation of at least two GPI-anchored proteins is recommended to exclude the possibility that the clinical process is a consequence of an inherited or acquired isolated deficiency of a single GPI-AP.

More sensitive tests have been developed that raise questions about the relationship between the two different diseases [51]. Aerolysin is a toxin that binds to cells via the anchor proteins encoded by the *PIG-A* gene. Since PNH red cells cannot bind the toxin, they survive in an aerolysin-based assay [fluorescent aerolysin (FLAER) assay]. Using this assay, residual PNH cells can be detected in samples from PNH patients but not in cells from patients with myelodysplasia or in normal control samples. However, as many as 60% of the cells obtained from patients who have idiopathic AA and not PNH by flow cytometry before treatment with the aerolysin toxin show characteristics of PNH after treatment [51,52] (Table 47.1). This sensitive test indicates that previously undetectable PNH cells of different lineages circulate in patients with untreated AA, and points to the possibility of clonality in the early stages of AA.

Non-transplant treatment of PNH

There are no widely accepted evidence-based indications for treatment of PNH. In classic PNH, eculizumab should be considered for patients with disabling fatigue, thromboses, transfusion dependence, frequent pain paroxysms, renal insufficiency, or other end-organ complications from disease. Watchful waiting is appropriate for asymptomatic patients or those with mild symptoms. In patients with AA-PNH, therapy should be directed towards the underlying bone marrow failure; patients who meet criteria for severe AA should be managed with either allogeneic bone marrow transplantation (BMT) or immunosuppressive therapy depending on the age of the patient and the availability of a suitable HLA-matched sibling donor. Immunosuppressive therapy using antithymocyte globulin (ATG)/cyclosporine does not eradicate the abnormal PNH clone, and patients may continue to be symptomatic from the disease [53–56]. In a small number of patients, high-dose cyclophosphamide (CY) without marrow infusion has resulted in autologous marrow recovery without persistence of the PNH clone [57].

Eculizumab

By far the most significant advance in non-transplant therapy for PNH is the recent development of eculizumab, a humanized monoclonal antibody against the complement protein C5, thereby blocking terminal complement activation as shown in the right-hand diagram in Figure 47.1. Eculizumab prevents hemolysis in patients with PNH. In a randomized placebo-controlled, multicenter phase III trial, 21 of 43 patients assigned to eculizumab achieved stabilization of hemoglobin levels and transfusion independence, whereas none of 44 patients assigned to placebo achieved those outcomes. Eculizumab reduced intravascular hemolysis by 86% and was associated with improved quality of life measures [58]. After treatment, serum LDH concentration returns to normal or near normal, with approximately half to two-thirds of patients achieving transfusion independence; however, mild to moderate anemia, hyperbilirubinemia, and reticulocytosis persist in essentially all treated patients.

Accumulating data also suggest that eculizumab may protect against other complications of hemolytic PNH, such as deteriorating renal function, pulmonary hypertension, and thromboembolism [59–61]. Treatment with eculizumab appears to have a favorable impact on survival, because a recent study of 79 patients treated between 2002 and 2010 showed the same survival rates as those of age- and gender-matched controls from the general population [48,62]. However, the impact of eculizumab on survival cannot be accurately quantified, owing to the lack of a control patient group in that study.

There are, however, limitations in the treatment with eculizumab; it is expensive (~$400,000/year in the United States) and has no effect on either the underlying stem-cell abnormality or on the associated BM failure. Consequently, treatment must continue indefinitely and leukopenia, thrombocytopenia, and reticulocytopenia, if present, persist. Suboptimal response to treatment with eculizumab may occur as a result of breakthrough intravascular hemolysis (i.e., increases in LDH and development of constitutional symptoms) near the end of a treatment cycle, inappropriately low erythropoietin levels, decreased iron stores, and/or extravascular hemolysis as C3 was found bound to a portion of the PNH erythrocytes (i.e., the CD59-deficient population after treatment with eculizumab) [30].

Corticosteroids

Corticosteroids as treatment for both chronic hemolysis and acute hemolytic exacerbations is a subject of debate. Nonetheless, some patients appear to respond rapidly and dramatically to glucocorticoids (given in the dosage range 0.25–1.0 mg/kg/day of prednisone). The rapid response (often within 24 hours of initiating therapy) suggests that complement inhibition accounts for the antihemolytic activity of glucocorticoid therapy. Such an effect could be direct (the result of inhibition of the activity of some component of the alternative pathway of complement) or indirect (the result of damping a process, such as inflammation, that stimulates activation of complement). Hence the main value of corticosteroids may be in attenuating acute hemolytic exacerbations. Under these circumstances, brief pulses of prednisone may reduce the severity and duration of the crisis while avoiding the untoward consequences associated with long-term use.

Androgens

Androgen therapy, either alone or in combination with steroids, has been used successfully to treat the anemia of PNH. As with glucocorticoids, the mechanism by which androgenic steroids ameliorate the anemia of PNH is not fully understood, although the rapid onset of action is consistent with complement inhibition [63].

Iron replacement

Patients with PNH frequently become deficient in iron as a result of both hemoglobinuria and hemosiderinuria. Clinically important iron loss from hemosiderinuria can occur even in the absence of gross hemoglobinuria. Replacement is often associated with exacerbation of hemolysis, regardless of the route of administration [63,64]. Compared with parenteral replacement, oral administration of iron may be accompanied by less severe hemolytic exacerbations, but urinary iron loss may be so great that repletion cannot be achieved through this mechanism. If a hemolytic exacerbation occurs in the setting of iron repletion, the episode can be controlled by treatment with corticosteroids or androgens or by suppression of erythropoiesis by transfusion.

Transfusion

In addition to increasing the hemoglobin concentration, transfusion may ameliorate hemolysis by suppressing erythropoiesis. Concerns about inducing a hemolytic exacerbation as a consequence of infusion of small amounts of donor plasma that may contaminate RBC preparations appear unwarranted [65]. However, hemofiltration is recommended to prevent transfusion reaction arising from the interaction between donor leukocytes and recipient antibodies. Transfusion-related iron overload from chronic transfusion may be delayed in patients with PNH due to iron loss from hemoglobinuria/hemosiderinuria [64]. However, iron overload remains a concern in patients who require chronic transfusion when the anemia is primarily a consequence of marrow failure rather than hemolysis.

Transplantation for PNH

Before the availability of eculizumab, the primary indications for transplantation for PNH were bone failure, recurrent life-threatening thrombosis, and uncontrollable hemolysis. The last process can be eliminated by treatment with eculizumab and the thrombophilia of PNH may also respond to inhibition of intravascular hemolysis by eculizumab [59]. Nonetheless, transplantation is the only curative therapy for PNH, and the availability of molecularly defined, matched unrelated donors, less toxic conditioning regimens, reductions in transplantation-related morbidity and mortality, and improvements in post-transplantation supportive care make this option a viable alternative to medical management. The decision as to who should receive a transplantation and when it should be performed is complex.

Hematopoietic cell transplantation (HCT) from an HLA-identical sibling for a patient with PNH was first reported in 1973 [66]. This patient was found to have PNH at the time of presentation of severe AA and had a positive sucrose hemolysis or Ham's test. In subsequent reports, most patients had life-threatening disease from

Table 47.2 Published reports of transplantation for paroxysmal nocturnal hemoglobinuria (PNH)

Study	No of patients	Type of donor and No. of each	Conditioning regimen	PNH after transplant
Storb [66]	1	Allogeneic 1	Yes	No
Fefer [67]	1	Syngeneic 1	No	Yes
Kolb [68]	2	Allogeneic 1, syngeneic 1	Yes, no	No, yes
Antin [69]	4	Allogeneic 4	Yes	No
Szer [70]	4	Allogeneic 3, syngeneic 1	Yes, no	No, yes
Kawahara[a] [71]	9	Allogeneic 7, syngeneic 2	Yes, no	No[b], yes
Saso [72]	57	Allogeneic 55, syngeneic 2	Yes, no/yes	No, yes
Bemba [73]	16	Allogeneic 16	Yes	No
Raiola [74]	7	Allogeneic 7	Yes	No
Graham [75]	1	Syngeneic 1	Yes	No
Hershko [76]	1	Syngeneic 1	No	Yes
Endo [77]	1	Syngeneic 1	No	Yes
Woodard [78]	3	Allogeneic 3	Yes	No
Suenaga [79]	1	Allogeneic	Yes – RI	No
Markiewicz [80]	2	Allogeneic	Yes	No
Van den Heuvel-Eibrink [81]	5	Allogeneic	Yes	No
Lee [82]	1	Allogeneic	Yes – RI	No
Ditschkowski [83]	1	Allogeneic	Yes	No
Lee [84]	5	Allogeneic	Yes – RI	No
Hegenbart [85]	7	Allogeneic	Yes – RI	No
Flotho [86]	2	Allogeneic	Yes	No
Takahashi [87]	5	Allogeneic	Yes – RI	No
Grosskreutz [88]	1	Allogeneic	Yes – RI	No

RI, reduced-intensity conditioning regimen.

[a] Ref. [71] contains cases previously reported in [66,67,70].

[b] One allogeneic recipient (patient 8; Table 47.3) had a return of the PNH clone 2.7 years after transplantation and is asymptomatic.

marrow aplasia, and hemolysis played a less important role at the time of HCT [67–70]. Subsequently, patients with hemolysis or thrombosis were evaluated for transplantation. Overall, the number of individuals who have been transplanted is small (Table 47.2).

The experience in Seattle with transplantation for nine patients was summarized in 1992 [71]. Subsequently, 19 additional patients have been transplanted, and the results are updated in Table 47.3. Among the total of 28 patients, 12 presented with the severe AA phase of PNH and had severely hypocellular marrows. The remaining 16 patients presented with hemolysis or thrombosis and had moderately cellular to hypercellular marrows. One of these (patient 28) also had documented thromboses associated with the presence of the factor V Leiden abnormality. Data from Table 47.3 are summarized in Table 47.4.

Conditioning for grafting of the patients with AA who had HLA-identical sibling donors consisted of 200 mg/kg CY with or without ATG in five patients, and 16 mg/kg busulfan (BU) with 60 mg/kg CY in two patients. One of these patients with AA received two grafts from the same donor. The patient with AA and the haploidentical parental graft was conditioned with 120 mg/kg CY and 1200 cGy total body irradiation (TBI). The syngeneic recipient with AA received an infusion of marrow without conditioning. One patient with AA received an unrelated umbilical cord blood HCT after myeloablative conditioning with ATG, BU, and 1350 cGy TBI. Two patients received matched unrelated peripheral blood stem cells following conditioning with fludarabine 90 mg/m^2 body surface area and 200 cGy TBI.

The conditioning regimens for the HLA-identical siblings with thrombotic or hemolytic presentations of PNH consisted of BU CY plus ATG in four patients and BU CY in three patients. One patient with a history of idiopathic chronic myelofibrosis, hemolysis, and pancytopenia received an HLA-identical sibling peripheral blood stem-cell graft after BU, fludarabine 120 mg/m^2 body surface area, and ATG. The recipients of the parental donor graft and the child donor graft received BU CY plus ATG, or BU CY, respectively. The mismatched sibling received BU CY and ATG. Among three recipients of unrelated donor marrow, two were conditioned with BU CY and ATG, and one with BU CY. One patient received CY, ATG, and 1350 cGy TBI for an unrelated umbilical cord blood graft. The syngeneic marrow recipient did not receive conditioning.

Fifteen of the 28 patients developed acute graft-versus-host disease (GVHD) (10 grade II, three grade III, and two grade IV), and 10 subsequently developed clinically extensive chronic GVHD. PNH recurred 9–17 years after transplant in both syngeneic recipients and 2.7 years after transplantation in one allogeneic recipient [71]. Fourteen patients died. The causes of death included GVHD complicated by infection in two patients, fungal pneumonia in two patients, and a gastrointestinal bleed in one patient. The last patient had thromboses of the portal and splenic veins from PNH, which led to bleeding varices. One patient rejected the haploidentical parental graft and died of pulmonary hemorrhage shortly after a second transplant from another haploidentical family member. One patient died of a post-transplant lymphoproliferative disorder 169 days after an unrelated umbilical cord blood transplant. One died from human immunodeficiency virus (HIV) infection 9.3 years after transplantation. One died of hepatitis C 14.6 years after transplant, and one syngeneic recipient died 25.2 years after transplantation as a result of complications of a liver transplant for hepatitis C. One developed myelodysplasia in the donor cells 26.9 years after transplant and had a second successful myeloablative transplant from a different HLA-identical sibling following BU/CY, but committed suicide about 1 year later, 28.5 years after the first transplant. One each died of probable cerebral hemorrhage, myocardial infarction, and respiratory failure of unknown cause 176 days, 1.7 years, and 4.1 years, respectively, after HCT. Fourteen patients are surviving more than 1–35 (median 7.6) years after transplantation. Six of these individuals are well, and eight have clinically extensive chronic GVHD.

These results show that patients with the AA presentation of PNH do well; none of the eight AA patients who received HLA-identical sibling or syngeneic grafts died from the complications of transplantation (Figure 47.2). One of these died later from HIV infection before the era of blood screening for HIV. In contrast, eight of the 16 patients who received a transplant from any type of donor for the hemolytic or thrombotic presentations of PNH died. Five of these patients died within 120 days after transplantation of complications from GVHD with non-fungal infection, fungal infection, or variceal bleeding. Three died later, one each of hepatitis C, post-transplant lymphoproliferative disorder, and respiratory failure presumably related to chronic GVHD.

The International Bone Marrow Transplant Registry has reported the results of 57 patients with PNH who were transplanted at 31 different centers [72]. Thirty-nine of these patients presented with PNH as the only diagnosis. Sixteen evolved from PNH into an AA presentation by the time of HCT, and two developed PNH syndrome after initially presenting with AA. The donors were HLA-identical siblings in 48 cases, syngeneic donors in two cases, a haploidentical family member in one case, and HLA phenotypically identical, unrelated donors in six cases. These patients received conditioning consisting of BU/CY for 30 patients, CY/TBI for 12,

Table 47.3 Patients in the Seattle transplant program

Patient No.	Age (years)	HCT donor	Paroxysmal nocturnal hemoglobinuria presentation	Conditioning regimen	GVHD acute grade/chronic	Status	Survival post-transplant at last contact	Cause of death
1	23	HLA ID SIB	A	CY	0/No	A	>35.0 years	
2	19	Syngeneic	A	None	0/No	D	25.2 years	Hepatitis C
3	14	HLA ID SIB	A	PA PA PA BC CY BU CY	II/Limited	D	28.5 years	Suicide
4	16	HLA ID SIB	A	CY	0/No	A	>27.7 years	
5	29	Syngeneic	H	None	0/0	A	>25.2 years	
6	38	HLA ID SIB	A	BC CY	II/Clin Ext	D	9.3 years	HIV
7	20	Haplo parent/ haplo SIB	A	CY TBI ATG BC CY	No/NA	D	39 days	Pulmonary hemorrhage
8	37	HLA ID SIB	T	BU CY	0/Clin Ext	A	>16.6 years	
9	22	HLA ID SIB	H	BU CY	II/No	D	14.6 years	Hepatitis C
10	25	HLA ID SIB	H	BU CY	II/NA	D	105 days	Fungal pneumonia
11	32	HLA ID SIB	H	ATG BU CY	IV/Clin Ext	D	83 days	GVHD infection
12	29	Matched Unrel	H	ATG BU CY	IV/NA	D	58 days	GVHD infection
13	33	1Ag MM parent	T, H	ATG BU CY	III/NA	D	81 days	Fungal pneumonia
14	21	HLA ID SIB	A	ATG BU CY	II/No	A	>10.9 years	
15	42	HLA ID SIB	T	ATG BU CY	0/NA	D	11 days	Bleeding varices
16	39	HLA ID SIB	H	ATG BU CY	0/Subclin	A	>5.0 years	
17	41	1Ag MM child	H	BU CY	II/Clin Ext	A	>8.1 years	
18	19	MM SIB	H	ATG BU CY	III/Clin Ext	D	4.1 years	Respiratory failure
19	21	HLA ID SIB	H	ATG BU CY	0/Sub Clin	A	>7.0 years	
20	35	Unrel cord blood	T	ATG CY TBI 1350	III/Clin Ext	D	169 days	Post-transplant lymphoproliferative disorder
21	36	Matched Unrel	T	ATG BU CY	II/Clin Ext	A	>6.5 years	
22	33	HLA ID SIB	A	ATG CY	II/Limited	A	>6.1 years	
23	18	HLA ID SIB	A	ATG BU CY	III/Clin Ext	A	>6.1 years	
24	32	Matched Unrel	H	BU CY	0/0	A	>1.0 years	
25	14	Unrel cord blood	A	ATG CY TBI 1350	II/Clin Ext	D	1.7 years	Myocardial infarction
26	67	Matched Unrel PBSC	A	FLU TBI 200	II/Clin Ext	A	>1.0 yrs	
27	41	HLA ID SIB PBSC	H, A, ICMF	BU FLU ATG	II/0	A	>1.6 yrs	
28	31	Matched Unrel PBSC	A	FLU TBI 200	0/0	D	176 days	Possible cerebral hemorrhage

GVHD, graft-versus-host disease.

HCT donor: 1Ag, 1 antigen; haplo, haploidentical; HLA ID SIB, human leukocyte antigen-identical sibling; MM, mismatched; PBSC, peripheral blood stem cells; Unrel, unrelated.

Paroxysmal nocturnal hemoglobinuria presentation: A, AA; H, hemolysis; ICMF, idiopathic chronic myelofibrosis; T, thrombosis.

Conditioning regimen: ATG, antithymocyte globulin; BC, donor buffy coat; BU, busulfan; CY, cyclophosphamide; FLU, fludarabine; PA, procarbazine + ATG; TBI 1350, 1350 cGy total body irradiation; TBI 200, 200 cGy total body irradiation.

GVHD acute grade/chronic: Clin Ext, clinical extensive; NA, not applicable; Subclin, subclinical.

Status: A, alive; D, dead.

limited field irradiation and CY for 11, and CY alone for three patients. One identical twin recipient did not receive any pretransplant conditioning. GVHD prophylaxis consisted of methotrexate or cyclosporine plus methotrexate in 39 patients, and cyclosporine plus corticosteroids in 11 patients. Six patients received T-cell-depleted grafts, and one identical twin recipient did not receive prophylaxis.

Graft failure occurred in 11 of 48 patients evaluable for engraftment. Of those with engraftment, 34% developed grade II or greater acute GVHD, and 33% of patients surviving beyond 90 days developed chronic GVHD. Overall, 30 of the 57 patients survived. Among those 48 patients receiving HLA-identical sibling grafts, 27 (56%) survived compared with one survivor among seven patients (14%) receiving HLA-non-identical family member or unrelated donor grafts. Six patients in three reports had matched sibling donors for hemolytic or aplastic presentation, and five survived transplantation with myeloablative conditioning regimens without PNH recurrence [81–83].

One report described three patients with the AA presentation of PNH given unrelated donor bone marrow grafts following T-cell depletion of marrow. The post-transplant course was complicated by an Epstein–Barr virus-positive lymphoproliferative disorder that responded to cytotoxic T-cell therapy specific for this virus. Another patient had venocclusive disease and another severe hemolytic uremic syndrome and hemorrhagic cystitis. However, they all recovered and were alive without PNH 2.5–5.1 years after transplantation [78]. Three reports describe seven unrelated donor transplant recipients with myeloablative conditioning regimens for aplastic or hemolytic presentations of PNH, five of whom survived without subsequent recurrence of their disease [80,81,86].

These data suggest that survival after HCT for the aplastic phase of PNH using HLA-identical sibling donors is fairly good. However, transplantation is successful in only about half of the cases with non-aplastic presentations of PNH using HLA-identical sibling donors. Results using partially matched family member donors are poor. Finally, although the results using matched unrelated donors are encouraging, the reports are few.

A recent study by the European Group for Blood and Marrow Transplantation (EBMT) evaluated the outcome and risk factors affecting survival after allogeneic HCT in the largest cohort ever studied of 211 PNH patients [89]. They also conducted a formal survival comparison of this cohort of transplanted PNH patients

Table 47.4 Summary of patients in the Seattle transplant program ($n = 28$): donor type, conditioning regimen, and survival

Aplastic anemia presentation	12
HLA identical sibling	
CY ± ATG	5
BU CY	2
Haploidentical family	
TBI 1200 CY	1
Unrelated umbilical cord	
TBI 1350 BU ATG	1
Unrelated donor reduced intensity	
TBI 200 FLU	2
Syngeneic	
None	1
Survival 100 days post-transplant	11
Survival at last contact	6
Alive and well	3
Chronic GVHD	3
Hemolytic thrombotic presentation	16
HLA-identical sibling	
BU CY ± ATG	7
BU FLU ATG	1
Haploidentical family	
BU CY ± ATG	2
Mismatched sibling	
BU CY ATG	1
Unrelated umbilical cord	
TBI 1350 CY ATG	1
Unrelated donor	
BU CY ± ATG	3
Syngeneic	
None	1
Survival 100 days post-transplant	9
Survival at last contact	8
Alive and well	3
Chronic GVHD	5

ATG, antithymocyte globulin; BU, busulfan; CY, cyclophosphamide; FLU, fludarabine; GVHD, graft-versus-host disease; TBI 1200, 1200 cGy total body irradiation; TBI 1350, 1350 cGy total body irradiation.

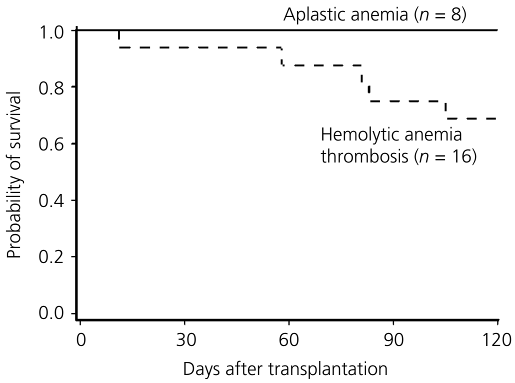

Figure 47.2 Survival 4 months after transplant. The solid line represents eight patients with the AA presentation of paroxysmal nocturnal hemoglobinuria (PNH) who had HLA-identical sibling or syngeneic donors compared with 16 patients with the hemolytic or thrombotic presentations with any type of donor (dashed line).

with a matched cohort of non-transplanted PNH patients reported to, and previously published by, the French Society of Hematology. After a median follow-up of 5 years, the 5-year overall survival rate ± SE (%) was 68 ± 3 in the transplanted group (54 ± 7 in case of thromboembolism, 69 ± 5 in case of AA without thromboembolism, and 86 ± 6 in case of recurrent hemolytic anemia without thromboembolism or AA). Only thromboembolism indication for transplantation was associated with worse outcome ($p = 0.03$). The study identified 24 pairs of transplanted and non-transplanted patients with thromboembolism for the matched comparison, with worse overall survival for the transplanted patients {HR 10.0 [95% confidence interval (CI) 1.3–78.1], $p = 0.007$}. This was confirmed by the global matching procedure ($p = 0.03$). Concerning AA without thromboembolism, 30 pairs were identified for matched comparison. Not significantly worse overall survival was observed for transplanted patients [HR 4.0 (95% CI 0.9–18.9), $p = 0.06$]. The global matching procedure was not feasible. One of the conclusions of the authors was that allogeneic stem-cell transplantation is probably not a suitable treatment option for life-threatening thromboembolism in PNH.

Transplants following reduced-intensity conditioning

Because of reduced toxicity, non-myeloablative or reduced-intensity conditioning regimens are appealing. The reduced-intensity conditioning regimens often used may be insufficient for sustained donor engraftment in patients with non-aplastic presentations because even those syngeneic recipients of marrow for the aplastic phase of PNH required conditioning regimens to achieve sustained donor engraftment (Table 47.2) [67,68,71,72,75–77,79]. Furthermore, syngeneic recipients who did not receive pre-transplant conditioning eventually had a return of PNH disease years later [70,71].

Except for one patient, recipients of allogeneic stem cells had sustained elimination of the disease (Table 47.3) [71]. These results suggest that a graft-versus-PNH type of effect in either a myeloablative or reduced-intensity allogeneic transplant procedure makes an important contribution toward eliminating the PNH clone. There have been reports of individual cases treated by reduced-intensity HCT approaches with successful elimination of PNH that support the idea that immunologically based eradication of PNH cells is possible following reduced-intensity conditioning regimens (Table 47.2). Further studies of the reduced-intensity approach are warranted, especially for patients who cannot undergo conventional HCT because of an increased risk of transplant-related mortality due to pre-transplant organ dysfunction.

Use of eculizumab during HCT

PNH patients who are deemed candidates for allogeneic HCT may have been treated with eculizumab prior to HCT. The question may arise of whether eculizumab would be required or helpful in the management of PNH during HCT. There is no published evidence to support the use of eclulizumab; however, for patients with a large PNH clone, there is a risk of significant intravascular hemolysis, which is potentially massive due to withdrawal of eculizumab. Active hemolysis can be associated with renal dysfunctions and thrombosis, and therefore may adversely impact on the transplant outcomes. Hence it would be reasonable to consider the use of continued eculizumab until donor-derived hematopoiesis is established with none or minimal residual PNH cells.

In addition, Kwan et al. recently reported that total body irradiation caused upregulation and activation of alternative pathway complement components by recipient antigen-presenting cells (APCs) [90]. Allo-HCT with DASF-null (*Daf1–/–*) host BM and *Daf1–/–* donor lymphocytes led to exacerbated GVHD outcome and resulted in splenic and organ-infiltrating T-cell expansion. T cells deficient in C3a receptor (C3aR) and/or C5a receptor (C5aR) responded weakly in allogeneic hosts and exhibited limited ability to induce GVHD. The study also showed that pharmacological C5aR blockade reduced GVHD morbidity.

Autologous transplantation for PNH

An interesting hypothesis has been put forward by Musto et al. [91]. They demonstrated that flow cytometry could select CD34$^+$/CD59$^+$ cells from PNH patients who had a mixed population of circulating normal and abnormal cells. If these cells do not bear the mutation in the *PIG-A* gene, a rationale to mobilize and select autologous normal hematopoietic stem cells for autologous transplantation exists, although no autologous transplant cases have been reported [56].

Finally, indications for bone marrow/stem cell transplantation and management issues for patients with PNH are presented in Table 47.5.

Table 47.5 Bone marrow/stem cell transplantation for patients with PNH

Indications for consideration of transplantation
- Bone marrow failure
  - Decision on transplantation is based on the severity and prognosis of underlying marrow abnormality (e.g., AA, MDS)[a]
- Major complication of PNH[b,c]
  - Recurrent, life-threatening thromboembolic disease
  - Refractory, transfusion-dependent hemolytic anemia

PNH-specific transplant-related issues
- Conditioning regimens
  - Cyclophosphamide/ATG (or other regimens used for AA)
- For patients with Classic PNH
  - more myeloablative regimen is indicated[d] (i.e. BU CY, BU FLU, FLU MEL). Additional investigation is required to define the role of non-myeloablative regimens
- Syngeneic twin transplants
  - a myeloablative conditioning regimen is recommended to prevent recurrence of PNH

Other complications/outcomes
- There are no PNH-specific adverse events associated with transplantation; severe acute graft-versus-host disease (GVHD) occurs in more than one-third of the patients and the incidence of chronic GVHD is roughly 35%. Overall survival for unselected PNH patients who undergo transplantation using an HLA-matched sibling donor is in the range 50–60%
- Consider eculizumab during BMT (i.e. until donor engraftment) to prevent withdrawal hemolysis

[a] If the dominant clinical abnormalities are a consequence of bone marrow failure (e.g., hypoproliferative anemia, neutropenia with recurrent infections, thrombocytopenia with major bleeding complications), the decision to recommend transplantation should be based on guidelines for management of the specific marrow failure syndrome.

[b] Factors including age, comorbid conditions, availability of an HLA-matched sibling donor, and time from initial diagnosis should be considered before recommending transplantation for major complications of PNH.

[c] The availability of new treatment options (e.g., eculizumab) may influence the decision to recommend transplantation.

[d] Radiation or busulfan based.

Source: Parker et al. 2005 [4]. Reproduced with permission of the American Society of Hematology.

Conclusion

The diagnosis of PNH alone should not be the sole indication for HCT therapy. Defining the risk of a fatal complication in an individual PNH patient is still the most important determination to make before recommending HCT. Eculizumab reduces the risk of hemolysis although it is unlikely it will affect the aplastic phase. Accumulating evidence suggests that eculizumab may reduce the risk of thrombotic complications and may improve the survival outcome of this disease.

When the patient has a life-threatening presentation with AA and an HLA-identical sibling or identical twin donor is available, HCT is recommended. Unrelated donors have been used to achieve successful grafts in patients with idiopathic AA, and HCT with a matched, unrelated donor for the life-threatening aplastic phase of PNH is warranted, although the experience is small. Reduced-intensity conditioning for HCT should be explored further, especially for patients with severe PNH who cannot tolerate a full myeloablative transplant due to comorbid medical conditions. In all other phases of disease, the care should be supportive, and transplantation should be performed when the disease becomes life threatening, or reliable predictors are developed to identify which patients with the non-aplastic phases of PNH are at particularly high risk of death from PNH.

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CHAPTER 48

Hematopoietic Stem Cell Transplantation for Chronic Myeloid Leukemia

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Introduction

Chronic myeloid leukemia (CML) is a malignant disorder of hematopoiesis resulting from the clonal expansion of a primitive hematopoietic cell. At the outset of disease, the CML stem cell retains the capacity to differentiate, leading to marked marrow hyperplasia and increased numbers of myeloid cells and platelets in the peripheral blood. The natural history of untreated CML is a relatively benign chronic phase (CP) lasting on average approximately 3–5 years followed by an accelerated phase (AP) lasting several months and then eventually terminating in a rapidly fatal blast crisis (BC).

CML is relatively rare, with an incidence of about two cases per 100,000 per year [1], yet it can be argued that it is the best example of "bench to bedside" in oncology. Consider these major milestones: first, CML was the first malignant disease found to be consistently associated with a specific cytogenetic abnormality, the Philadelphia (Ph) chromosome; second, it was the first leukemia where transplantation became the initial curative option; third, it was the first (and best) example of "targeted" therapy with the use of tyrosine kinase inhibitors.

Nowell and Hungerford in 1960 [2] described the presence of a small chromosome in metaphase preparations of marrows from patients with CML. This abnormal chromosome, termed the Philadelphia chromosome (after the city where it was discovered), was later shown by Rowley to be the result of a translocation between chromosomes 9 and 22 [t(9; 22)(q34;q11)] [3]. The translocation is the fusion of the *BCR* (breakpoint cluster region) gene on chromosome 22 to the *ABL* (Abelson leukemia virus) gene on chromosome 9, and results in the production of an abnormal BCR-ABL fusion protein, which is a constitutively active cytoplasmic tyrosine kinase [4,5]. Thus, the unique BCR-ABL translocation serves as both a target of therapy (with the suppression of ABL by tyrosine kinase inhibitors) and monitoring [of the Ph by cytogenetics, BCR-ABL DNA by fluorescence in situ hybridization (FISH), or BCR-ABL mRNA by reverse transcriptase polymerase chain reaction (RT-PCR)].

Without therapy, CML evolves from a CP to an AP and eventually to BC. Various definitions of AP have been developed. Two of the more commonly employed are those of Sokal et al. and the International Bone Marrow Transplant Registry (IBMTR) [6] (Table 48.1). In general, AP is characterized by symptoms of fever, night sweats, weight loss, bone pain, difficulty in controlling counts using conventional therapy, increased numbers of blasts and early myeloid cells in marrow and peripheral blood, and evidence of karyotypic evolution. The most common cytogenetic changes associated with disease evolution are an additional Ph chromosome, trisomy 8, isochrome i(17q), and trisomy 19. Blast crisis has been defined as having more than 30% blasts and promyelocytes in the bone marrow or peripheral blood, or the development of extramedullary blastic infiltrates, although a reclassification by the WHO has lowered the blast cutoff to 20%. In approximately two-thirds of patients, the blasts are predominately myeloid, whereas in the remaining one-third the blasts have a lymphoid phenotype [6,7].

A number of prognostic scoring systems have been developed with the goal of predicting the length of chronic phase in individual patients, the best known and widely used is by Sokal et al. [6]. In an algorithm, spleen size, percentage of circulating blasts, platelet count, and age were identified as prognostic factors for chronic phase patients. However, the Sokal scale was based on therapies considered archaic today (busulfan, splenectomy), and newer systems for patients treated with interferon resulted in newer prognostic scoring systems [8].

The past: allogeneic hematopoietic cell transplantation as curative therapy in CML

Tyrosine kinase inhibitors (TKIs) have replaced transplantation as front-line therapy, but it must be remembered that before TKIs, allogeneic transplantation of CML was a profound success, particularly in CP. Indeed, CML was unique in that before TKIs, allogeneic transplantation was offered as soon as possible to patients with available donors (initially matched related, and later unrelated donors). To understand the place of transplantation in the modern era of TKI therapy, it is useful to review the outcomes of transplantation, and the factors that influence its effect.

Phase of disease

As with all treatments of CML, outcomes are far superior for hematopoietic cell transplantation (HCT) for patients in chronic phase compared to advanced phase disease. Studies by the Seattle, Hammersmith, and German consortium groups show 3–5-year survival rates of 80–90% for patients treated in CP (Figure 48.1) [9–11] A report on long-term outcomes of 2444 CML patients in CP who receive allogeneic myeloablative HCT between 1978 and 1998 and had survived in continuous complete remission for at

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least 5 years confirms the excellent outcome for these patients [12]. Overall survival rates at 15 years were 88% for sibling HCT and 87% for unrelated donor HCT, with corresponding cumulative incidences of relapse of 8 and 2%, respectively. In comparison with age-, race-, and gender-adjusted normal populations, the mortality of HCT recipients was significantly higher until 14 years post-HCT; thereafter, mortality rates were similar to those of the general population.

Outcomes in advanced phase disease (AP and BC) are inferior to CP, with the 4-year probabilities of overall survival (OS) and event-free survival (EFS) being 49 and 43%, respectively [13,14]. A study comparing outcomes of imatinib versus allogeneic HCT for AP CML showed similar survival in “low-risk” patients (those without adverse prognostic factors of disease duration >12 months, hemoglobin <10 g/L, and peripheral blood blasts >5%), but allogeneic HCT conferred a significant survival advantage for patients with these adverse prognostic factors [15]. In virtually all studies, the outcome of transplantation for patients in BC is very poor, because of both a high risk of disease recurrence and a high incidence of transplant-related deaths with survival of approximately 20% or less. A report from the Center for International Blood and Marrow Transplantation Research (CIBMTR) for 449 allogeneic HCTs performed from 1999 to 2004 in advanced phase CML confirmed that disease stage remained the strongest determinant of transplant outcomes, even in the imatinib era [16].

Table 48.1 Definition of accelerated phase chronic myeloid leukemia

Sokal criteria	IBMTR criteria
Peripheral blood or marrow blasts ≥5%	Leukocyte count difficult to control with conventional therapy
Basophils >20%	Rapid doubling time of leukocytes (<5 days)
Platelet count ≥1000 × 10^9/L despite adequate therapy	Peripheral blood or marrow blasts ≥10%
Karyotype evolution	Peripheral blood or marrow blasts plus promyelocytes ≥20%
Frequent Pelger–Huet-like neutrophils; nucleated erythrocytes, megakaryocytic nuclear fragments	Peripheral blood basophils and eosinophils ≥20%
Marrow collagen fibrosis	Anemia or thrombocytopenia unresponsive to therapy
Anemia or thrombocytopenia unrelated to therapy	Persistent thrombocytosis
Progressive splenomegaly	Karyotypic evolution
Leukocyte doubling time <5 days	Progressive splenomegaly
Fever not otherwise explained	Development of chloromas or myelofibrosis

IBMTR, International Bone Marrow Transplant Registry.

Ablative preparative regimens

The majority of patients treated in the early 1980s received a preparative regimen of 120 mg/kg cyclophosphamide (CY), followed by total body irradiation (TBI) of 2.0 Gy on each of six successive days. Subsequent studies showed that regimens containing 16 mg/kg busulphan (BU) administered over 4 days combined with 60 mg/kg CY on each of two successive days showed no differences between the CY–TBI and BU–CY treatment groups in survival at 3 years (80% for both), relapse (13% for both), or EFS (68% for CY–TBI and 71% for BU–CY)[17]. An update in 2002 shows OS of 78% at 10 years with BU–CY vs. 64% with CY–TBI [18]. The advance of BU monitoring to achieve a steady-state BU concentration at 900–1200 ng/mL was shown in a study of CP patients transplanted with human leukocyte antigen (HLA)-identical relatives, to offer a 3-year survival of 86%, a relapse rate of only 8%, and a non-relapse mortality rate of 14% [10]. Other preparative regimens have also been reported to provide excellent disease control with acceptable toxicities in the treatment of CP CML, including the regimen of TBI plus etoposide reported by the City of Hope National Medical Center and Stanford University groups and the use of CY plus 500 cGy TBI given as a single dose at a high dose rate [19].

Reduced-intensity conditioning (RIC) in CML

Given the power of the graft-versus-leukemia (GVL) effect following standard transplantation for CML, reduced-intensity or non-ablative transplant approaches have been developed in an attempt to avoid the toxicities of high-dose preparative regimens while retaining the potentially powerful GVL effects. These approaches (discussed in more detail in Chapter 20) are of particular relevance for patients with CML given that their median age at diagnosis is 67 years. The Seattle consortium published results with a non-myeloablative (NMA) regimen of Flu–TBI or 2 Gy TBI in 24 patients with CML first CP, second first CP, or AP. All patients received peripheral blood progenitor cells (PBPCs) from matched related donors [20]. Five of 25 patients died from non-relapse mortality (NRM), and the 100-day NRM rate was 4%. The 2-year estimates of OS were 70% for patients in first CP and 56% for patients beyond first CP. However, results using unrelated donors were not as initially encouraging because of high rates of graft rejection (45%). Investigators at Hadassah-Hebrew University Hospital published results for a younger cohort of patients (median age 35 years) using an RIC regimen of Flu 180 mg/m^2, oral BU 8 mg/kg, and ATG 20–40 mg/kg, in 24 patients with CP CML. Nineteen patients received minimal

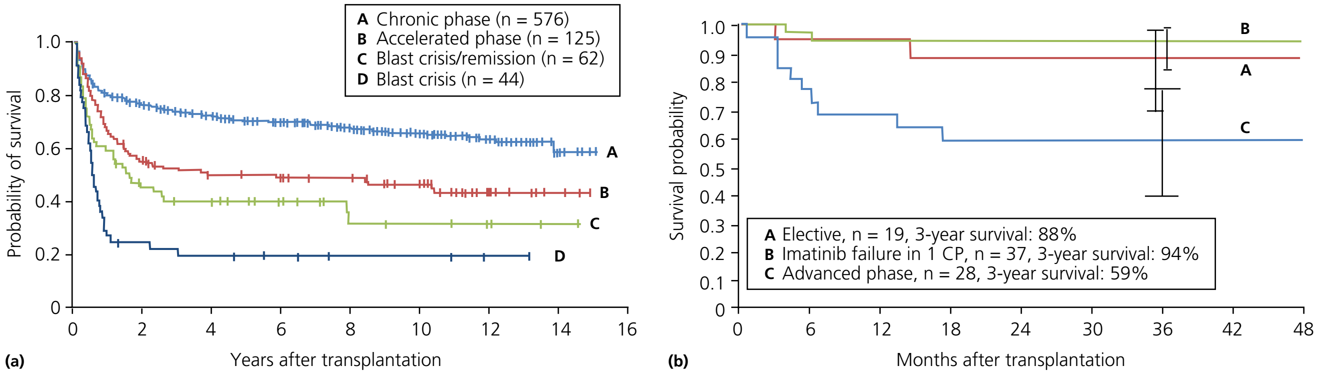

Figure 48.1 (a) Survival of CML patients receiving allografts at the Fred Hutchinson Cancer Research Center, from 1995 to present (includes both matched related and unrelated donors). Source: reproduced with permission of Dr. Ted Gooley. (b) Survival of patients from the German CML IV study for chronic and advanced phase CML. Source: Saussele et al. 2010 [11]. Reproduced with permission of the American Society of Hematology.

residual disease (MRD) monitoring and five received unrelated donor PBPCs [21]. The 100-day NRM was 0% and there were only three mortalities in the series, all secondary to GVHD. The estimated 5-year OS and relapse-free survival (RFS) were 85%. At a median of 42 months from HCT, all patients had a complete molecular response.

The European Group for Blood and Marrow Transplantation (EBMT) has reported results on 186 patients with CML (median age 50 years) who received RIC [22]. Patients with all stages of CML were included, with various RIC regimens. In addition, there was a mix of stem-cell product source [PBSC vs. bone marrow (BM)], and donors (HLA-matched and -mismatched related and unrelated donors). The 100-day NRM was 6.1% and the 2-year NRM was 23.3%. The 3-year OS and RFS were 58 and 37%, respectively. At least 40% of patients attained a complete molecular response and at least 62% a complete cytogenetic response. In an analysis of 306 CML patients aged 40 years and older undergoing RIC or NMA HCT reported to the CIBMTR from 2001 to 2007, outcomes were similar for patients aged 40–49, 50–59, and over 60 years in terms of the OS at 3 years (54, 52, and 41%, respectively) and treatment-related mortality at 1 year (18, 20, and 13%, respectively). For CP patients, RFS and disease-free survival (DFS) were similar across all ages [23]. These studies indicate that allogeneic RIC HCT is a viable strategy in patients with CML, and that RIC for older CP CML patients treated with TKIs can control relapse with acceptable toxicity and survival.

Donor type

Only approximately one-third of patients have HLA-matched family members to serve as donors. Hence considerable research into the use of alternative donors for transplantation in CML has been conducted. In Chapter 45, details concerning the use of unrelated donors for transplantation in CML are presented. As discussed in that chapter, although early results with matched unrelated donor transplantation in CML suggested results somewhat poorer than seen with matched siblings [24], advances in donor selection, GVHD prophylaxis, and supportive care have resulted in continued improvements in outcome so that now selected single institutions are reporting results almost equivalent to those seen with matched siblings, and registry data are showing 65% survival at 5 years among younger patients transplanted within 1 year of diagnosis [25,26]. In the Seattle experience, survival in CP, AP, and BC are similar between matched-related and unrelated donors (with a caveat that unrelated donor transplants have a lower patient age exclusion). Details about the use of mismatched related donors are presented in Chapter 44, and the use of cord-blood transplants is included in Chapter 39. The experience with autologous transplantation in CML is summarized briefly below.

Source of hematopoietic stem cells

Two separate large randomized trials involving patients with a variety of hematologic malignancies receiving transplants from matched siblings have been published demonstrating that the use of granulocyte-colony-stimulating factor (G-CSF)-mobilized peripheral blood hematopoietic cells, when compared with bone marrow, leads to more rapid myeloid and platelet recovery, no significant difference in acute or chronic GVHD, and an OS advantage. In the studies by Bensinger et al. [27] and Couban et al. [28], there was a trend towards improved survival in CML patients with the use of peripheral blood. A randomized study of CP CML showed no statistically significant differences in outcome between the bone marrow and peripheral blood groups. Relapse rates were lower in the peripheral blood group, but chronic GVHD was higher (and subsequent complications thereof), balancing the survival [29].

Patient age

Initial studies of transplantation for CML in CP from HLA-matched siblings showed that the results deteriorated with increasing patient age. As methods for GVHD prophylaxis and supportive care were improved, the impact of age on outcome appeared to diminish, with no clear effect up to age 65 years using matched related donors, although age seems to be more clearly related to transplant outcome in the unrelated donor setting [9,10,25].

Relapse following transplantation

Graft-versus-leukemia effect in CML

Although evidence for a GVL effect can be found in many settings, nowhere is it as strong as in the setting of allogeneic transplantation for CML. Evidence in support of such an effect includes, first, the higher rates of relapse following syngeneic and T-cell-depleted transplants compared with non-modified allogeneic transplants [30,31]. Second, following non-T-cell-depleted transplants, there is a strong inverse association between acute and chronic GVHD and relapse [32]. Third, response rates to donor lymphocyte infusions to treat post-transplant relapse range from 50 to 100% in various reports, higher than in any other malignancy [33,34]. The markedly increased relapse rates seen with T-cell depletion argue that it is largely a T-cell-mediated process. The targets of T cells might include minor histocompatibility antigens shared by most cells in the body, thus accounting for the association of GVL with GVHD. Alternatively, there may be polymorphic minor histocompatibility antigens with expression limited to hematopoietic tissue. A number of such antigens have been identified [35] and their role as targets for GVL could explain the observation that relapse rates are less following non-T-cell-depleted transplants than with T-cell-depleted transplants even in patients who develop no signs of GVHD. A third possible category of targets for the GVL effect in CML is the over-expression of protein targets in CML cells. T-cell responses could be directed at the tumor-specific peptides spanning the BCR-ABL fusion region, but there is little evidence for the presence of high numbers of T cells with specificity against BCR-ABL in transplant patients. Understanding the cells and their targets responsible for the potent GVL effect seen in CML will be critical to the development of more effective, less toxic transplant-based therapies in the future.

Monitoring minimal residual disease

The fact that CML is always associated with the BCR-ABL translocation coupled with the development of PCR techniques capable of detecting and quantifying extraordinarily small numbers of abnormal cells among a large population of normal ones makes CML an ideal setting to test the capabilities of MRD monitoring to provide insight into the disease and direct therapies. Details about MRD detection are provided in Chapter 25. With non-quantitative PCR techniques, it is possible to detect a single CML cell in a population of 10^5–10^6 cells. Such a qualitative assay was found to be predictive of relapse in 346 patients tested post-transplantation [36]. In over 40% of patients, PCR assays were positive for residual disease at 3 months post-transplant, but this finding was not predictive of outcome, suggesting that eradication of the CML clone post-transplant takes an extended period of time. In contrast, at 6 or 12 months post-transplant, the risk of being PCR positive dropped to ~25%, and here the assay was a powerful predictor of outcome, as 42% of PCR-positive patients subsequently relapsed, compared with 3% of PCR-negative patients ($p < 0.0001$). The predictive power of PCR among long-term survivors is weaker. When studied at 18 months or more post-transplant, only 1% of 289 BCR-ABL-negative patients subsequently relapsed compared with 14% of 90 BCR-ABL-positive patients [37]. The phenomenon of long-lived PCR positivity without relapse has been observed by several groups, and is discussed under “dormancy” in Chapter 25.

The advent of quantitative PCR assays allowed fine tuning of the association of MRD and relapse. Accordingly, attempts have been made to develop quantitative PCR assays and apply them to the post-transplant CML setting. Olavarria et al. [38] studied 138 patients who received allografts, using a quantitative RT-PCR assay performed at 3–5 months post-transplant, and were able to define patients as having a low risk (16%) of relapse, an intermediate risk (43%), or a high risk (86%) based on the quantification of the PCR signal [39]. A more complete discussion of MRD in CML can be found in Chapter 25.

Treatment of post-transplant relapse

The pace of disease progression after post-transplant relapse is variable. Indeed, if relapse is defined at a molecular (PCR) level, some patients may never progress, or at least may not progress for a very long time [37]. Similarly, patients whose only evidence of relapse is detection of low levels of Ph-positive metaphases may remain stable for many years, and even patients with clinical relapse may not progress rapidly. Two retrospective EBMT studies have examined CML patients who relapsed after transplant [40,41]. Stage at transplant and relapse, time from diagnosis to transplantation and from transplantation to relapse, and type of donor (matched sibling vs. volunteer) were all shown to affect survival. Specifically, recipients of matched sibling grafts for CP disease with a short interval from diagnosis to transplant but a long interval from transplant to relapse did particularly well, with a likelihood of being alive at 10 years after post-transplant relapse of 42%. An appreciation of the likely tempo of progression is necessary when considering treatment interventions.

Several potential interventions are available to the patient who has relapsed after allogeneic transplantation for CML. Prior to the introduction of TKIs, treatment with interferon (IFN) was found to produce both clinical and cytogenetic remissions in relapsed patients [42]. More commonly, donor leukocyte infusion (DLI) was used to promote a GVL effect, as detailed in Chapter 70. There have been a large number of studies demonstrating cytogenetic complete response rates of 50–100% in patients treated for clinically relapsed CP CML (reviewed in [43]). Response rates tend to be higher for patients treated earlier at the time of cytogenetic relapse and lower for patients in AP. There is a trend towards improved response rates in patients treated within 2 years after transplantation [44]. The two major complications of DLI are transient marrow failure and the development of GVHD. Most early studies of DLI involved a single infusion of a relatively large number of donor T cells. DLI is better tolerated with less GVHD if administered in a fractionated fashion rather than as a single bulk dose, starting at lower doses of lymphocytes and escalating dosage as required. In a report of 328 patients with relapsed CML who received DLI, 122 (38%) developed some form of GVHD. DLI-related mortality was 11%, disease-related mortality was 20%, and OS for all patients was 69% at 5 years [45].

TKIs have emerged as an initial option for relapsing CML post-transplant [46–52]. As with IFN and DLI, the effectiveness is largely determined by the phase of disease at time of relapse. Responses have been very good in CP patients with high rates (>60%) of both hematologic and cytogenetic remissions [46–49,53]. Fewer studies have evaluated the effect of TKI therapy in patients with advanced disease after HCT, and only a minority of patients achieved a durable remission with TKIs alone [46–48,53]. Post-transplant imatinib treatment is generally well tolerated, with the main toxicity being pancytopenia, which can often be treated with either a temporary discontinuation or dose adjustment. There are limited data with regard to the use of second-generation TKIs for post-transplant relapse [54–57]. The limited data with dasatinib suggest greater toxicity than with imatinib, including gastrointestinal bleeding and pulmonary complications, in addition to the expected hematologic toxicity.

The rationale of combining a TKI with DLI is attractive and has been the focus of a few small studies [50,51]. Although some did not show an improved outcome of combination therapy compared with imatinib alone, one study of 37 patients suggested a beneficial effect of the DLI–imatinib combination compared with the use of either agent alone, as the rates of DFS with DLI–imatinib, imatinib, and DLI were 100, 54, and 43%, respectively.

The optimal time of initiation of TKI therapy after transplant, optimal dose, and duration of TKI therapy for relapsed CML after HCT are unclear. In most post-transplant studies, imatinib at doses of 400–800 mg daily was continued for periods from 9 to 14 months [46–49]. Palandri et al. [58] continued imatinib for longer periods with a median treatment duration of 31 months. In total, 12 of 16 (75%) patients with hematologic, cytogenetic, or molecular relapse of CP or AP/BC achieved molecular CR. This suggests that prolonged treatment with imatinib is effective and feasible in the post-transplant period and can be safely administered over longer periods.

TKIs can be given prophylactically to prevent relapse in high-risk cases [46,59–61].The approach of early TKI therapy, coupled with sensitive molecular monitoring, may allow for prevention or early intervention of disease reoccurrence in CML, and may improve outcomes for transplant in AP disease. However several questions regarding the use of TKIs to treat or prevent relapse post-transplantation remain, including the choice between the use of different TKIs post-transplantation, whether pre-transplant response and BCR-ABL mutations predict for TKI-responsive post-transplantation, and how long treatment needs to be continued after HCT to avoid disease recurrence.

Second transplants have been used to treat patients who have developed recurrent CML following a first transplant. In general, preparative regimens based on chemotherapy only have been used for patients previously exposed to TBI, and TBI-based regimens have been used when the first regimen relied only on chemotherapy. The numbers of second transplants reported in CML are small, yet the data are consistent, reporting regimen-related mortality of ~40%, with long-term DFS of ~25% [62]. As expected, survival in BCs is rare. With the availability of DLI and TKIs, the use of second transplants has diminished substantially.

Autologous transplantation

Experimental and clinical evidence for persistence of polyclonal Ph^- progenitors provided the rationale for autologous transplantation in CML patients [63–65]. Transplantation of autologous Ph^- cells could allow restoration of Ph^- hematopoiesis. Initial studies using unmanipulated autologous marrow or blood cells collected from patients in CP indicated that autologous transplantation could re-establish CP in patients with advanced disease and induce transient cytogenetic responses in a small proportion of patients [66–69]. Subsequent attempts to improve the results of autologous transplantation by ex vivo purging of malignant progenitors from infused cells prior to transplantation showed that ex vivo graft manipulation resulted in depletion of Ph^+ progenitors and was associated with cytogenetic remission post-transplant. However, remissions were of relatively short duration and delayed or partial engraftment was a significant problem. Another approach to depleting malignant cells from the graft was to treat patients with intensive chemotherapy prior to harvesting the autograft (in vivo purging). Autografting of a selected group of CML patients with good cytogenetic responses to intensive treatment was associated with a high incidence of cytogenetic responses post-transplantation [70–72], although other reports were less compelling [73–75]. Moreover, high-dose chemotherapy was often associated with significant toxicity and delayed recovery. An alternative approach to in vivo purging has been to collect PBSCs by growth factor mobilization following induction of cytogenetic responses by IFN-α [76,77] or TKI treatment [78]. The PBSC collection process is feasible and well tolerated. The collection of Ph collections is more consistent than with chemotherapy and IFN-based strategies. However, a subset of patients fail to reach target $CD34^+$ cell collection, and many patients mobilize suboptimally and require multiple collections, possibly as a result of effects of imatinib on normal hematopoiesis and the mobilization process [79]. Collection of PBSCs after a brief discontinuation of imatinib appears to result in greater success. Collections are usually Ph^- by routine cytogenetics and FISH. However, in most patients residual BCR/ABL^+ cells can be detected using sensitive PCR assays. Although the role of autologous HCT has been the subject of a number of studies over more than 25 years, clear evidence of benefit has not been forthcoming. A meta-analysis of six trials in which patients were randomly allocated to receive autologous HCT or an IFN-based regimen did not show an advantage for HCT. Historically, autologous transplant was considered as a treatment option for CML patients without a suitable matched donor. However, in view of the excellent results of imatinib treatment, and the availability of second- and third-generation kinase inhibitors with significant activity in imatinib-resistant CML patients, autologous HCT for CML is not being actively pursued at present.

Awareness of the above factors, along with other contributions of supportive care and GVHD prophylaxis, has made transplantation in CML more successful over time. The pre-transplant prognostic score devised by Gratwohl et al. uses HLA matching, stage, age, gender of donor/recipient, and time from diagnosis to transplant [14]. The scoring system is effective in defining post-transplant outcomes following an ablative transplant, with survival in EBMT data >70% for the best score to <20% for the worst score (Figure 48.2). This scoring was subsequently applied again to EBMT cases transplanted in three cohorts, 1980–1990, 1991–1999, and 2000–2002 [9]. In all risk groups, survival improved over time (overall improvement of >50%), largely from halving of the regimen-related mortality.

However, the dramatic success of TKI therapy has made transplantation a very rare event in CML therapy. Figure 48.3 shows the number of transplants for CML in Seattle and in Europe; the rate descends quickly after the introduction of imatinib in clinical trials, and continues downwards after imatinib achieved regulatory approval in 2001.

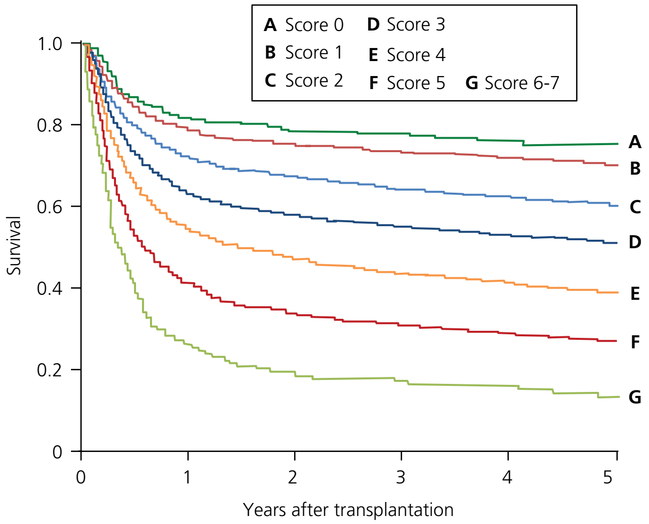

Figure 48.2 Overall survival after allogeneic transplants based on risk assessment score [14]. Components of the score include age, time from diagnosis to transplant, donor type, and donor–host gender.

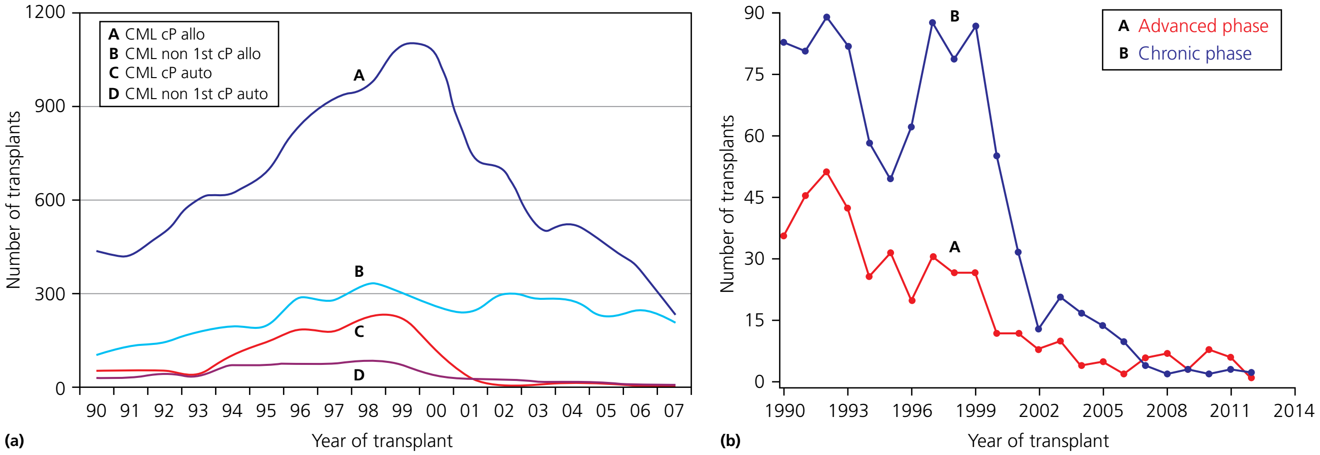

Figure 48.3 Number of allograft transplants for CML (a) in Europe (EBMT data source: courtesy of Dr. A. Gratwohl [14]) and (b) at the Fred Hutchinson Cancer Research Center (source: data courtesy of Dr. Ted Gooley).

The Present: TKI therapy and the more limited role of transplantation

The rapid decline of CML transplants reflects the enthusiasm over the clinical success of imatinib (and later, the "second generation TKIs" such as nilotinib and dasatinib). After a decade of TKI use, detailed treatment guidelines have been produced (and constantly updated) by the National Comprehensive Cancer Network (NCCN) (www.nccn.org) and the European Leukemia Network (ELN) (www.leukemia-net.org). Both the NCCN and ELN guidelines are available in print and on the web, and readers are referred there for detailed and current information. However, the highlights of treatment strategies and expected outcomes are outlined below in order to give context to the question of *why and when* transplant should be used in the TKI era.

Front-line TKIs in newly diagnosed CML

The advent of TKIs changed the treatment course in CP CML. To summarize the salient points of TKI therapy: (1) Primary therapy for CP is highly effective, and can produce a complete cytogenetic remission (CCyR) in ~80% of cases. For these patients who achieve CCyR, survival is approximately 90% approaching a decade of follow-up. However, approximately 20–30% of cases will fail primary therapy, from either intolerance, relapse, or progression to advanced phase disease. (2) For patients receiving secondary therapy for resistant disease, approximately 50% will achieve a CCyR. The survival for these patients is ~80% at 3 years. Those who do not response have a relatively short time to progression to advanced phase disease. (3) Patients with AP or BC can respond TKI therapy, but this does not appear to be associated with long-term progression free survival (PFS).

The first approved TKI was imatinib, and much of what we know about response, relapse, and monitoring stems from the large phase III IRIS trial of newly diagnosed CP CML, randomized to imatinib or IFN plus cytarabine. At 12 months, CCyR occurred in ~70% of the imatinib-treated patients versus 7% with IFN–cytarabine therapy. Disease progression was seen in 1.4% of the imatinib group versus 10.3% of the IFN patients. A subsequent 5-year follow-up showed that nearly 70% of imatinib-treated patients remained in complete cytogenetic remission, with an OS of over 85% [80,81].

Two new "second-generation" TKIs have subsequently been approved by the US Food and Drug Administration (FDA) for the treatment of newly diagnosed CP CML. Dasatinib (DAS) is a broad-spectrum kinase inhibitor, which inhibits tyrosine kinases, src kinases, c-KIT, and PDGFR-β, whereas nilotinib (NIL) is a narrow-spectrum TKI, engineered from the structure of imatinib to provide stronger tyrosine kinase inhibition. Phase II and III studies have been remarkably unified in showing that these second-generation TKIs have stronger short-term activity than imatinib (IM) 400 mg/day, including improvements in 12 month CCyR (approximately 80% with NIL or DAS, compared with ~70% with IM) and major molecular response (MMR) rates (~40–50% with the second-generation TKIs vs. 20–30% with IM), higher complete molecular response (CMR) rates, and lower progression to advanced phase disease (1–2% for DAS/NIL vs. 3–4% for IM) (Table 48.2) [82–84]. However, no study has shown a benefit in OS with the second-generation agents compared with IM. It is not clear if this is because there is no difference, or if the benefit is sufficiently small to require greater study numbers, and more follow-up time. At any rate, guidelines by the European Leukemia Network (ELN) and the National Comprehensive Cancer Network (NCCN) both allow for up-front therapy with IM, DAS, and NIL, at the discretion of the physician and patient [85,86].

Table 48.2 12-month responses (%) in ENESTnd [84], DASISION [82], and US Intergroup [83] trials[a]

Response	NIL 300 BID	NIL 400 BID	IM 400	DAS 100	IM 400	DAS 100	IM 400
CCyR	80	78	65	83	72	84	69
MMR	44	43	22	46	28	59	43
AP/BC	0.7	0.4	4.2	1.9	3.5	1	3

CCyR, complete cytogenetic remission; MMR, major molecular response; AP/BC, patients progressing to accelerated phase and blast crisis.

[a] Doses in mg/day unless indicated otherwise.

Second-line TKI therapy after imatinib failure or intolerance

Resistance to imatinib can be either primary (intrinsic), where patients fail to respond to treatment, or secondary (acquired), where resistance develops after an initial response [87]. In the IRIS trial, an estimated 16% of patients with newly diagnosed CP CML failed to achieve a major cytogenetic response (MCyR) after 12 months of imatinib and approximately 24% failed to achieve a CCyR after 18 months of imatinib therapy. Acquired resistance was also observed in 17% of cases, with 7% of patients progressing to AP or BC CML [80].

Primary resistance to imatinib may involve BCR-ABL-independent mechanisms, such as alterations in drug transporter influx (Oct-1) and efflux (ABC proteins), or other signaling pathways besides BCR-ABL, such as the SRC-family kinases (SFKs), which are substrates and downstream mediators of BCR-ABL signaling [88–91]. Several mechanisms underlie "acquired" (secondary) imatinib resistance, including amplification of the Ph, and the acquisition of point mutations in the ABL kinase domain (KD) of the BCR-ABL fusion protein [92,93]. BCR-ABL mutations are the most common cause of imatinib resistance (40–90%) and inhibit imatinib binding and efficacy by either inducing a structural change towards the active conformation of the protein, causing a shift in the equilibrium of BCR-ABL from inactive (which imatinib binds) to active (which imatinib is unable to bind), or by mutating critical binding sites in the molecule. More than 100 different BCR-ABL KD mutations have been found [94]. The likelihood of acquiring a BCR-ABL KD mutation increases with the phase of disease and length of time from diagnosis to treatment. Thus, the acquisition of KD mutations increases with advanced phase disease and in CP patients with a longer time (>1 year) from diagnosis to initiating imatinib therapy [95].

Four TKIs (nilotinib, dasatinib, ponatinib, and bosutinib) are now available and are recommended for the treatment of patients who are intolerant or resistant to imatinib [85,86]. In addition, the cetaxine from the Chinese plum yew tree, omacetaxine (formerly homoharringtonine), has shown modest activity in cases that have failed multiple TKIs. All the TKIs have a remarkably similar response in imatinib-intolerant and -resistant cases, producing CCyR in ~70% of cases of imatinib intolerance and 40–50% CCyR in cases treated for resistance [96,97]. The choice of which TKI to use as second-line therapy is based on several factors, including (a) existing comorbidities (e.g., cardiac risks in nilotinib, pulmonary with dasatinib), and (b) the specific BCR-ABL KD mutation. Each of these TKIs has a different spectrum of activity to different point mutations, as determined by in vitro drug inhibitory assays. Of note is that of the approved TKIs, only ponatinib has activity against the T315I "gatekeeper" mutation. The last important factor in TKI selection is the physician's familiarity with use. Since CML is a rare disease, physicians often have more comfort with one TKI than another; if there is no clear drug preference based on comorbidity or mutation, experience is an important factor in TKI choice.

Before the introduction of second-line TKIs, imatinib resistance was associated with a relatively rapid progression to advanced phase disease, especially with mutations the P-loop region of ABL [98–100]. The 3-year survival rate for imatinib-resistant patients with CP CML was 72%, falling to 30% for patients with AP CML and 7% for BC CML [99]. Second-generation TKIs have activity in advanced disease; for example, 27 and 43% of myeloid and lymphoid BC, respectively, achieved a CCyR on dasatinib therapy as a single agent. Unfortunately, the response was short-lived, as the median PFS was 5 and 3 months for myeloid and lymphoid BC, respectively [101,102].

Monitoring disease response to TKIs

Several layers of monitoring can be used CML, each associated with a level of disease response, and linked to treatment guidelines of the ELN and NCCN. *Complete hematologic response* (CHR) refers to the normalization of peripheral blood counts. In CP CML, all of the TKIs are associated with a >90% CHR rate by 3 months of therapy, and failure to achieve such a response is considered an indicator of a treatment failure. *Cytogenetic response* refers to the number of Ph chromosome metaphases among at least 20 metaphase preparations, and is the strongest prognostic factor for predicting long-term response to TKIs once patients have achieved a CHR. Achieving a CCyR is an independent prognostic factor for survival, and is the next major a goal of therapy after CHR. Failure to achieve any reduction in the number of Ph+ cells after 6 months of imatinib therapy and failure to achieve an MCyR response after 12 months of imatinib therapy predicts a <20% chance of ever achieving a CCyR [103]. Additionally, studies have shown that achieving an MCyR at 3 months is associated with prolonged time to disease progression in patients with late CP and AP CML [104,105]. FISH (see Chapter 25) has a somewhat limited role in BCR-ABL monitoring. At diagnosis, it should not replace conventional cytogenetics, since it cannot detect other chromosomal changes indicative of AP disease. It can, however, be used in the diagnostic setting if the patient has an inaspirable bone marrow. FISH is not adequately sensitive for following low levels of residual disease once a patient has achieved a CCyR. To study disease levels below that detectable by cytogenetics, *molecular response* using quantitative RT-PCR (see Chapter 25) of the chimeric BCR-ABL mRNA transcript is the most sensitive method to monitor disease burden, and can be combined with cytogenetics to predict outcome. Results from the IRIS trial showed that patients who had achieved a CCyR after 12 months of imatinib had a significantly lower risk of disease progression than patients without a CCyR. Patients who had both a CCyR and "major molecular response" (or MMR, a >3 log reduction of BCR-ABL transcript level from an aggregate baseline of 30 newly diagnosed patients) at 12 months of imatinib therapy had a 100% probability of remaining progression free at 24 months. In comparison, patients with a CCyR and <3 log reduction of BCR-ABL had 95% PFS, and patients who did not achieve a CCyR by 12 months had an 86% PFS [106]. Five-year follow-up data from this study revealed that no patient who achieved an MMR by 12 months had progressed to advanced disease [80].

Earlier responses after 3–6 months of TKI therapy may also be a predictor of long-term outcome. Several studies have shown that both OS and PFS are improved in patients who achieve a cytogenetic response at 3 or 6 months [105,107]. In addition, early molecular responses have also been shown to be associated with better outcome, as those patients who fail to achieve a 1–2 log reduction after 3 months of therapy are less likely to ever achieve an MMR [108–110]. In a large study of CP cases, it was shown that the achievement of an MMR at 3, 6, and 12 months was highly correlated with the duration of a CCyR. For example, for those patients who achieved a CCyR after 3 months of therapy, the subset who also achieved an MMR at that time had a 0% risk of relapse in the next 2 years, compared with a 20% risk for those without an MMR. This trend also held true for those in a CCyR at 6 and 12 months [109]. The significance of a poor response at 3 months appears strongest for those patients who fail to achieve a reduction in BCR-ABL to <10% International Scale (IS); this association of poor initial response and CCyR, OS, and EFS is evident in patients treated with both low- and high-dose imatinib, dasatinib, or nilotinib [111–113]. For example, a large study of 483 newly diagnosed CP cases showed that the EFS for cases with a BCR-ABL <10% at 3 months was 61%, compared with >95% in cases with a superior molecular response [114].

Both the ELN and the NCCN have published guidelines with respect to patient monitoring, treatment milestones, and therapeutic options. Thankfully, the recommendations are fairly similar. For example, the NCCN sets treatment goals as the achievement of a complete hematologic remission and a minor cytogentic response and/or the achievement of a BCR-ABL transcript value of <10% IS by 3 months, and a complete cytogenetic response by 12 months. The recommended monitoring schedule is based on the above treatment milestones. Thus in the NCCN guidelines, cytogenetic testing of bone marrow is recommended diagnosis, and at 3 and 12 months after the start of therapy. PCR testing for BCR-ABL begins at diagnosis and continues at 3-month intervals. Once a CCyR has been achieved, monitoring continues by peripheral blood BCR-ABL PCR testing alone.

Peripheral blood BCR-ABL is used both to access the degree of response, and also to detect early resistance. A rise in BCR-ABL is considered significant if BCR-ABL increases 5–10-fold on repeat measurements 1 month apart. If this occurs, this should trigger (a) inquiries about adherence to therapy (which is remarkably and surprisingly poor with TKI) [115,116], and (b) mutation studies of BCR-ABL.

As noted above, the detection of specific ABL point mutations can inform which other TKIs should be used as second-line therapy, based on in vitro drug inhibition studies [117]. The most common clinically significant mutations, and the appropriate drug choice, are shown in Table 48.3. Note that ponatinib appears to have activity for all ABL mutations so far detected. Resistance in the absence of a BCR-ABL KD mutation may be significant, since it suggests that resistance is occurring through a mechanism not dependent on BCR-ABL signaling (since in the absence of a mutation, it should be inhibited by the TKI).

Table 48.3 Options for specific BCR-ABL tyrosine kinase domain mutations[a]

Mutation	Treatment options
T315I	Ponatinib, HCT, omacetaxine, clinical trial
V299L	Nilotinib, ponatinib, omacetaxine
T315A	Nilotinib, ponatinib, imatinib, bosutinib, omacetaxine
F317L/V/I/C	Nilotinib, ponatinib, bosutinib, omacetaxine
Y253H, E255K/V, F359V/C/I	Dasatinib, ponatinib, bosutinib, omacetaxine

[a] As discussed in the text, preferences are based on the activity of the TKI versus the specific agent, patient comorbidities, and familiarity of the medication to the prescribing physician. A special exception is the T315I mutation, where the only available TKI with activity is ponatinib.

Who and when do we transplant?

Given the above summary of the TKI treatment experience, and the contemporary results of transplant, can we develop general "rules of engagement" for to whom and when we should offer transplantation? While this strategy is evolving, we can ask and answer some basic questions that can clarify our treatment course.

Should we ever offer transplantation as a first option?

Given the great results of front-line imatinib in newly diagnosed CP CML, the situations where transplantation should be offered first seem few. However, in health systems where a TKI cannot be obtained (owing to financial limitations or other bureaucratic/infrastructure restrictions), then transplant would be a good option for initial therapy of CP disease [118].

Very few CML cases present with advanced phase disease. It is important to remember that although CML is categorized in three phases, in reality the disease is a continuum, hence it is not sensible to consider an AP case with 19% blasts differently from a BCs case with 20% blasts. For BC patients, initial therapy with a TKI is warranted as a "bridge" to transplantation, which should be performed as expeditiously as possible. There is not as clear a consensus for patients presenting with AP disease, partly because of the wide variety of definitions of that clinical state. It is reasonable to start all newly diagnosed AP patients on a TKI, with a quick consultation with the transplant team. Patients with AP disease only by cytogenetic criteria (i.e., low blast counts and another cytogenetic abnormality besides the Ph) may be observed to see if they "behave" like a CP patient (since many of the TKI trials categorized these cases as CP), whereas AP patients who look more like BC cases (high blast count, large spleen, etc.) should be transplanted sooner rather than later.

When should transplantation be used after initial TKI failure?

There is increasing evidence that resistance may last forever. Fewer than 50% of cases of IM failure achieve a CCyR with secondary TKI therapy [101,119]. Moreover, patients who do fail secondary therapy often have a new mutation [120–122]. Hence secondary TKI therapy may be an unfortunate example of the Darwinian selection of fit clones that survive each successive therapeutic attack. Moreover, molecular data have shown than the biological states of resistance and progression have a large overlap [123–125]. Given these facts, it is reasonable to prepare patients for transplantation at the time of first-line failure. This means a consultation at a transplant center, and lining up a donor, either sibling or unrelated.

It is certainly reasonable, and sensible, to start a secondary TKI at the time of resistance, for two reasons: first, in case the patient is one of the fortunate ones who obtain a CCyR on secondary therapy; and second, a donor search can take months (especially for an unrelated donor), so therapy is needed to blunt the progression of the disease. Efforts have been made to identify clinical or laboratory variables that will predict outcome after the initiation of secondary TKI therapy. At present, patients with early cytogenetic response to a secondary TKI seem to do well. Thus, patients who are placed on a secondary TKI, but who fail to have any cytogenetic response after 3 months of therapy, are very unlikely ever to achieve a CCyR. Likewise, failure to achieve at least a minor cytogenetic response by 6 months, or an MCyR by 12 months of secondary therapy, bodes poorly for sustained disease control [126]. A scoring system based on the best cytogenetic score on imatinib therapy, Sokal score, and a history of neutropenia during imatinib therapy has been proposed by the Hammersmith group. Patients with a low score (few adverse features) had a >90% chance of achieving a CCyR on a secondary agent, compared with <20% for cases with a high score (several adverse features) [126,127].

There are a few special circumstances where a move to transplant is warranted after front-line TKI failure. If a patient has relapsed with a T315I mutation, ponatinib can be used if available (it is not approved in all countries as of this printing, but if ponatinib is not available, then transplantation should be offered once a donor has been secured. In addition, patients who progress to advanced phase disease while on a TKI have a very poor prognosis (median survival 10 months), and these patients should be moved to transplant as soon as possible. If mutation analysis points to the use of a particular TKI, it can be used as a bridge to transplant.

As noted above, patients who have a particularly poor response after 3 months of therapy have a comparatively poor PFS. While the NCCN suggests that these patients switch their TKI, it is not known if that will change the outcome for these patients. Therefore, they may benefit from a transplant discussion, initiation of HLA typing, and perhaps more frequent molecular monitoring once the switch to the new TKI has been made.

It is very unusual for patients to be intolerant of all the available TKIs, although it does rarely occur. If patients cannot be adequately dosed with a TKI, owing to either hematologic or non-hematologic toxicity, then transplantation should be considered.

Does prior treatment with TKIs affect transplant results?

There have been several studies on the effect of prior imatinib and transplant outcomes. Early reports warned of an increase in regimen-related toxicity and mortality, especially from hepatic causes [128]. Larger studies have failed to show a deleterious effect of pre-transplant imatinib [129,130]. A study of 140 CML patients showed no difference in regimen-related mortality, survival, or relapse in patients who received imatinib versus 200 historical controls [131]. Indeed, an IBMTR study of 409 patients who received imatinib therapy transplant compared with 900 patients who did not suggested that those CP patients who received imatinib enjoyed a slightly better outcome following transplant, although this effect seemed to be limited in cases who moved to transplantation because they could not tolerate imatinib, rather than treatment failure [132]. There was no positive or negative effect of imatinib on the outcome for advanced phase patients. So far, there is no evidence that resistant patients with Abl mutations have a poorer outcome following transplantation.

In a study of imatinib-resistant CML patients who underwent HCT and had BCR-ABL mutation analysis, 32 of 47 transplanted patients (68%) had at least a major molecular response. The 2-year EFS was 36 and 58% and OS was 44 and 76%, for patients with or without BCR-ABL mutations, respectively [133]. Recent results suggest that HCT is an effective strategy for patients with CML who have the T315I mutation [134]. Results for 84 patients who underwent transplantation in the randomized German CML Study IV according to predefined criteria (low EBMT score, imatinib failure, and advanced disease), showed that 3-year survival of 56 CP patients at 30 months was 91%, and of 28 AP patients 59%. The complete molecular remission rate after HCT was 88% [11]. These results suggest that HCT is an important salvage option for TKI-resistant patients with or without BCR-ABL mutations, including the T315I mutation, particularly in earlier stages.

The future of CML treatment

CML serves as an excellent model for how insights into the molecular basis of a malignant disease can be translated into powerful diagnostic tools and highly effective therapies. We now have a near "embarrassment of riches" with regard to effective treatment options, but there are still questions that point to future improvements of care:

1. Can we predict the "bad actors" prior to therapy, and pick the right agent for the job? Efforts to achieve this by clinical and genomic methods have been mixed. The reason for the uneven success is unclear, but may be largely due to the poor adherence of TKI therapy, as patients may do not respond because they are poorly compliant but have a responder genotype, who may be misclassified. However, one hopes that pretreatment prediction, added to an early kinetic assessment by PCR, might quickly suggest which patients need a more aggressive approach than a TKI alone.
2. Will early switching after 3–6 months of ineffective therapy improve outcomes? It is hoped that trials will quickly enlighten us. Although it is optimistic to think that switching to another TKI will help, it may well be that these cases have a biology that will require something more – transplantation, perhaps, or combination therapy in the context of a clinical trial.
3. Will patients be "cured" with TKIs? There has recently been great interest in discontinuing TKI treatment in patients with a prolonged CMR. It appears that roughly 40% of patients with prolonged TKI exposure may reach sustained CMR; once TKI treatment is stopped, roughly 40% will remain in CMR off TKIs. Thus, a "functional cure" may be achievable in 0.40 × 0.40 = 16% of CML cases. How will we make this better? One might imagine the use of combination therapies, particularly immunologic approaches, to push patients from the MMR range of disease burden into CMR. Indeed, once the community demands drug-free molecular negativity, transplant popularity may increase, as one can imagine potent TKI therapy followed by an NMA regimen as a strong combined approach in select cases.

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CHAPTER 49

Hematopoietic Cell Transplantation for Juvenile Myelomonocytic Leukemia

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Introduction

Heterogeneity in presentation has long impaired a rational approach to the clinical management of children with juvenile myelomonocytic leukemia (JMML). The recent discovery that approximately 90% of children with JMML have either somatic or germline mutations in genes involved in the RAS signal transduction pathway, such as *PTPN11* (the gene encoding SHP-2), *CBL*, *NRAS*, *KRAS*, or *NF1*, has greatly improved our understanding of the molecular pathogenesis of this disorder. Further, it enormously facilitated diagnosis by allowing mutational analyses. With clinical diagnosis confirmed by molecular markers, allogeneic hematopoietic cell transplantation (HCT) can be performed early in the course of the disease. In addition, monitoring of minimal residual disease following transplantation has become feasible for many cases. This chapter summarizes the clinical and molecular features of JMML and reviews the role of HCT.

Classification and epidemiology

JMML is a clonal hematopoietic disorder of early childhood characterized by excessive proliferation of monocytic and granulocytic cells. Following a first case description in 1924 [1], Jean Bernard and his co-workers in Paris carefully described the clinical picture in a group of 20 children in 1962 [2]. They referred to the disorder as subacute and chronic myelomonocytic leukemia and identified prognostic factors for survival [3]. Following the demonstration that the Philadelphia chromosome was absent in infants with chronic myelomonocytic leukemia, Hardisty et al. coined the term “juvenile CML” (jCML) [4], and it became common practice to contrast JMML with Philadelphia chromosome-positive chronic myelogenous leukemia (CML) [5]. At the same time, British colleagues proposed a separate entity in children with myeloproliferative disorders and monosomy 7. This infantile monosomy 7 syndrome had clinical features similar to those of jCML, but low hemoglobin F (HbF) levels [6]. Meanwhile, the French–American–British (FAB) group had rationalized the morphologic classification of myelodysplastic syndrome (MDS) in adults [7]. Some investigators argued for the application of modified FAB criteria in children and used the term chronic myelomonocytic leukemia (CMML) instead of jCML [8]. To avoid further confusion, in 1996, an international working group under the direction of Robert Castleberry proposed the term juvenile myelomonocytic leukemia (JMML) and established criteria for confirming diagnosis [9]. JMML incorporates those disorders previously referred to as jCML or CMML of infancy, and also some cases of the infantile monosomy 7 syndrome [10]. To account for both the myelodysplastic and proliferative features noted in JMML, the WHO classification placed the entity in the group of myelodysplastic/myeloproliferative disorders [11,12].

JMML accounts for about 2–3% of all childhood hematologic malignancies [13]. Incidence studies from Denmark and British Columbia showed a JMML incidence of 1.2 per million children per year [13], while a much lower incidence of 0.6 per million has been reported from the United Kingdom [14]. It is unknown whether these differences are due to different geographic incidence rates or to variations in reporting.

Clinical presentation and differential diagnosis

JMML predominates in infants with a median age at diagnosis of 2 years [3,8]. About 9% of patients are diagnosed by the age of 4 months, whereas only 8% are 6 years or older. There is a male predominance, with a male-to-female ratio of 2:1.

Pallor, fever, infection, skin bleeding, and cough are the most commonly presenting symptoms [8]. Marked splenomegaly and hepatomegaly are generally present and may give rise to abdominal discomfort. Occasionally, spleen size is normal at diagnosis, but it rapidly increases thereafter. Gut infiltrates may predispose to diarrhea, sometimes with bloody features, and gastrointestinal infections. About half the patients have lymphadenopathy [8,15]. In addition, leukemic infiltrates may give rise to markedly enlarged tonsils. Dry cough, tachypnea, and interstitial infiltrates on chest X-ray are signs of peribronchial and interstitial pulmonary infiltrates. JMML rarely involves the central nervous system, although a small number of patients with local leukemic infiltration and ocular chloroma, diabetes insipidus, or facial palsy have been described [8].

Leukemic skin infiltrates are common. Most often they present as eczematous eruptions or indurated raised lesions with a central clearing. In addition to these often non-specific lesions, juvenile xanthogranulomas may be seen. In some children, xanthogranulomas are associated with multiple café au lait spots and the clinical diagnosis of neurofibromatosis type 1 (NF1); in children with

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germline CBL syndrome, a Noonan syndrome (NS)-like phenotype, one or two café au lait spots may be present [16].

Signs of autoimmunity such as antinuclear antibodies or positive antiglobin test can be noted in up 25% of children with JMML, while hypergammaglobulinemia is present in more than half of them [8]. In occasional cases with RAS mutations, autoimmunity, and indolent clinical course, the diagnosis of autoimmune lymphoproliferative syndrome (ALPS) has been entertained [17,18]. Although ALPS is generally characterized by a sustained elevation of αβ double negative T cells and leukopenia rather than myeloproliferation, there might be overlap cases.

The clinical picture of JMML can be mimicked by a number of viral infections, such as Epstein–Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus-6 (HHV-6), or severe bacterial disease, such as endocarditis [19–21]. More interestingly, some children with leukocyte adhesion deficiency [21], infantile malignant osteopetrosis [21], or Wiskott–Aldrich syndrome (WAS) [22] can present with a picture morphologically and clinically resembling JMML. Hence the latter disorders need to be excluded in infants with myeloproliferation in the absence of a molecular lesion of the RAS signal transduction pathway. Myeloproliferation with monosomy 7 but absence of splenomegaly can be noted in rare cases of GATA2-haploinsufficiency syndrome [23]. In some JMML cases, striking hemophagocytosis is observed; although hemophagocytic lymphohistiocytosis (HLH) is characterized by a low rather than elevated white blood cell (WBC) count, the differential diagnosis between these two rare disorders can be puzzling [24].

JMML in neurofibromatosis type 1

The diagnosis of NF1 with multiple café au lait spots can be established in up to 11% of children with JMML [3,6]. As estimated from these data, the risk of developing JMML for patients with NF1 is 200–350-fold higher than in patients without NF1 [25]. A positive family history for NF1 is known in about half of cases of NF1 and JMML. Although clinical parameters generally do not differ between patients with or without NF1, NF1 is more common among the few patients who are diagnosed after the age of 5 years. In addition, children with NF1 have a higher platelet count and higher percentage of blasts in the bone marrow (BM) than patients without NF1 [8].

Myeloproliferation and JMML in Noonan syndrome and related rasopathies

Germline mutations in a number of genes coding components of the RAS-MAP kinase-signaling pathway have led to the definition of a new group of autosomal dominant, genetic disorders, the so-called neuro-cardio-facio-cutaneous syndromes or RASopathies [26]. They comprise NF1 (see above), Noonan syndrome (NS), and NS-related disorders. NS is characterized by short stature, distinct facial anomalies, a typical spectrum of congenital heart defects, including stenosis of the pulmonary artery, hypertrophic cardiomyopathy, and septal defects, mild coagulopathy, and often developmental delay, in addition to failure to thrive. Approximately 50% of patients with NS have germline mutations in the *PTPN11* gene encoding for SHP-2, a tyrosine phosphatase protein [27].

Some infants with NS develop a myeloproliferative disorder within the first months of life that is clinically and hematologically indistinguishable from JMML [28]. On a molecular level, these children harbor germline *PTPN11* mutations, which exhibit a weaker "gain of function" effect of the phosphatase SHP-2 than the acquired somatic mutants found in JMML [29,30] (see the later section Molecular pathogenesis). Most children with NS and a myeloproliferative disorder show spontaneous resolution of their myeloproliferation [28]. They are, however, often critically ill and may benefit from intermittent cytoreductive therapy, for example, with 6-mercaptopurine [31]. Few patients with NS and myeloproliferation acquire additional lesions such as cytogenetic aberrations and thus experience an aggressive clinical course [28].

In rare cases, NS can be caused by constitutional *KRAS* [32] or *NRAS* mutations [33], and some of these patients may present with a myeloproliferative disorder resembling JMML [32,34,35].

Another less common rasopathy with NS-like clinical features is caused by constitutional mutations in *CBL* which functions as an E3 ubiquitin ligase [16]. Interestingly, children with CBL syndrome and JMML may show 1–2 café au lait spots and xanthogranuloma [16].

Hematologic features

Leukocytosis, thrombocytopenia, and anemia are common findings in JMML patients. The median WBC count is 33×10^9/L [3,8,15]. In contrast to Philadelphia-positive CML, the WBC count rarely exceeds 100×10^9/L. A presenting WBC count under 10×10^9/L is occasionally noted, particularly in children with monosomy 7 [6,8].

Microscopy of the peripheral blood smear is the most important step in establishing the diagnosis. Immature monocytes, along with myelocytes, metamyelocytes, and nucleated red blood cells, are usually evident. Almost all cases show a striking monocytosis, often with dysplastic cell forms, and an absolute monocyte count exceeding 1×10^9/L is required for the diagnosis of JMML (Table 49.1) [9]. Often, a few blasts can be noted on peripheral blood smears, but they rarely exceed 20% [8]. Thrombocytopenia is a common finding, with about 14% of children having a platelet count at diagnosis below 20×10^9/L. Most patients have a hemoglobin concentration between 7 and 11 g per 100 mL. Red blood cells are generally normocytic, while macrocytosis is noted in some patients with monosomy 7 [8].

BM findings in JMML are not by themselves diagnostic, but rather are consistent with the diagnosis. The aspirate shows a high cell number with predominance of granulocytic cells at all stages of

Table 49.1 Diagnostic criteria of JMML

I. Clinical and hematological features (all three features mandatory)
  - Peripheral blood monocyte count $>1 \times 10^9$/L
  - Blast percentage in peripheral blood and BM <20%
  - Splenomegaly

II. Oncogenetic studies (1 sufficient)
  - Somatic mutation in *PTPN11*[a] or *KRAS*[a] or *NRAS*[a,b]
  - Clinical diagnosis of NF1 or germline *NF1* mutation
  - Germline *CBL* mutation and loss of heterozygosity of *CBL*[c]

III. In the absence of one parameter listed under II, the following criteria have to be fulfilled:
  - Absence of Philadelphia chromosome (*BCR/ABL* rearrangement)
  - Absence of GATA-2 germline mutation
  - And at least two of the following criteria:
    - Spontaneous growth or GM-CSF hypersensitivity in colony assay
    - Hemoglobin F increased for age
    - Myeloid precursors on peripheral blood smear
    - White blood cell count $>10 \times 10^9$/L
    - Monosomy 7 or other chromosomal abnormality

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[a] Germline mutations (indicating Noonan syndrome) need to be excluded.

Diagnosis of JMML but spontaneous regression of myeloproliferation may be noted in:

[b] Very few patients with NRAS mutation and normal HbF.

[c] Patients with *CBL* germline mutation and loss of heterozygosity.

Source: adapted from Gratwohl et al. 2009 [9].

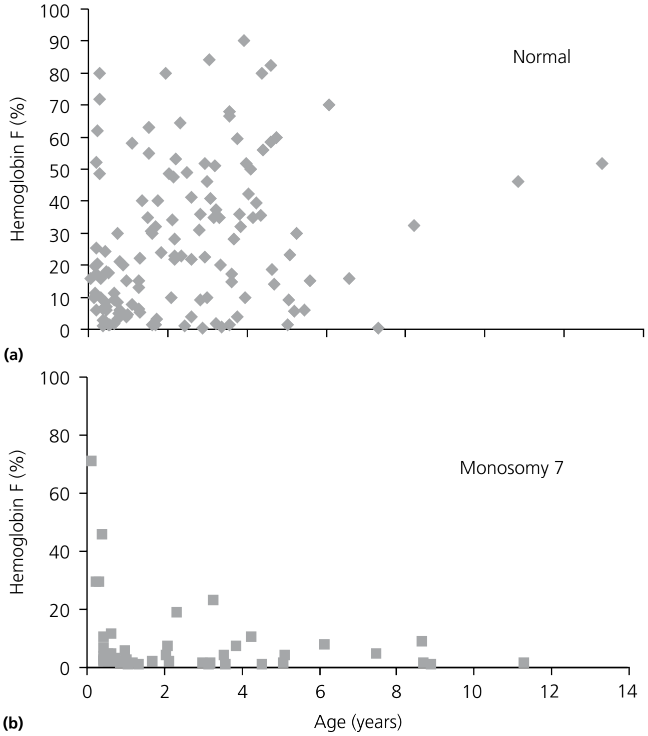

Figure 49.1 Concentration of hemoglobin F for patients with JMML and either normal karyotype (a) or monosomy 7 (b).

maturation, except for the few cases in which the erythroid series predominates. Monocytosis in BM is generally less impressive than that in peripheral blood [3,8]. The marrow blast count is moderately elevated, but never reaches the level seen in acute leukemia. Megakaryocytes are reduced in number or absent in about two-thirds of cases. A remarkable feature of many JMML cases with a normal karyotype is a markedly increased synthesis of HbF (Figure 49.1), resulting from a high number of circulating F cells [36].

Chromosomal abnormalities

Chromosomal studies of leukemic cells show monosomy 7 in approximately 25% of JMML patients, with other abnormalities in 10%, but the majority (65%) have a normal karyotype [6,8,15]. Although the clinical characteristics of patients with monosomy 7 do not differ from those of patients with a normal karyotype, patients with monosomy 7 display some characteristic hematologic features [8]. In addition to a lower median WBC count, red blood cells are often macrocytic, and erythropoiesis in BM is more pronounced than in cases with a normal karyotype. In addition, patients with monosomy 7 present with a normal or only moderately elevated HbF, which is often elevated in patients with normal karyotype (Figure 49.1). Among the chromosomal abnormalities other than monosomy 7, loss of material on the long arm of chromosome 7 is frequent [8]. As mentioned before, JMML lacks the Philadelphia chromosome and the *BCR-ABL* fusion gene.

Molecular pathogenesis

A hallmark feature of JMML is the formation of abnormally high numbers of granulocyte–macrophage colony-forming units (CFU-GM) in methylcellulose cultures containing low concentrations of granulocyte-macrophage colony-stimulating factor (GM-CSF) [37]. Thus, GM-CSF hypersensitivity of myeloid progenitors had been utilized as an important diagnostic tool. Phosphorylation of the βc chain of the GM-CSF receptor creates docking sites for adapters and signal relay molecules, resulting in activation of the RAS pathway.

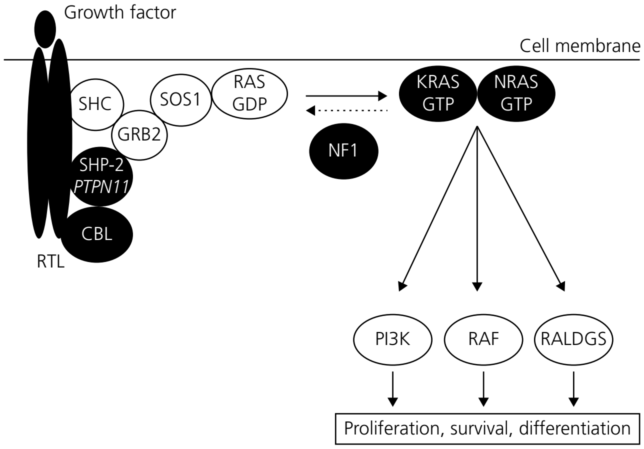

Figure 49.2 Simplified RAS signaling pathway. Proteins involved in the pathogenesis of JMML (due to biallelic inactivation of *NF1*, *CBL*, or heterozygous somatic mutations in *PTPN11*, *KRAS*, or *NRAS*) are indicated in black.

Studies on human leukemic cells and mouse models provided evidence that GM-CSF hypersensitivity is due to a selective inability to downregulate RAS-dependent signaling pathways (Figure 49.2). In 90% of JMML cases, the pathologic inactivation of the RAS signaling cascade results from oncogenic molecular alterations in *NF1*, *PTPN11*, *NRAS*, *KRAS*, or *CBL*. Downstream of RAS, the activation of the Raf/MEK/ERK pathway, the phosphoinositide-3 kinase (PI3K)/Akt/mTOR cascade, and RALGDS are implicated in malignant transformation and tumor maintenance [38] (Figure 49.1).

Dominant somatic mutations of *KRAS* and *NRAS*

Approximately 25% of JMML cases harbor dominant somatic mutations of *NRAS* or *KRAS* in hematopoietic cells [39,40]. Members of the RAS family of signaling proteins regulate cellular proliferation by cycling between an active guanosine triphosphate (GTP)-bound state (RAS-GTP) and an inactive guanosine diphosphate (GDP)-bound state (RAS-GDP). Highly conserved regions within RAS that are crucial for the function of RAS as a molecular switch include the phosphate-binding (P) loop (codons 11–16) and the switch I (codons 33–37), and switch II (codons 59–66) regions [41]. Oncogenic *RAS* mutations typically introduce amino acid substitutions at positions Gly12, Gly13, or Gln61. Mutant *RAS* alleles encode proteins that are locked in the GTP-bound conformation.

Expressing oncogenic *KRAS*^{G12D} is sufficient to initiate a rapidly fatal myeloproliferative disorder in mice associated with hyperproliferation, tissue infiltration, and aberrant responses to growth factors [42,43]. While the myeloproliferative disorder initiating activity is restricted to hematopoietic stem cells that acquire a competitive proliferative advantage over wild-type cells [44], cooperation with additional genetic events is needed to produce frank malignancy [45]. Children with JMML and somatic *KRAS* mutations generally have a fatal clinical course [39], although Japanese investigators reported some cases with long-term spontaneous improvements

[46,47]. Uniparenteral disomy of *KRAS* can contribute to aggressive leukemic transformation [48].

Mouse models demonstrate clear phenotypic differences between mutant KRAS and NRAS [38,49]. Expressing *NRAS*^{G12D} from its endogenous genetic locus produces an indolent myeloproliferative disorder in mice, which ultimately die of a diverse spectrum of hematologic cancers [50]. Similarly, in an HSCT model harboring the *NRAS*^{G12D} mutation, recipient mice develop myeloproliferative disease with a prolonged latency and acquisition of multiple genetic alterations, including uniparental disomy of the oncogenic *NRAS* allele [51]. Children with JMML and somatic *NRAS* mutations generally have a fatal course unless HCT is performed. However, in a small number of infants with *NRAS* alterations and normal HbF spontaneous resolution of myeloproliferation despite persistence of the *NRAS* mutated clone, and long-term survival in the absence of HCT was been observed [52].

Germline mutations in *NF1* and loss of heterozygosity

The conversion from active RAS-GTP to the inactive RAS-GDP state is facilitated by GTPase-activating proteins (GAPs). Neurofibromin, the protein encoded by the gene for NF1, functions as a GAP and negatively regulates *RAS*. As described above, 11% of children with JMML have constitutional NF1 [3]. These patients carry one intact and one deficient allele of the *NF1* tumor suppressor gene in the germline. Loss of the normal *NF1* allele is common in JMML cells from children with NF1, resulting in severely deregulated RAS signaling with aberrant growth of hematopoietic progenitor colonies in vitro. Two studies indicated that a common mechanism of NF1 inactivation is loss of heterozygosity based on uniparental disomy (UPD) replacing the wild-type *NF1* allele by the second copy bearing the *NF1* mutation [53,54]. In a mouse model, some heterozygous *Nf1* mutant animals develop a myeloproliferative disorder [55] and recipient mice reconstituted with *Nf1*$^{-/-}$ fetal hematopoietic cells succumb to a rapidly fatal disease that models JMML [56].

Dominant somatic mutations in *PTPN11*

Shortly after the discovery of mutations in protein tyrosine phosphatase non-receptor type 11 (*PTPN11*) in about 50% of patients with NS, specific germline *PTPN11* alterations were identified in the young children with NS syndrome who developed a myeloproliferative disorder within the first few weeks of life [29,57]. Subsequently, somatic *PTPN11* mutations were uncovered in JMML cells from 35% of children with JMML [29,57]. The *PTPN11* gene encodes for SHP-2, a tyrosine phosphatase protein that relays growth signals from activated growth factor receptors to other signaling molecules, including RAS. Most *PTPN11* mutations are predicted to disrupt the autoinhibition of the catalytic protein tyrosine phosphatase (PTPase) domain by the N-terminal src-homology 2 (N-SH2) domain, thereby promoting the active conformation of the protein [58]. Somatic mutations observed in patients with JMML differ from those mutations found in patients with NS and JMML-like myeloproliferation and from those detected in patients with NS alone [30]. In elegant functional experiments, several groups have shown that somatic *PTPN11* mutations associated with sporadic JMML exhibit stronger biochemical and biological effects than germline *PTPN11* mutations, leading to the concept that only milder SHP-2 activation may be tolerated during embryonic development [59–61]. Interestingly, in addition to JMML, gain-of-function mutations in *PTPN11* have been rarely associated with other hematologic malignancies in children or adults [62].

PTPase SHP2 plays a crucial role in physiologic hematopoiesis and hematopoietic stem-cell function; endogenous expression of gain-of-function mutants in mice reduces hematopoietic stem-cell repopulating activity and promotes mobilization of stem cells from the bone marrow to the spleen [62]. Whereas the endogenous expression of gain-of-function Shp2D61Y induces myeloproliferation, but not acute leukemia [63], the gain-of-function Shp2E76K mutation results in a myeloproliferative disease that progresses to acute leukemia of multiple lineages [64].

Germline *CBL* mutations and loss of heterozygosity

CBL was originally discovered as the cellular homolog of the *V-Cbl* oncogene, the transforming gene of the Cas NS-1 murine retrovirus that causes myeloid leukemia in mice. It encodes a member of a family of RING finger proteins, which functions as an E3 ubiquitin ligase. CBL negatively regulates signaling by tyrosine kinases and functions as an adapter protein to regulate signaling positively [65]. Using single nucleotide polymorphism arrays, copy-neutral loss of heterozygosity based on UPD at chromosome 11q identified *CBL* mutations in myeloid neoplasm and JMML [66]. In JMML, the *CBL* mutations were found to target preferentially amino acid Y371 in the linker region, whereas mutations affecting this amino acid are rare in other myeloid neoplasms [67]. The exclusivity of *CBL* mutations with respect to other RAS pathway-associated mutations indicated that *CBL* plays a role in deregulating the RAS pathway in JMML [66,68,69].

Subsequently, it was shown that children with JMML and *CBL* mutations have germline missense *CBL* mutations and display several congenital anomalies that overlap with NF1, NS, and Legius syndrome [16]. Individuals with germline *CBL* mutations are at increased risk of developing loss of heterozygosity for the *CBL* locus in hematopoietic stem or progenitor cells. The resulting myeloproliferative disorder either may be self-resolving or follow an aggressive clinical course [16,66,70,71].

Secondary mutations

While mutually largely exclusive mutations in *NF1*, *PTPN11*, *KRAS*, *NRAS*, and *CBL* are noted in 90% of CMML patients, alterations seen in other myeloproliferative disorders such as JAK-2 [72–74], TET-2 [75,76], ASXL2 [76,77], SETBP1 [78], and JAK-3 [78] are rare, as are *FLT3* mutations [79,80].

Natural course and prognostic factors

JMML is a rapidly fatal disorder for most children if left untreated. Low platelet count, age above 2 years at diagnosis, and high HbF at diagnosis are the main predictors of short survival (Figure 49.3) [3,6,8]. In a retrospective series of 110 cases, all children presenting with a platelet count of 33×10^9/L or less had died within 1 year from diagnosis, whereas those with higher counts and age less than 2 years at diagnosis had a median survival of 3 years [8]. British investigators devised a scoring system, where HbF of 10% or higher and a platelet count of 33×10^9/L or less negatively affect prognosis [6]. Blastic transformation is infrequent in JMML, and most untreated patients succumb to respiratory failure due to pulmonary infiltration of mature leukemic cells.

As outlined above, there are rare patients with somatic mutations in *NRAS* [52] and possibly *KRAS* [46,47] who experience spontaneous resolution of their JMML despite persistence of the mutated clone. Low HbF and high platelet counts will be helpful in distinguishing these few cases from the generally fatal courses of JMML.

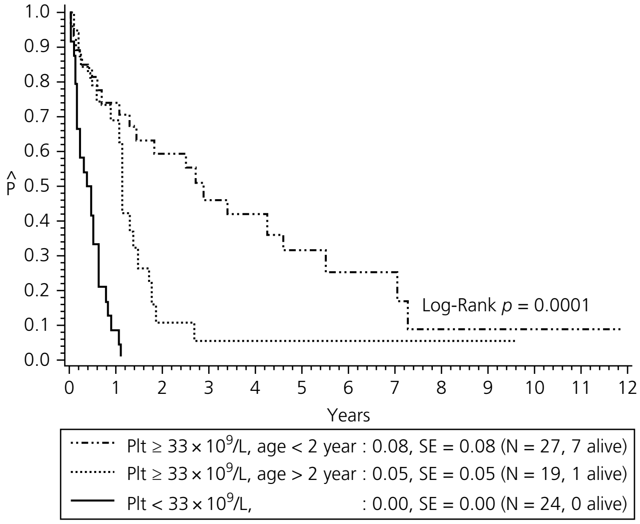

Figure 49.3 Survival of patients without bone marrow transplantation according to platelet count and age at diagnosis [8].

In addition, children with germline *PTPN11*, *NRAS* and *KRAS* mutations and myeloproliferation generally show amelioration with time and do not require HSCT [28]. The same applies to most children with germline *CBL* alterations.

On a molecular level, gene expression studies found that patients with JMML could be separated into two prognostic groups. Interestingly, the profile of JMML cells in cases with poor prognosis was reminiscent of that of acute myeloid leukemia (AML) cells. The 10-year event-free survival (EFS) after HCT was 6% for AML-like and 63% for non-AML-like patients [81]. Furthermore, epigenetic alterations were shown to be associated with outcome [82]. Using MassARRAY, JMML cells were analyzed for aberrant DNA methylation at four gene loci. Hypermethylation was noted in 13–54% of JMML cases and, in multivariate analysis, this epigenetic characteristic retained prognostic value for survival and relapse post-HCT independently of other clinical risk factors [82].

Non-transplant approaches

To ameliorate disease activity prior to HCT, a great variety of antineoplastic drugs and combination-type therapies have been applied in JMML in the past [31]. Clinical and hematologic responses in JMML have most consistently been obtained with mercaptopurine, administered either as a single agent or in combination with low-dose cytarabine or etoposide. There are, however, no data indicating that therapy with mercaptopurine influences the duration of survival.

Clinical remissions and long-term survival after AML-type combination therapy have been reported in small series [31]. Study CCG 2891 of the Children's Cancer Study Group for treatment of childhood AML, MDS, and JMML included 13 children with JMML [83]. Seven of the 12 JMML patients receiving intensive induction chemotherapy achieved hematologic remission. Other investigators have pointed out that intensive chemotherapy is notably unsuccessful in patients with aggressive disease, and durable remissions may not be achievable [31]. A recent phase II trial sponsored by the Children's Oncology Group (COG) utilized cytoreductive therapy consisting of fludarabine and high-dose cytarabine concomitantly [40].

Data from culture studies showing inhibition of JMML colony formation prompted the clinical utilization of interferon-α and 13-*cis*-retinoic acid [84,85]. While a prospective study with interferon-α sponsored by the Pediatric Oncology Group was stopped owing to excessive toxicity [86], preliminary results on 10 children with JMML treated with isoretinoin 100 mg/m^2 daily were promising [87]. However, a large proportion of these children were below the age of 2 years, a factor known to characterize patients with a less aggressive course. In a subsequent phase II window trial of the COG, 22 evaluable patients were accrued: five complete and four partial responses were noted [88]. Other investigators did not observe significant clinical responses with retinoic acid [89], and its value in JMML remains questionable.

Because aberrant DNA methylation is a strong predictor of poor outcome in JMML [82], epigenetic therapeutic approaches may be promising. One of the agents, azacitidine, is believed to exert its antineoplastic effects by multiple mechanisms including cytotoxicity on abnormal hematopoietic cells in the BM and hypomethylation of DNA. The cytotoxic effects of azacitidine may result from multiple mechanisms, including inhibition of DNA, RNA, and protein synthesis, incorporation into RNA and DNA, and activation of DNA damage pathways. Incorporation of azacitidine into DNA results in the inactivation of DNA methyltransferases, leading to hypomethylation of DNA. DNA hypomethylation of aberrantly methylated genes involved in normal cell cycle regulation, differentiation, and death pathways may result in gene re-expression and restoration of cancer-suppressing functions to cancer cells. Following an intriguing case of JMML and clonal monosomy 7 with complete hematologic and molecular response to azacytidine [90], a phase I/II study with azacitidine was initiated by the European Working Group of Myelodysplastic Syndromes in Childhood (EWOG-MDS) and the Consortium for Innovative Therapies for Children with Cancer (ITCC) (NTR2578). Azacytidine may be a viable option for JMML patients, either as a pre-transplant window or in the absence of a suitable HCT donor.

In summary, most investigators prescribe 6-mercaptopurine for pre-transplant therapy in children with JMML. Single doses of low-dose cytarabine are added if more cytoreduction is needed. In critically ill children, the combination of fludarabine and high-dose cytarabine can be lifesaving, although rapid leukemic regrowth is common. The response to demethylating agents is promising and will need confirmation in clinical studies.

Therapy targeting Ras-dependent pathways

The understanding of the important role of GM-CSF and the downstream RAS signaling transduction pathway has led to the design of therapeutic strategies targeting individual components of the pathway. The GM-CSF receptor has been targeted by the GM-CSF analog E21R. In a child with JMML relapsing after HCT, the administration of two cycles of E21R resulted in a transient clinical and hematologic response lasting for about 60 days [91]. RAS proteins are synthesized as precursor molecules in the cytoplasm, becoming activated in part by the addition of farnesyl moiety catalyzed by the enzyme farnesyl-protein transferase. Farnesyltransferase inhibitors (FTIs) can reduce JMML colony formation in vitro but did not inhibit leukemic infiltration in *Nf1*$^{-/-}$ mice [92]. In an up-front phase II window the Children's Cancer Group (CCG) studied the effects of the FTI tipifarnib (FTI R115777) in newly diagnosed patients with JMML [93]. The overall response rate was based on changes in WBC count and organomegaly. Among the 38 patients receiving more than two courses, 22 had a complete or partial response. There was no relationship between farnesyl-protein transferase inhibition or response and the presence of *RAS/PTPN11* mutation or inhibition of prenylation in vitro [93]. Furthermore, there was no impact on survival [40].

The two effector pathways of RAS signaling, the Raf/MEK/ERK cascade and the P*i*3K/Akt/mTOR pathway, are logical potential therapeutic targets in JMML. Agents specifically designed to target components of these cascades are still to be studied in the future. Although these strategies may not be sufficient to abolish the malignant clone by themselves, they could have an important role in future regimens of multimodality therapy. In this respect, rapamycin, an mTOR inhibitor, is integrated as immunosuppressive therapy and minimal residual agents post-HCT in the current COG trial ASCT1221 [40].

Hematopoietic cell transplantation

Early experiences

The first HCT in JMML was reported by the group in Seattle in 1979 [94]. Subsequently, in 1988, the same investigators published data on a series of 14 children including the first case. These early experiences indicated that HCT was capable of curing approximately one-third of the children with JMML. Relapse was the major cause of treatment failure and occurred early in the post-transplant course. In contrast, non-relapse mortality (NRM) in this young patient population was low. Partially matched family member donors and unrelated donors (URDs) were introduced in JMML transplantation in the early 1980s. In a retrospective multicenter review of 43 children treated with HCT between 1987 and 1995, and registered to the EWOG-MDS database, the probability of EFS for 18 patients transplanted from a matched URD or a two- to three-antigen-disparate relative was 22% at 5 years, compared with 38% for 25 patients transplanted from an HLA-identical sibling donor ($p < 0.5$) [95]. With the exclusive application of molecular typing the outcome of HCT with URD has improved considerably.

Preparative regimen

During the early experiences, most children with JMML undergoing HCT received total body irradiation (TBI) as part of their preparative regimen. There have been a number of reports indicating more deleterious long-term morbidity in young children after TBI compared with busulfan (BU)-based regimens [96]. Since there are no reports indicating that TBI offers an advantage over a BU-containing preparative regimen, to avoid severe radiation-induced growth retardation, endocrine and neuropsychologic sequelae, and second malignancies, several groups introduced non-TBI preparative regimens for HCT in JMML [97–100]. Most investigators chose BU and cyclophosphamide (CY) combined with etoposide (VP16) [100] or melphalan [95,97,101], whereas others employed BU, cytarabine (Ara-C), and melphalan [102].

In a retrospective study on 27 children with JMML transplanted between 1990 and 1997 in Japan, the probability of EFS at 4 years was 54%, with no difference in outcome between TBI and non-TBI regimens. In contrast, the retrospective EWOG-MDS study demonstrated for the group of children given HCT from an HLA-identical sibling or a one-antigen-disparate relative that the use of BU was associated with a lower probability of relapse (38% versus 78%, respectively), which resulted in a significantly better EFS in comparison with the use of TBI (62% versus 11%, respectively, $p < 0.01$) [95]. A significantly higher relapse rate in patients prepared with TBI was also demonstrated in a literature review on 65 children with JMML transplanted prior to 2000 [103].

The EWOG-MDS chose a preparative regimen consisting of the three alkylating agents BU, CY, and melphalan [97]. Melphalan had been shown to increase the antileukemic efficacy of a BU–CY regimen in children given an unpurged autologous HCT [104]. In addition, a preparative regimen consisting of three alkylating drugs that have non-cell-cycle-specific action appears potentially capable of eradicating stem-cell disorders, such as JMML, in which at least a portion of clonogenic cells are dormant out of cell cycle. In the currently largest prospective study, 100 consecutive JMML patients registered at EWOG-MDS were given unmanipulated grafts after a preparative regimen with BU 16 mg/kg, CY 120 mg/kg, and melphalan 140 mg/m^2. All but three children experienced prompt engraftment. The 5-year probability of EFS for 48 patients given a graft from an HLA-identical sibling and for 52 children transplanted from an HLA-matched or one antigen/allele-disparate URD was 55% [95% confidence interval (CI) 41–70%] and 49% (95% CI 5–63%), respectively (p = NS) [101]. With a cumulative incidence of NRM of 13%, and no significant difference between sibling and URD transplants, this result indicates that the preparative regimen was safe. In addition, it confirmed that, in the last 15 years, the use of URDs has offered minimal or possibly no significant disadvantage compared with employing an HLA-identical sibling [99]. Figure 49.4 gives an update for EFS, NRM, and relapse incidence for the EWOG-MDS study [101] after a median observation time of patients alive of 5.8 years (range 2.3–12 years). Based on the results of EWOG-MDS, the North American Children's Oncology Group (COG) is currently studying whether a conditioning regimen with BU and fludarabine will have less NRM and comparable EFS compared with the BU–CY–melphalan preparative regimen.

Non-relapse mortality

With current supportive care, recent multicenter trials indicated that the probability of NRM in patients transplanted for JMML is not influenced by the use of either an HLA-identical sibling donor

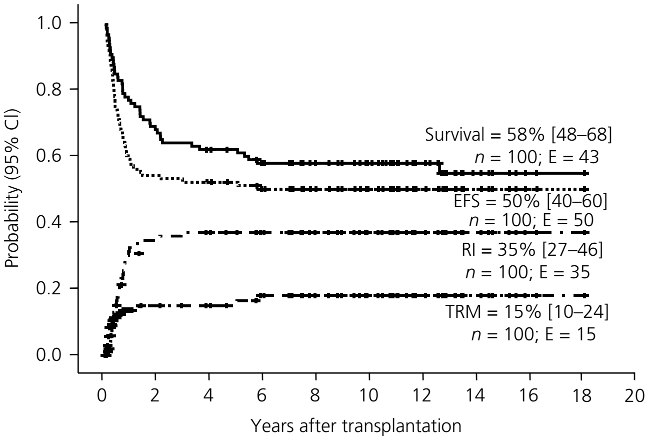

Figure 49.4 Five-year overall survival, event-free survival (EFS), relapse incidence (RI), and transplant-related mortality (NRM) of 100 children with JMML transplanted after a conditioning regimen including busulfan, cyclophosphamide, and melphalan from a matched related ($n = 48$) or a matched unrelated ($n = 52$) donor. Update with a median observation time of patients alive of 11.1 years (range 3.9–18.5 years) of data published by Locatelli et al. [101].

or a URD, with results of 11% [99], 13% [101], and 18% [105]. A similar NRM of 16% has been reported from the large retrospective analysis of umbilical cord (UCB) transplantation for children transplanted after 2003 [106]. This surprisingly low mortality in a generally much compromised patient population transplanted with an intensive preparative regimen might be explained by the young patient age and the experience of centers dedicated to HCT in infants.

Relapse

Relapse is the major cause of treatment failure in patients with JMML undergoing HCT [95,99,101,105]. It generally occurs within the first year after transplantation, the peak being observed after a median time of 2–6 months [101,105,106]. Older age [95,99,101], increased percentage of HbF [101,105], abnormal karyotype [99,106], and blast percentage above 20% [101] have been identified as patient-specific risk factors for disease recurrence. Age above 2 years at diagnosis and a high HbF have been found to predict short survival also in studies analyzing the natural history of JMML in patients not treated with HCT [3,6,8].

In the multivariate analysis of the prospective EWOG-MDS study, age remained the major significant factor for prediction of disease recurrence. The cumulative incidence of relapse for children less than 2 years of age at diagnosis, 2–4 years, and older than 4 years was 18% (95% CI 10–32%), 46% (95% CI 30–70%), and 73% (95% CI 53–98%), respectively, resulting in a probability of EFS of 64% (95% CI 51–76%), 50% (95% CI 31–70%), and 16% (95% CI 0–34%), respectively [101]. An update on these data with a median observation time of patients alive of 5.8 years (range 2.3–12 years) is shown in Figure 49.5. The poor outcome for older children with JMML demands new strategies such as a better preservation of a graft-versus leukemia (GVL) effect. It also points to the fact that HCT shortly after diagnosis is indicated even in patients less than 1 year of age.

As mentioned above, an AML-like gene expression profile and an aberrant hypermethylation of certain genes predict an increased risk of disease recurrence [87,88].

Graft-versus-host-disease and graft-versus-leukemia-effect

The most frequently used regimens for graft-versus-host disease (GVHD) prophylaxis consist of cyclosporine (CSP) for patients transplanted from an HLA-identical sibling donor, and of CSP with methotrexate and antithymocyte globulin for URD transplantation [101,106]. The prospective EWOG-MDS study reported a cumulative incidence at day 100 of grade II–IV GVHD of 40% (range 31–51%), whereas that of grade III–IV acute GVHD was 17% (range 11–26%) [101]. There was no difference in the incidence of grade II-IV GVHD between matched sibling donor (46%) and URD donor (35%) transplant recipients (p = NS). No patient- or transplantation-related variable was significantly associated with the development of acute GVHD in multivariate analysis [101]. Acute GVHD grade III–IV has been associated with poor overall survival in JMML patients [99,105].

Owing to the young age of JMML patients, incidence rates for acute and chronic GVHD tend to be lower than in other leukemic diseases. Chronic GVHD in children transplanted for JMML has been reported in 17% of children at risk in the prospective EWOG-MDS study [101], in 25% of patients in the NMDP cohort [105], and in 10 of 24 cases in the Japanese review [99]. Interestingly, in contrast to EWOG-MDS reporting the lowest cumulative incidence of chronic GVHD, investigators from the United States and Japan noted a better overall survival for children suffering from chronic GVHD compared with those without [99,105]. These data suggest a possible GVL effect directed against JMML cells. A number of reports have described the efficacy of withdrawal of immunosuppressive therapy in patients with incipient relapse of JMML [103]. In addition, less intensive GVHD prophylaxis is generally employed during second HCT, a procedure resulting in long-term survival similar to that of first HCT despite the use of the same donor [107]. Taken together, there is sufficient evidence that GVL plays a major part in eradicating JMML cells following allogeneic transplantation. Not surprisingly, HCT after in vitro depletion of donor T-lymphocytes is hampered by a high relapse rate (EWOG-MDS Registry, unpublished observation).

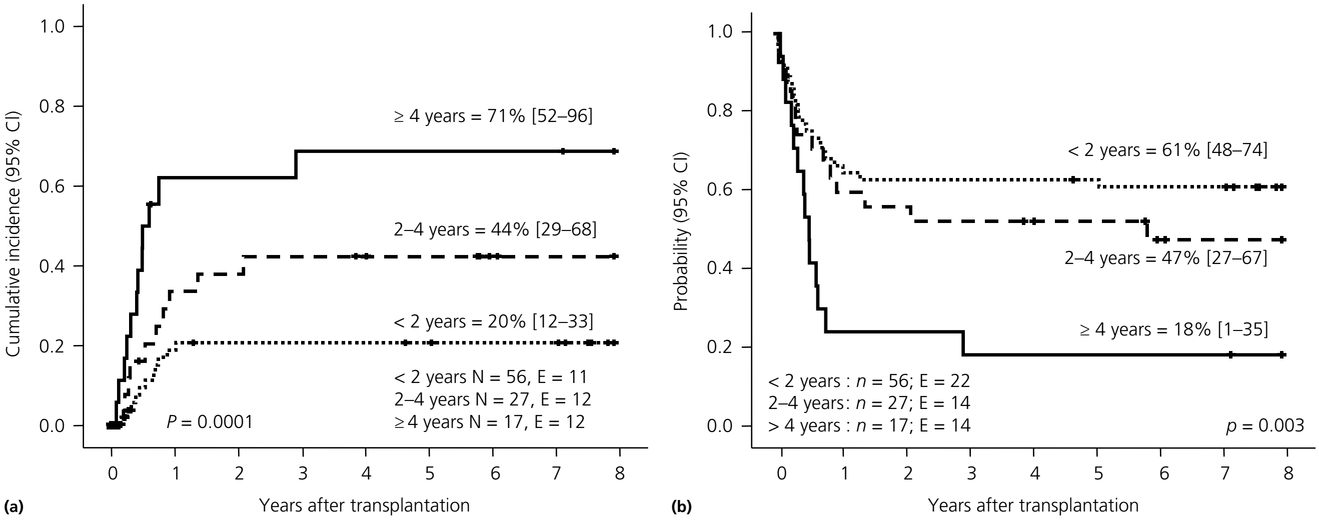

Figure 49.5 Relapse incidence (a) and event-free survival (b) by age at diagnosis of 100 children with JMML transplanted after a conditioning regimen including busulfan, cyclophosphamide, and melphalan from a matched related ($n = 48$) or a matched unrelated ($n = 52$) donor. Update with a median observation time of patients alive of 11.1 years (range 3.9–18.5 years) of data published by Locatelli et al. [101].

Stem-cell source and umbilical cord blood transplantation

Most HCT in JMML has been performed with unmanipulated BM cells. HCT with peripheral blood hematopoietic cells does not result in a better overall survival or lower relapse rate [101]. In the last two decades, UCB from a URD has been increasingly used as an alternative source of hematopoietic stem cells. Major advantages of UCB compared with BM in this young patient population generally in urgent need for HCT are rapid availability of stem cells and the possibility of using donors with HLA disparity with respect to the recipient. Although UCB transplantation in JMML had been successfully performed [101,108,109], there was concern that it may have an insufficient GVL effect for this aggressive form of leukemia. A recent retrospective analysis on behalf of Eurocord, the European Group for Blood and Marrow Transplantation, EWOG-MDS, and the Center for International Blood and Marrow Transplant Research on 110 children with JMML given a single-unit unrelated donor UCB demonstrated outcomes similar to what has been reported with different sources of donors of hematopoietic stem cells [106]. With a median follow-up of 64 months, the 5-year probability of overall survival was 52% (95% CI 47–59%). Better outcome was associated with age at diagnosis younger than the median age of 1.4 years, use of donor with either 0 or 1 HLA disparity with the recipient, and a karyotype other than monosomy 7 [106]. Despite a greater HLA disparity, the probabilities of grade II–IV acute GVHD (41%, 95% CI 37–45%) and chronic GVHD (15%, 95% CI 11–19%) were similar to those reported in studies employing HLA-matched BM or peripheral blood cells. There was a trend for an increased risk for NRM of patients given UCB transplantation before 2003, the 95% CI of NRM being 11–21% for children receiving grafts after 2003 and 23–35% for those given UCB transplantation before 2003 [106]. Thirty-seven patients relapsed at a median of 2.6 months; the 5-year cumulative incidence of relapse was 33% (95% CI 28–38%) [106], a value similar to what has been reported in studies using BM grafts. Because there is experimental evidence that the presence of two competing UCB grafts elicits powerful cytotoxic reactions during early post-HCT phase, double cord transplantation had been advocated in cases of disease occurrence following a first allograft [110]. Overall, the available evidence indicates that UCBT is a suitable option for a large proportion of children with JMML lacking an HLA-identical sibling donor. A search for a suitable unrelated cord blood unit should be initiated simultaneously with that for locating an HLA-matched unrelated BM donor.

Chimerism studies and MRD

As in other hematologic malignancies, serial quantitative chimerism studies using a small number of tandem repeat markers have been shown to identify patients with increasing mixed chimerism who are at high risk for relapse following transplantation for JMML [111]. Because it could be demonstrated that rapidly increasing mixed chimerism is invariably followed by relapse of JMML [111], immediate withdrawal of immunosuppressive therapy is indicated after the first detection of mixed chimerism. This maneuver appears safe and effective, although a number of cases of successfully sustained remission have been reported to be associated with chronic GVHD [112]. In some patients, rapid progression of disease occurs in the absence of a previously documented mixed chimerism status. More sensitive methods, such as the application of disease-specific markers, may increase sensitivity, thus allowing the detection of a smaller percentage of residual leukemic cells. For this purpose, a fluorescently based, allele-specific polymerase chain reaction assay to detect the most common *RAS* or *PTPN11* mutations was developed [113]. Prospective studies will be required to demonstrate whether this method will allow modification of post-transplant immunosuppression early enough to prevent hematologic relapse.

Donor lymphocyte infusion

Treatment options for JMML patients relapsing after HCT are limited. Withdrawal of immunosuppressive drugs is usually the first measure, which by itself can control leukemia in a limited number of patients [103,112,114], suggesting a GVL effect in JMML. In case of non-response and for patients suffering disease recurrence after discontinuation of immunosuppressive agents, donor lymphocyte infusion (DLI) or second HCT may be considered. There are several reports on successful therapy with DLI of relapsed JMML [112,115–117]. Two of three patients with chromosomal studies reported had a normal karyotype, one monosomy 7. Following chemotherapy ($n=2$) or splenectomy ($n=1$), DLI from the matched ($n=2$) or one HLA locus-mismatched ($n=1$) URD had been administered. GVHD was observed in two patients and BM failure in one, and all patients were alive at the time of reporting. These case reports suggest that at least some relapsed JMML patients can benefit from DLI. However, reporting bias is likely to exaggerate the benefit of this form of therapy in single successful cases. In this respect, in a retrospective study from EWOG-MDS, the efficacy of DLI was studied in 21 JMML patients receiving DLI for mixed chimerism ($n=7$) or relapse ($n=14$) [114]. Response rates were significantly higher in patients receiving a higher total T-cell dose ($\geq 1 \times 10^7$/kg) and in patients with an abnormal karyotype. None of the six patients receiving DLI from an HLA-identical sibling donor responded. In total, six of the 21 patients responded to DLI, but only one of the responders remained alive in remission, two relapsed, and three died of complications. Although JMML can be sensitive to DLI, the overall outcome of patients responding to DLI is poor due to complications and relapse.

Second transplantation

Several large reports on the results of second HCT for relapse after first HCT in patients with various types of leukemia have been published. These analyses generally show a poor outcome with a high rate of relapse and NRM. Because relapse remains the major cause of transplant failure in children with JMML, second HCT had been attempted in a number of patients. Chang et al. reviewed the reports on 13 patients undergoing second transplantation, seven of whom were alive and disease free with a median follow-up of 53 months [118]. A retrospective review of EWOG-MDS confirmed that second HCT can be an effective salvage therapy for children with JMML relapsing after a first grafting procedure [107]. Twenty-four patients were given a second allograft for relapse diagnosed at a median of 160 days after first HCT. The median time between first and second HCT was 260 days. The same donor was employed for both first and second HCT in 19 of the 24 patients. Ten patients (43%) were alive in remission after a median observation time of 3.3 years. The probability for leukemia-free survival at 3 and 5 years was 50% (95% CI 30–70%) and 32% (95% CI 8–56%), respectively. Although none of the six patients who developed chronic GVHD after the second HCT experienced leukemia recurrence, eight of 14 patients without chronic GVHD relapsed after the second HCT ($p=0.04$). It is reasonable to speculate that reduced immunosuppressive therapy employed during the second grafting procedure resulted in a stronger GVL effect and favorable outcome. Eighteen of the 24 patients had received a BU-containing regimen for first

HCT and a TBI-based regimen for second HCT, and nine of 18 were alive in remission. A report on HCT in JMML from the Seattle group included six patients who underwent a second allograft, using TBI–CY ± BU and BU–CY regimens for the first and second HCT, respectively [119]. Only one of the six patients was alive in remission after the second HCT. Promising, although still preliminary, results have been obtained using treosulfan-based regimens (EWOG-MDS Registry, unpublished results). In conclusion, current results on second HCT in JMML are superior to those obtained with DLI and, surprisingly, similar to those reported for first HCT.

Splenectomy and other treatment prior to transplantation

Although some investigators have suggested that aggressive chemotherapy can ameliorate the disease in some children with JMML [83], there is no evidence that it reduces relapse incidence or improves survival following transplantation [101]. Therefore, intensive chemotherapy prior to HCT for JMML is not recommended by most investigators. Likewise, none of the other therapy approaches has been shown to have an impact on post-transplant survival. Whether epigenetic drugs, such as 5-azacytidine, or agents targeting the RAS pathway prior to HCT may be beneficial is currently unknown. Splenectomy remains a controversial intervention for children with JMML. In Philadelphia chromosome-positive CML, it has not been shown to improve survival after allogeneic HCT. However, gross enlargement of the spleen may give rise to abdominal discomfort and excessive transfusion requirements. Therefore, early splenectomy has been recommended for amelioration of the disease, and in the last study of the COG, all clinically stable patients were scheduled for splenectomy. In a retrospective study of 72 children with JMML who received HCT, splenectomy prolonged survival independently of other risk factors (authors' own unpublished observations). Although it has been common practice to remove large spleens prior to HCT [95], the benefit of splenectomy for prevention of post-transplant relapse is unknown [98]. In the prospective EWOG-MDS study, splenectomy before HCT, and also spleen size at time of the allograft, did not have an impact on post-transplantation survival, relapse rate, or time to engraftment [101]. Similarly, splenectomy did not influence survival in the large retrospective analyses on UCB transplantation in JMML [106]. Results of these large cohorts are not in favor of the indiscriminate use of splenectomy before transplantation. Splenectomy at a young age is associated with an increased risk of life-threatening infections. The indication of performing splenectomy has to be carefully evaluated for each individual child. The presence of massive splenomegaly with evidence of hypersplenism and/or refractoriness to platelet transfusion can be an argument for considering this procedure in order to promote engraftment, to hasten hematologic recovery, and to lower the risk of hemorrhagic complications.

Outlook

Currently, about 60% of children with JMML can be cured following first or second HCT. Reduction of relapse incidence for older children continues be one of the major challenges for future clinical trials. Less immune-suppressive therapy for GVHD prophylaxis and careful monitoring of MRD following transplantation could be instrumental in reducing the relapse risk. Preparative regimens with better antileukemic efficacy can be designed, but major advances in transplant biology in children with JMML may depend on new insights into the etiology and molecular genesis of the disease.

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CHAPTER 50

Hematopoietic Cell Transplantation for Adult Acute Myeloid Leukemia

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Introduction

Acute myeloid leukemia (AML) defines a spectrum of myeloid malignancies with many common features but also considerable variability in underlying biology and clinical outcomes. Because the majority of patients will not be cured with conventional chemotherapy, hematopoietic cell transplantation (HCT) plays an important role in the management of this disease. However, given the multiple forms of transplantation available (allogeneic related, allogeneic unrelated, autologous, reduced intensity, etc.), together with the variability in AML, careful evaluation and planning are required to integrate HCT optimally into the care of each patient.

Epidemiology and etiology

In 2015, approximately 20,830 Americans will develop AML. The incidence of AML is roughly constant over the first three decades of life and then increases almost exponentially [1]. In most cases, no clear etiology can be found. A genetic predisposition exists in some individuals. The concordance rate between identical twins approaches 100% if AML develops within the first year of life, but subsequently declines with age. “Pure familial leukemias” are rare syndromes without other manifestations caused by single mutations in several genes, including *RUNX1*, *CEBPA*, and *GATA2*. An increased incidence of AML is seen in the RNA-ribosomal bone marrow failure syndromes, Diamond–Blackfan, Shwachman–Diamond, and dyskeratosis congenita, and also in syndromes associated with defective DNA repair, including Fanconi anemia and Bloom syndrome. AML is also increased in Li–Fraumeni syndrome and in syndromes associated with abnormal chromosome numbers, including 21+ and XXY.

Environmental exposure to benzene or benzene-containing solvents has been associated with an increased incidence of myelodysplasia and AML. The incidence of AML is doubled in cigarette smokers. With increasing use of radiation and chemotherapy to treat malignancy, treatment-related AML has become more frequent and now accounts for 6–8% of AML cases [2]. AML following alkylating agent exposure typically has a latency period of 5–7 years and first appears as a myelodysplastic syndrome (MDS) with abnormalities of chromosomes 5 and 7. AML following topoisomerase II exposure has a shorter latency without an MDS prodrome and often has chromosomal abnormalities involving 11q23. However, following combination chemotherapy, many cases of treatment related AML fail to fall into a discrete category. In general, patients with treatment-related AML respond less well to therapy than patients with de novo AML, even after adjusting for other known risk factors.

Other myeloid malignancies eventually evolve into AML. This commonly occurs in the MDSs, occasionally happens with polycythemia vera and idiopathic myelofibrosis, and rarely is seen in essential thrombocythemia. AML arising from an antecedent hematologic malignancy usually has trilineage dysplasia and frequently has unfavorable cytogenetic changes. Approximately 3% of AMLs in patients under age 60 years and as many as 15% of cases over age 60 years arise from a prior myeloid malignancy.

Molecular and cellular biology

Cell of origin

AML is a clonal disorder, with all leukemia cells in an individual patient descending from a common precursor. The initial proof of clonality in AML came from studies of the disease in females who were heterozygotic for the X-linked glucose-6-phosphate dehydrogenase isoenzyme. Whereas normal hematopoietic cells in such individuals would be a 50:50 mix of the two isoenzymes, leukemia cells are all of one isoenzyme or the other, indicating their clonal origin. Recent genome-wide analyses of AML have demonstrated considerable complexity with a number of recurrent potential “driver” mutations in each individual case. Further studies of individual cells within single cases of AML have found considerable mutational variability from cell to cell, suggesting that the development of AML is not a simple progressive accumulation of mutations in a liner direction, but rather a dynamic process with multiple cycles of mutation acquisition and clonal selection [3].

Within the clonal population of AML, there must be a subpopulation of cells capable of both self-renewal and proliferation. Efforts to identify the nature of such “AML stem cells” have been based on the ability of a cell to establish leukemia when transferred to an immunodeficient mouse. Such studies indicate that leukemia stem cells make up a relatively rare subpopulation of the leukemia, accounting for only 0.2–100/10^6 leukemic cells, and have a primitive CD34^{++}CD38^{-} phenotype, no matter what the degree of differentiation of the rest of the leukemia cells is [4]. However, defining a leukemic stem cell by its ability to engraft in an immunodeficient mouse is admittedly arbitrary and may not identify a true AML stem cell.

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Pathology

Although the leukemia stem cell may have a primitive phenotype, the bulk of leukemia cells in any individual will exhibit morphologic evidence of some differentiation. For more than three decades, the French–American–British (FAB) system was used to classify AML, dividing cases among eight categories (M0 to M7) according to the degree of differentiation and lineage [5]. The prior FAB system has since been incorporated as part of the newer World Health Organization (WHO) Classification of AML (see Table 50.1). AML morphology is of little significance once the underlying molecular abnormality is taken into consideration.

Immunophenotype

AML blasts can be defined according to the combination of myeloid-associated antigens expressed on the cell surface. The most immature cases express CD34, CD117, and HLA-DR. CD13 and CD33 are pan-myeloid markers and are present on the surface of most cases of AML. CD14, CD15, and CD11b are associated with monocytic differentiation. CD36 and CD71 are associated with erythroid leukemia whereas CD41a and CD61 are associated with megakaryocytic leukemia. In 10–20% of cases, AML blasts express a single B- or T-cell-associated antigen (CD19 or CD2), but this is of no apparent clinical significance. However, in rare cases, AML blasts express multiple lymphoid markers. Such cases of mixed-phenotype acute leukemia have a poor prognosis. Immunophenotyping of AML has several uses. It can confirm the distinction between AML and acute lymphocytic leukemia, and it has some weak prognostic power. Most commonly, however, since AML cells virtually always have an immunophenotype distinct from that of normal cells, immunophenotyping in the context of multidimensional flow cytometry (MFC) is used as a method of detecting minimal residual disease (MRD).

Table 50.1 World Health Organization classification of acute leukemia with corresponding FAB classification subtypes (2008)

- AML with recurrent genetic abnormalities
  - AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
  - AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
  - APL with t(15;17)(q22;q12); *PML-RARA*
  - AML with t(9;11)(p22;q23); *MLLT3-MLL*
  - AML with t(6;9)(p23;q34); *DEK-NUP214*
  - AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
  - AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*
  - *Provisional entity: AML with mutated NPM1*
  - *Provisional entity: AML with mutated CEBPA*
- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- Acute myeloid leukemia, not otherwise specified
  - AML with minimal differentiation
  - AML without maturation
  - AML with maturation
  - Acute myelomonocytic leukemia
  - Acute monoblastic/monocytic leukemia
  - Acute erythroid leukemia
    - Pure erythroid leukemia
    - Erythroleukemia, erythroid/myeloid
  - Acute megakaryoblastic leukemia
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis
- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
  - Transient abnormal myelopoiesis
  - Myeloid leukemia associated with Down syndrome
- Blastic plasmacytoid dendritic cell neoplasm

Molecular pathology and cytogenetics

Animal models of AML suggest that most cases require multiple mutations for full expression of the disease, including mutations that block differentiation and others that result in inappropriate proliferation. These mutations can be divided into those that are recognizable by particular chromosomal abnormalities and those that require other molecular studies for their recognition.

Recurrent cytogenetic abnormalities

Core binding factor (CBF) translocations

CBFα (also known as RUNX-1) and CBFβ normally combine to form a heterodimeric transcription factor required for hematopoietic differentiation. The t(8;21) abnormality seen in 8% of adult AML cases results in fusion of the gene encoding CBFα on chromosome 8 (*RUNX*-1) with a gene (*MTG8*) on chromosome 21. Inv(16) seen in approximately 7% of AMLs, results in the fusion of the gene encoding CBFβ to the smooth muscle myosin heavy-chain gene. In both cases, the resulting abnormal fusion protein acts as a dominant negative regulator of transcription. As will be discussed in more detail, CBF AMLs tend to occur in younger individuals and have a relatively favorable prognosis [6].

Retinoic acid receptor α translocations

Acute promyelocytic leukemia (APL) represents about 8% of adult AML cases and is essentially always associated with t(15;17), a translocation which fuses the promyelocytic leukemia gene (*PML*) on chromosome 15 with the retinoic acid receptor alpha gene (*RARα*) on chromosome 17. The resultant PML/RARα fusion protein acts as a dominant negative, in part by recruiting nuclear corepressor and the molecules sin3 and histone deacetylase [7]. All-*trans*-retinoic acid (ATRA) appears to have unique activity in this disease by virtue of its ability to bind to the fusion protein, resulting in a change in its configuration and release of the attached nuclear corepressors. Other rare translocations involving *RARα*, including t(11;17) and t(5;17), give rise to leukemia that clinically resembles APL but is much less responsive to ATRA.

Mixed-lineage leukemia mutations

Translocations involving 11q23 are seen in approximately 7% of adult AML cases. Although t(9;11) is the most common, t(6;11), t(10;11), t(11;17), and t(11;19) are also seen. Murine studies suggest that the *MLL* gene product positively regulates homeodomain (*HOX*) genes, which are required for normal development. Thus, a current hypothesis is that MLL fusion proteins result in increased expression of *HOX* genes resulting in increased self-renewal [8].

Chromosomal abnormalities involving 5q, 7q, and 20q

Abnormalities in 5q, 7q, or 20q are particularly frequent in AML in the elderly, AML secondary to prior exposure to alkylating agents, and AML following MDS [9]. These leukemias have a relatively poor prognosis. Although it has been hypothesized that the recurrent appearance of these abnormalities might be explained by the existence of classic tumor suppressor genes at these locations, no such genes have been identified.

Mutations not detectable on routine cytogenetics

Tyrosine kinase receptor mutations

Receptor tyrosine kinases are a family of proteins with extracellular, transmembrane, and intracellular domains with tyrosine kinase activity. Ligand binding of these proteins leads to receptor dimerization and subsequent phosphorylation of adaptive proteins, which in turn activate RAS and other downstream proteins. Activating mutations in *FLT3*, a prototypic receptor tyrosine kinase, are found in 30–35% of AML patients. The majority of mutations are internal tandem duplications (ITDs), and approximately 25% of mutations are point mutations. The incidence of *FLT3* mutations is higher in older individuals and those with higher white blood cell (WBC) counts at diagnosis. The presence of *FLT3* mutation implies a poorer outcome with conventional chemotherapy, and this trend is increased with increasing size of the ITD and with higher allelic ratios of mutated to wild-type genes in marrow specimens. Mutations in other receptor tyrosine kinases are sometimes seen in AML, including mutations in *FMS* and *KIT* [10].

RAS mutations

RAS is a guanosine diphosphate-binding protein activated by various tyrosine kinases. Activation of RAS has multiple effects depending on the cell and its particular state, but can result in cellular proliferation and transformation. Mutations in RAS are found in 15–20% of AML cases. Such mutations prevent hydrolysis of RAS-GTP, thereby keeping RAS in the "on" position. For normal function, RAS must have a farnesyl or geranylgeranyl lipid attached, so compounds that inhibit farnesylation have been studied as possible therapies in AML.

NPM1 mutations

NPM1 encodes a nucleolar phosphoprotein with multiple hypothesized functions. *NPM1* is mutated in 30% of AML cases and is associated with monocytic differentiation, normal cytogenetics, and an improved overall prognosis.

C/EBP alpha mutations

C/EBP alpha stands for CCAAT/enhancer binding protein alpha, which is a transcription factor required for normal granulocyte differentiation. C/EBP alpha is mutated in 6–10% of AMLs, and such cases tend to have M1 or M2 morphology, intermediate risk cytogenetics, and a favorable clinical outcome.

IDH1/IDH2 mutations

Mutations in *IDH1* and *IDH2* result in loss of their normal enzyme function and create a novel ability of the enzymes to convert α-ketoglutarate into 2-hydroxyglutarate, a possible oncogenic metabolite. Mutations in *IDH1*/*IDH2* are found in 15–20% of AMLs and seem to be associated with a worse outcome, especially in patients with otherwise favorable *NPM1* mutations.

Other non-random mutations in AML

Recently, the Cancer Genome Atlas Research Network performed whole exome sequencing on 200 cases of AML and found each case had, on average 13 mutations of which 8 were random while 5 were in genes recurrently mutated in AML [11]. The most commonly mutated genes were *FLT3*, *NPM1*, *DNMT3A*, *IDH2*, *TET2*, *RUNX1*, *TP53*, *NRAS*, *CEBPA*, and *WT1*.

Gene and microRNA expression Abnormal levels of expression of a number of single genes, including *BAALC*, *EV1*, *ERG*, and *MN1* has been reported in AML and linked with prognosis. Genome-wide expression profiling has identified distinct clusters of AML cases with distinct outcomes. Aberrant expression of multiple microRNAs has also been reported in AML. These studies, however, have not yet impacted standard clinical practice.

Classification

The WHO has proposed a classification schema for AML that includes as subgroups AML with the most common recurrent cytogenetic abnormalities, AMLs that evolve from MDS, AMLs that are therapy related, and, for those that do not fit into the first three categories, AML organized according to morphology (see Table 50.1) [12]. Although comprehensive, this classification system is not organized according to prognosis and therefore is not widely used in the development of treatment guidelines. Two decades ago, the Southwest Oncology Group (SWOG) and the UK Medical Research Council (MRC) each developed and adopted classification schemas that were based solely on cytogenetics [13,14]. The two systems, although not identical, were very similar, and defined the favorable risk group as t(15;17), t(8;21), and inv(16); the unfavorable group as abn(3q), del(5q)/−5, del(7q)/−7, t(6;9), and complex (≥3 unrelated abnormalities). All other cases were defined as intermediate. The MRC has since revised their schema slightly (see Table 50.2).

Although broadly used, these schemas do not include the non-cytogenetic mutational analyses that have more recently become available. More recently, classification systems that combine cytogenetics and molecular studies and which are based on treatment outcome have been developed. One widely used schema has been published by the European LeukemiaNet (ELN) (Table 50.3) [15]. Briefly, the favorable risk group includes the core binding factor AMLs t(8;21) and inv(16), and also those cases with normal cytogenetics and mutated *CEBPA*, or mutated *NPM1* and wild-type *FLT3*. The intermediate-1 category includes all those with a normal karyotype except those defined as favorable, and the intermediate-2 category includes all those with cytogenetic abnormalities not specifically defined as favorable or unfavorable. The unfavorable group includes those with inv(3) or t(3;3), t(6;9), t(v;11), or *MLL* rearrangements, −5 or del(5q), −7, abnl(17p), and complex karyotype. Figure 50.1 illustrates the overall survival of patients with AML according to ELN risk categories [16]. Others have reported similar results[17]. More complex systems that integrate additional mutations, and in some cases gene expression markers, have been published but have not yet gained wide usage.

Table 50.2 Cytogenetic classification of AML

Risk status[a]	SWOG [13]	MRC (1998) [14]	MRC (2010) [14]
Favorable	t(15;17), t(8;21), inv(16)/t(16;16)/ del(16q)	t(15;17), t(8;21), inv(16)/t(16;16)/ del(16q)	t(15;17)(q22;q21), t(8;21) (q22;q22), inv(16) (p13q22)/t(16;16)(p13;q22)
Intermediate	Normal, +8, +6, –Y, del(12p)	Normal, 11q23 abn, +8, del(9q), del(7q), +21, +22, all others	Abnormalities not classified as favorable or unfavorable
Unfavorable	abn(3q), del(5q)/–5, –7/ del(7q), t(6;9), t(9;22), 9q, 11q, 20q, 21q, 17p, complex (≥3 unrelated abnormalities)	abn(3q), del(5q)/–5, –7, complex (≥5 unrelated abnormalities)	abn(3q) [excluding t(3;5) (q21–25;q31–35)], inv(3) (q21q26)/t(3;3)(q21;q26), add(5q)/ del(5q)/–5, add(7q)/del(7q)/–7, t(6;11) (q27;q23), t(10;11) (p11–13;q23), t(11q23) [excluding t(9;11) (p21–22;q23) and t(11;19) (q23;p13)],t(9;22) (q34;q11), –17/ abn(17p), complex (≥4 unrelated abnormalities)
Unknown	All other abnormalities	Category not recognized	Category not recognized

[a] Risk for obtaining CR.

Table 50.3 *ELN* risk stratification for adult AML

Genetic group	Subsets
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD (normal karyotype)
	Mutated *CEBPA* (normal karyotype)
Intermediate-I	Mutated *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); *MLLT3-MLL*
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
	t(6;9)(p23;q34); *DEK-NUP214*
	t(v;11)(v;q23); *MLL* rearranged
	−5 or del(5q); −7; abnl(17p); complex karyotype

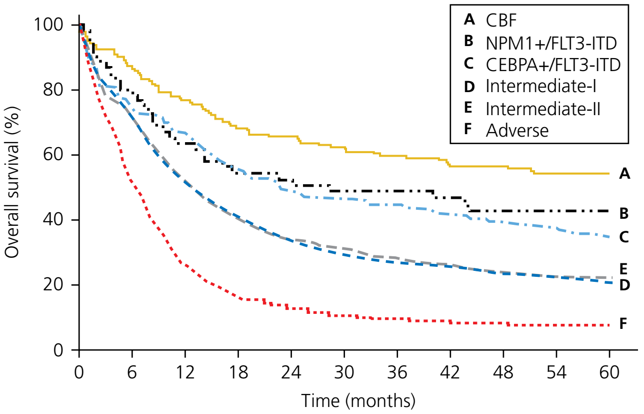


Figure 50.1 Overall survival of patients with AML according to the ELN risk categories. Source: Rollig et al. 2011 [16]. Reproduced with permission of the American Society of Clinical Oncology.

Clinical and laboratory presentation

The usual presenting symptoms of AML relate to decreased production of normal blood cells. Most patients are anemic at presentation and often complain of decreased exercise tolerance, fatigue, and headache. Thrombocytopenia is usually present and approximately one-third of patients note easy bruising, bleeding gums, or epistaxis. One-third of patients will present with significant infections, usually of bacterial origin. In addition to symptoms related to various cytopenias, diffuse bone pain and/or tenderness is noted by approximately 25% of patients. Leukemic infiltrates can give rise to chloromas, gingival hyperplasia, and leukemia cutis.

On presentation, most patients have a normochromic, normocytic anemia and are thrombocytopenic with 50% having platelet counts less than 50,000/mm^3. Most patients are granulocytopenic but the total WBC count is more variable, with 25% having a WBC count above 50,000/mm^3, 25% below 5000/mm^3, and the remainder in-between. The bone marrow is usually hypercellular and, by definition, must have >20% blasts to be considered AML as opposed to MDS. Patients with APL usually have prolonged partial thromboplastin and prothrombin times, reduced fibrinogen, and other evidence of disseminated intravascular coagulation. Blood chemistries in patients with newly diagnosed AML are usually normal, but some patients with very aggressive disease may have evidence of tumor lysis syndrome. Lumbar puncture will show unsuspected central nervous system (CNS) involvement with AML in 2% of cases. However, a randomized trial showed no advantage for CNS surveillance or prophylaxis in adult AML [18].

Non-transplant approach to treatment

Remission induction

If untreated, AML is usually a rapidly fatal disease, with death from bleeding or infection occurring within several months. Guidelines for initial therapy in AML often provide separate suggestions for patients younger or older than an arbitrary cutoff, usually around 60 years, based on the implicit assumption that older patients are less able to tolerate intensive induction therapy. Recent studies have examined the likelihood of early mortality after induction therapy and have found that although age plays a role, other factors, including performance status and prior treatment, make a larger contribution. hence the choice of initial induction therapy should be based on whether the patient is eligible for intensive therapy or not, and not on age per se. In general, intensive therapy is appropriate for patients at least up to age 75 years if they have a good performance status and limited comorbidities. The goal of induction therapy is the achievement of a morphologic complete remission (CR), which, according to the International Working Group recommendations, is defined as <5% marrow blasts with recovery of peripheral counts to an absolute neutrophil count of >1000/mm^3 and a platelet count of >100,000/mm^3 [19]. Standard induction therapy for patients up to age 65 years consists of an anthracycline such as daunorubicin, 60–90 mg/m^2/day for 3 days, or idarubicin, 12 mg/m^2/day for 3 days, combined with cytarabine given at a dose of 100–200 mg/m^2/day by bolus or continuous infusion for 7 days [20,21]. Patients above age 65 years with good performance status are generally treated similarly, but there is no advantage for anthracycline doses above 45–60 mg/m^2/day in this age group. With this approach, 65–80% of patients will achieve an initial remission. Approximately 5% of patients die of bleeding or infection during the period of pancytopenia, and approximately 20% will have persistent leukemia following intensive induction therapy. Numerous variations on the standard “3 + 7” theme have been studied, including other doses and schedules of anthracycline and cytarabine, the addition of other chemotherapeutic agents to the standard two-drug regimen, or the use of hematopoietic growth factors simultaneous with or following chemotherapy. None of these have convincingly improved outcome, although there are some recent encouraging results with the addition of gemtuzumab ozogamicin.

Postremission chemotherapy

If patients are given only induction chemotherapy, AML will inevitably recur, usually within 4–6 months. Therefore, some form of postremission therapy is required. Most contemporary protocols include repetitive cycles of high-dose cytarabine-containing consolidation chemotherapy for patients under age 65 years who are not transplanted in first remission. Among the more widely used regimens are those reported by the Cancer and Leukemia Group B [22], which consists of four cycles of cytarabine 3g/m^2 twice daily on days 1, 3, and 5, and the regimen of the MRC consisting of one cycle of standard “3 + 7” followed by a cycle of “MACE” chemotherapy that includes amsacrine, cytarabine, and etoposide, and a final cycle of high-dose cytarabine plus mitoxantrone [23]. Recently, consolidation with intermediate-dose cytarabine (1g/m^2 every 12 hours on days 1–6) was compared with high-dose cytarabine (3g/m^2 every 12 hours on days 1–6) and no advantage was seen with the higher dose regimen [24]. With standard consolidation therapy, approximately 35–40% of patients who achieve an initial CR can be expected to be alive in continuous first remission at 5 years from original diagnosis. As previously mentioned, the outcome of conventional chemotherapy is heavily influenced by cytogenetic and molecular markers of the disease.

Chemotherapy for recurrent AML

Patients who relapse after an initial remission achieve a second CR 30–50% of the time. Unfortunately, the median duration of second remission is generally short, averaging 6 months, and very few patients will remain in remission if treated only with chemotherapy. Mortality rates associated with reinduction have been 15–25%. Prognostic factors that have been consistently associated with an improved chance of achieving a second remission include younger age, favorable cytogenetic risk group and longer duration of first remission [25]. The choice of reinduction chemotherapy depends on the prior treatment received by the patient and also patient age and health. Younger patients with an initial remission of 6 months or longer are typically treated with a regimen containing an anthracycline plus high-dose cytarabine. The addition of cyclosporine to reverse multi-drug resistance was shown to be efficacious in one randomized trial [26]. For patients with shorter first remissions, alternative therapies, such as clofarabine-containing combinations, should be considered. A considerable number of agents are being studied for recurrent AML, including *FLT3* inhibitors, farnesyl transferase inhibitors, histone deacetylase inhibitors, antiangiogenic agents, and inducers of apoptosis, among others.

The special case of APL

Because APL is uniquely sensitivity to ATRA, arsenic trioxide, and anthracyclines, its treatment differs from that of other AML subtypes [27]. Initial induction therapy generally includes ATRA plus an anthracycline, with or without the addition of other chemotherapy. Complete response rates of 90–95% can be expected with such therapy. Induction failures are usually due to early bleeding events or, less commonly, drug resistance. Patients typically receive several cycles of consolidation therapy including ATRA and an anthracycline. A large prospective randomized trial showed that the further inclusion of two cycles of arsenic trioxide as consolidation therapy significantly improved event-free and overall survival [28]. In previous studies, maintenance with ATRA has also been found to be beneficial. With modern chemotherapy, at least 75% of patients with APL can be expected to be alive and disease free 3 years or more after diagnosis. Both age and WBC count at diagnosis are of prognostic importance, with the best results seen in patients aged less than 50 years presenting with a WBC count of less than 10,000/mm^3. The choice of treatment for recurrent APL depends on the specific clinical situation. Arsenic trioxide is the agent of choice for those patients who have not previously been exposed to the drug; in that setting, second CRs can be expected in 85% of cases. Patients resistant to ATRA and arsenic may respond to gemtuzumab ozogamicin.

Hematopoietic cell transplantation

Indications for allogeneic HCT in AML

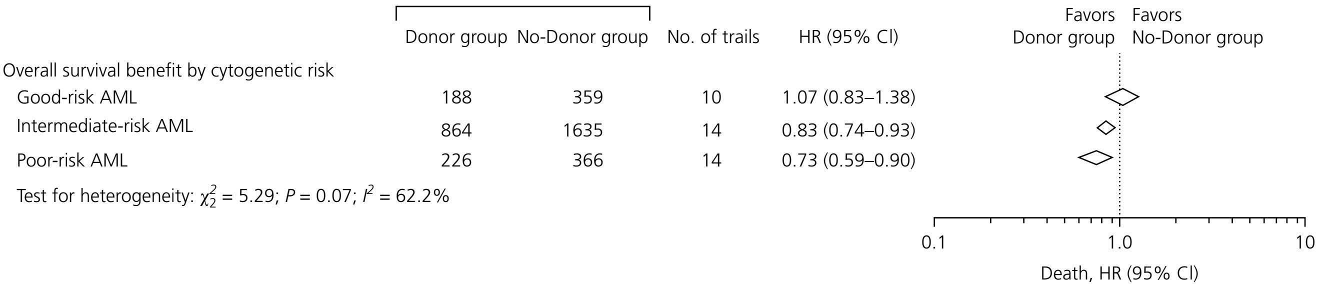
Primary induction failure

Patients who fail primary induction chemotherapy have essentially no chance of cure with alternative chemotherapies, but several studies have shown that long-term survival is possible if such patients undergo allogeneic transplantation from an HLA-matched sibling. For example, the European Group for Blood and Marrow Transplantation (EBMT) reported 21% 3-year disease-free survival in a group of 88 patients with AML who were transplanted after failing at least two cycles of induction chemotherapy [29]. Similar results have been published by others [30]. Because of the time required to perform a search, there is less experience with the use of unrelated donors for primary induction failure, but the limited data available demonstrate that cure is possible in approximately 20% of cases [31]. These results emphasize the importance of HLA typing patients and family members shortly after the diagnosis of AML so that valuable time is not lost in identifying suitable donors should remission attempts fail.

How quickly patients should be taken to transplant is often determined by logistical issues. However, most experts would suggest that there is little to be gained by persisting with standard chemotherapy if a CR is not achieved after two cycles of conventional dose induction, or one cycle containing high-dose cytarabine [32].

First remission

Multiple prospective studies have since been conducted in which patients who had achieved an initial CR were assigned to an allogeneic transplant if they had a matched sibling, or to either consolidation chemotherapy or autologous transplantation if they lacked a donor. Several meta-analyses have been conducted reviewing all such studies, provided that the studies reported overall survival, and were analyzed in an intent-to-treat manner. The meta-analyses found an overall survival advantage to allogeneic transplantation in first remission, compared with either chemotherapy or autologous transplantation [33,34]. If analyzed by cytogenetic risk group, this advantage was greatest for patients with unfavorable risk cytogenetics, was still present in those with intermediate risk disease, but was not present in those with favorable risk disease (see Figure 50.2). Based on these studies, allogeneic transplantation is not generally recommended for patients with favorable risk cytogenetics in first CR. There are, however, data suggesting that among the good-risk group, some subgroups do less well with standard chemotherapy, including those with mutations in *KIT* and those with treatment-related AML. Whether these subgroups do better with allogeneic HCT in first CR is unknown.



	No. of patients			
	Donor group	No-Donor group	No. of trails	HR (95% CI)
Overall survival benefit by cytogenetic risk				
Good-risk AML	188	359	10	1.07 (0.83–1.38)
Intermediate-risk AML	864	1635	14	0.83 (0.74–0.93)
Poor-risk AML	226	366	14	0.73 (0.59–0.90)

Test for heterogeneity: $\chi^2_2 = 5.29$; $P = 0.07$; $I^2 = 62.2\%$

Figure 50.2 Overall survival benefit of allogeneic HCT for AML in first CR by cytogenetic risk group. Source: Koreth et al. 2009 [34]. Reproduced with permission of the American Medical Association.

As noted earlier, patients without clonal cytogenetic abnormalities were previously included in the cytogenetically defined intermediate risk group, but more recent molecular studies have been able to segregate these patients into those with a more favorable outcome with chemotherapy (*CEBPA* mutant, or *NPM1* mutant without *FLT3* mutations) and those with a less favorable outcome (all others). Schlenk et al. published the results of a prospective study involving 872 patients with cytogenetically normal AML in first remission [35]. Those with a matched sibling were allocated to transplantation. Among those with mutant *NPM1* and wild-type *FLT3*, the outcomes were similar whether one had a matched sibling or not [hazard ratio (HR) for relapse or death during CR 0.92, 95% confidence interval (CI) 0.47–1.81], whereas there was a clear benefit for having a donor and proceeding to transplantation for all others (HR 0.61, 95% CI 0.40–0.94) (Figure 50.3).

While the benefit of matched related donor HCT for patients with intermediate and high-risk disease (using the ELN categorization) seems clear, only about one-third of all patients will have a matched relative to serve as donor. The development of unrelated donor registries makes it possible to find matched unrelated donors for many patients. Results from single centers, in addition to registry data, suggest that outcomes of transplantation for AML in first remission using matched related and matched unrelated donors are similar [36,37]. The Center for International Blood and Marrow Transplant Research (CIBMTR) recently compared the outcomes for 600 recipients of matched related HCT with those for 1156 recipients of matched unrelated HCT for AML, about half of whom were in first remission. Survival at 3 years was 39% in matched related recipients compared with 37% for unrelated recipients. In multivariate analysis, these results were indistinguishable [relative risk (RR) = 1.06] (see Figure 50.4) [37].

The National Marrow Donor Program estimates that it is now possible to identify an 8/8 allele matched donor for 45–70% of Caucasians, depending on their country of origin, 29–38% of Hispanics, and 12–20% of African-Americans. Compared with outcomes using a matched related donor, the use of a single antigen-mismatched donor increases the risk of mortality (HR 1.36, 1.19, 1.16, and 1.48 for mismatching at HLA-A, -B, -C and -DR, respectively) [38]. Results with partially matched double cord transplants have recently been compared with the outcomes of matched related and matched unrelated transplants in a study from Seattle and Minnesota [39]. Although transplant-related mortality was higher with double cord transplants, relapse rates were lower and overall survival was equivalent in all three groups. The studies showing relative equivalence of matched related, matched unrelated, and double cord blood transplants argue that the indications for transplantation should generally be the same with all three donor sources. However, these were retrospective comparisons and patient selection bias could have influenced these results. A prospective study has recently been published by the German–Austrian Group (AMLHD98A) in which patients with high-risk AML, defined either by cytogenetics or by failure to achieve a CR with induction therapy, were assigned to transplantation. The 5-year overall survival for those who were not transplanted was 6.5%, compared with 25.1% for those undergoing transplant. There was no difference in outcomes between the 62 recipients of matched related HCT and the 89 recipients of matched unrelated donor HCT [40].

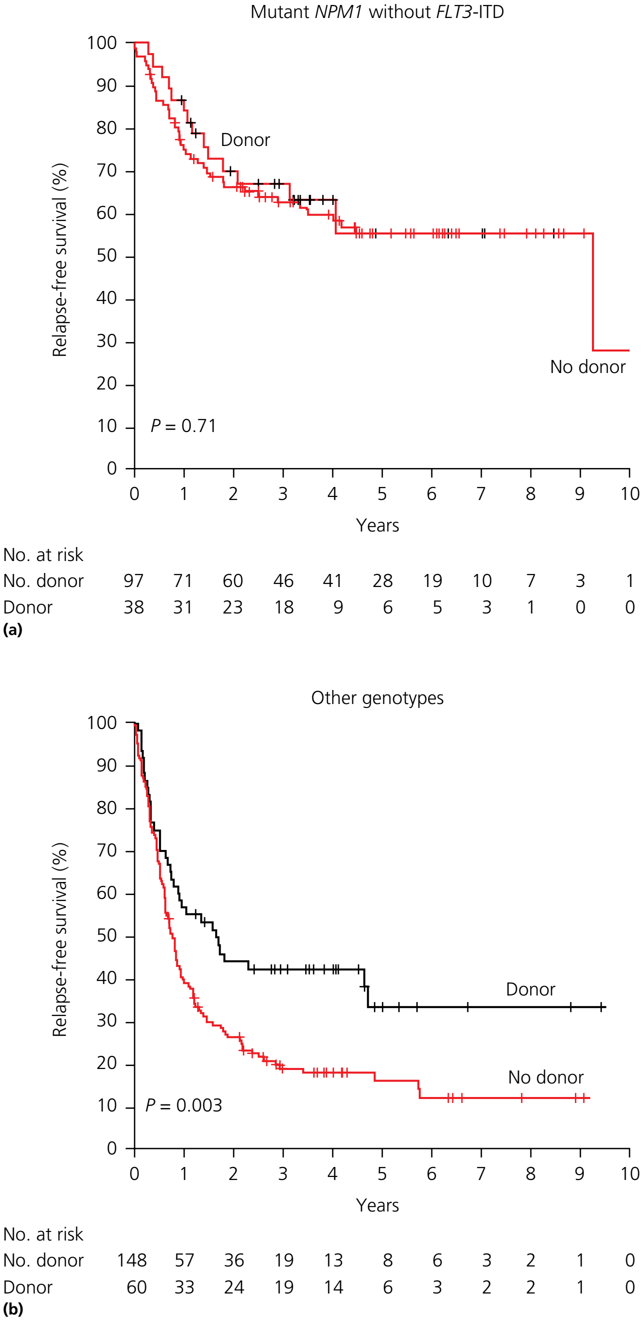


Figure 50.3 Relapse-free survival among patients with AML who achieved a first CR according to the availability of an HLA-matched related donor. Source: Schlenk et al. 2008 [35]. Reproduced with permission of the Massachusetts Medical Society.

The role of allogeneic transplantation for healthy but older patients with AML in first remission is less clear. There are inherent problems in attempting to conduct a prospective trial in this population, including a relative lack of healthy donors, and also patient and physician bias. Accordingly, no sufficiently sized prospective trial comparing transplantation with further chemotherapy has yet been reported. The Japanese National Cancer Center retrospectively examined the outcome of 1036 patients aged 50–70 years with AML who achieved a first CR and were then treated with either allogeneic HCT (n = 152) or chemotherapy (n = 884). Both relapse-free and overall survival were significantly improved in the HCT group, but of course patient selection bias could have influenced the results [41].

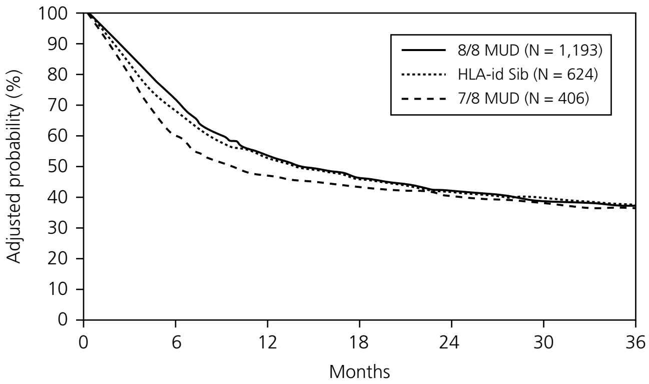


Figure 50.4 Adjusted probability of overall survival in 2223 adult AML patients by donor type. HLA-id Sib, human leukocyte antigen-identical sibling; MUD, matched unrelated donor. Source: Saber et al. 2012 [37]. Reproduced with permission of the American Society of Hematology.

Second or subsequent remission

If patients are not transplanted in first remission, the general consensus is that they should receive an allogeneic transplant in second CR if possible. Occasionally, chemotherapy-induced second remissions can be prolonged, but this is a relatively rare event restricted to patients with favorable risk cytogenetics and a long first remission. Generally, second remissions are short, averaging 6–9 months. Breems et al. developed a prognostic index for overall survival in patients with AML in first relapse and identified the duration of first remission, age, and cytogenetic risk group as the three most important factors predictive of longer survival. However, in this retrospective analysis, no matter what the risk category was, improved survival was seen in those patients who were able to receive a matched related allogeneic transplant [25].

Patients with AML in second CR (CR2) lacking matched siblings are candidates for either matched unrelated or autologous transplantation. The CIBMTR database reports 5-year survivals of 42 ± 2% for unrelated donor transplantation for AML in CR2 and 40 ± 3% with autologous transplantation [42]. The decision to pursue an unrelated versus an autologous transplant for the patients represented in the registry was undoubtedly affected by multiple considerations that could introduce considerable bias into any comparison between groups. No prospective randomized trial comparing autografting with unrelated donor transplants for AML in CR2 has been published. In an effort to reduce possible bias, single-institution and registry comparisons have been performed using either matched-pair analyses or proportional hazards regression adjusted for differences in prognostic variables [43,44]. These studies consistently showed decreased relapse rates but increased non-relapse mortality with unrelated transplantation. In none of the studies was the difference in leukemia-free survival statistically significantly different. None of these studies were designed to address whether particular subsets of patients might benefit from one approach or the other, but the improved control of leukemia with unrelated transplantation but increased non-relapse mortality suggests that unrelated transplantation might be favored in situations where there is a higher risk of relapse and a lower risk of toxicity (younger patients with shorter first remissions) whereas autologous transplantation might be favored in opposite settings (older patients with longer first remissions).

Untreated first relapse

Whether patients with AML in untreated first relapse who are transplant candidates should go directly to transplant or should first undergo chemotherapy in an attempt to induce a second remission prior to transplant is an unanswered question. Frequently the issue is moot, since non-medical issues including insurance clearance and transfer to a transplant center often require time and do not permit rapid initiation of the transplant procedure. However, with improving technologies applied to the detection of minimal residual disease, it is likely that in the near future an increasing number of patients will be diagnosed with incipient relapse, allowing greater lead time and thus making transplantation in untreated first relapse a more feasible approach.

To date, there have been only a limited number of published studies describing the outcome of transplantation in untreated first relapse. Studies from Seattle and elsewhere report survival rates at 5+ years of approximately 30% for matched related donor transplants for AML in untreated first relapse [45,46]. Only limited data exist on the outcome of allogeneic HCT for AML in untreated first relapse using other sources of marrow. A single study of autologous transplantation described 38 patients whose marrow had been stored in first remission who underwent autologous HCT at first relapse using a conditioning regimen of busulfan plus cyclophosphamide and post-transplant received IL-2 with or without lymphokine-activated killer cells [47]. Eight of the 38 patients were alive in remission at the time of the report. Because of the time required to identify and schedule an unrelated donor, there are almost no reports of unrelated donor transplants for AML in untreated first relapse, but the few reported cases suggest an outcome not markedly different from that seen with matched related donors [48].

Although the outcomes for transplantation in untreated first relapse are apparently not as favorable as outcomes in second remission, it should be remembered that reinduction attempts are successful in, at best, 50% of cases, and those who fail reinduction either never are transplanted or are transplanted in refractory relapse. A thought experiment conducted using available data suggests that among 100 patients subjected to reinduction, 50 would achieve a CR2 and if every one of those patients were transplanted, approximately 20 would be cured (50 × 0.40). Among the other 50, 15–20% would die or suffer complications during reinduction that would preclude subsequent transplant, and of the remaining 30, perhaps three (10%) would be cured. Hence this approach would yield 23 cured patients, which is less than the 30% cure rate reported for transplantation in untreated first relapse. Of course, considerable selection bias may have influenced those patients transplanted in untreated first relapse. In limited allogeneic experience of HCT in untreated first relapse, there was a trend towards better outcome in those who developed graft-versus-host disease (GVHD) and in those with less disease burden at the time of transplant, but no effect on the duration of first remission, whereas in the autologous experience, both increasing tumor burden and shorter first remission negatively impacted outcome. Available data are very limited, but based on what is currently known, patients with matched siblings or previously stored autologous stem cells might be considered for transplant in untreated first relapse if disease recurrence is detected at an early stage and logistical issues do not present a roadblock.

Refractory relapse

Various definitions of the term "refractory relapse" exist, making simple statements about treatment outcome for such patients based on published studies difficult. A strict and relatively unambiguous definition of patients in refractory relapse includes only those who have failed their last reinduction attempt. Although a second reinduction using an alternative regimen is sometimes tried, such efforts are rarely beneficial. For example, based on data from a retrospective study conducted by investigators at the M.D. Anderson Cancer Center, the likelihood of achieving a CR was <1% following administration of chemotherapy to patients whose AML failed to respond to the last initial reinduction attempt [49]. Results with transplantation in this strictly defined group of patients are also relatively poor. With matched sibling transplants, disease-free survival at 3 years or more was seen in only approximately 10% of such patients [50]. Similar results have been reported using unrelated donors, with 7% 5-year disease-free survival seen in 81 patients transplanted for refractory relapse [31]. Among those transplanted in refractory relapse, the results are marginally better if patients have a lower leukemia burden without circulating blasts. This has led to the approach of administering intensive chemotherapy in an attempt to clear circulating leukemic blasts prior to initiating a transplant procedure [51]. Whether these attempts at reducing tumor burden change the ultimate outcome of transplantation remains unknown.

Many reports use a less stringent definition of refractory relapse or have lumped together primary induction failure, untreated relapse, and refractory relapse. The CIBMTR reported on 1673 AML patients combining these different forms of relapse [52]. Survival 3 years after transplant was 19%; those with shorter first remissions, circulating blasts, poor performance status, poor risk cytogenetics, and receiving transplant from donors other than HLA-matched siblings not surprisingly did worse.

Indications for autologous HCT in AML

First remission

The comparative utility of autologous HCT versus chemotherapy for patients with AML in CR1 has been the subject of numerous randomized controlled trials. Between 1989 and 1998, six prospective randomized trials were conducted in which patients with AML in first remission were randomized to further chemotherapy or autologous HCT with bone marrow as the stem-cell source. A meta-analysis of these studies found that transplantation was associated with improved disease-free survival, but no improvement in overall survival [53]. In the following decade, three similar studies were conducted, but using peripheral blood rather than marrow as the source of autologous stem cells. The outcomes of those three trials were similar to the previous six, and a subsequent meta-analysis of all trials published through 2010 comparing autologous HCT (using either marrow or peripheral blood) with further chemotherapy concluded that autologous HCT was associated with improved disease-free survival without impacting overall survival [54].

Second or subsequent remission

As noted earlier, registry data report a 40 ± 3% disease-free survival at 5 years for patients with AML in second remission treated with autologous transplantation. These results appear very similar to those achieved with either matched related or matched unrelated donor transplantation, but patient selection bias could play a major role affecting these outcomes. These have been no prospective randomized trials comparing autologous with allogeneic transplantation for AML in second remission, so there are few objective data to guide the physician's choice.

HCT-specific issues

Preparative regimens

The general topic of transplant preparative regimens is reviewed in Chapter 20. The initial preparative regimens used for transplantation in AML were myeloablative, but more recently a large number of regimens of less intensity have been developed. In an effort to facilitate discussion on the topic, the CIBMTR has defined regimens on the basis of what would happen without transplantation of stem cells: (1) myeloablative (myeloid recovery is not expected), (2) reduced intensity (recovery will occur but only after a prolonged period), and (3) non-myeloablative (rapid and uneventful recovery is expected) [55]. A limited number of trials have explored the question of the optimal myeloablative regimen for patients with AML undergoing matched sibling HCT [56]. Two prospective randomized trials studied the issue of total body irradiation (TBI) dose when combined with a set dose of cyclophosphamide (Cy) in patients with AML in CR1. One study showed that 12 Gy of fractionated TBI was superior to 10Gy of TBI delivered as a single dose [57], whereas a second trial found that relapse rates were lower but non-relapse mortality was higher when the dose of fractionated irradiation was increased from 12 to 15.75Gy [56,58]. A single randomized trial explored the form of chemotherapy in combination with TBI and found melphalan and Cy to be equivalent [59]. A further randomized trial compared Cy plus TBI with busulfan (BU) plus Cy and found a significantly lower relapse rate with the TBI-containing regimen resulting in improved disease-free survival [60]. Subsequent retrospective registry data comparing 223 patients with AML in first CR transplanted using a Cy plus TBI regimen with a similar number of patients treated with BU plus Cy found relative equivalence of the two regimens [61]. Reasons for the discrepancy between the randomized trial outcome and registry data are not obvious.

As noted above, a large number of reduced-intensity and non-myeloablative regimens for allogeneic transplantation for AML have been developed. The initial impetus for their formation was the hope that benefits of reduced toxicity would at least balance the cost of increased relapse rates, especially in older patients and those with comorbidities. Despite the extensive literature on the topic, no large prospective randomized trials have been published to date. However, there have been a number of retrospective registry comparisons. Among the largest are reports from the EBMT and the CIBMTR. The EBMT compared 401 AML patients receiving reduced-intensity regimens with 1154 recipients of myeloablative transplants and found less relapse with myeloablative regimens, but lower non-relapse mortality in patients aged over 50 years [62]. These results favor higher intensity regimens in younger, but not older, patients. The study from the CIBMTR reached somewhat similar conclusions when examining the outcomes for 3731 recipients of myeloablative and 1448 recipients of reduced-intensity or non-myeloablative transplants [63]. BMTCTN 0901 was a prospective randomized trial comparing myeloablative to reduced intensity conditioning in the treatment of adults with AML or myelodysplasia. The study was recently stopped by the Data Safety Monitoring Board because of concern for an increased failure rate in the reduced-intensity arm.

Only a single, small randomized trial has compared high-dose preparative regimens prior to autologous transplantation and found relative equivalence between Cy plus TBI and BU plus Cy [61]. Registry data since confirmed the relative equivalence of Cy plus TBI and BU plus Cy for patients with AML undergoing autologous HCT in first CR [64].

Although a number of alternative and novel approaches to high-dose preparative regimen development have been reported, no randomized trials other than those noted above have been published, and insufficient numbers of patients have been treated with any one alternative to allow for retrospective comparisons that would be sufficiently meaningful to alter standard therapy.

Source of hematopoietic stem cells

The source of hematopoietic stem cells for transplantation can be defined both according to the relationship of the donor and recipient (autologous, matched sibling donor, matched unrelated donor) and according to their anatomic site (bone marrow, peripheral blood, umbilical cord blood). The broad topics of stem-cell source are dealt with in greater detail in Chapters 36–45. The following discussion is limited to specific issues of stem-cell source selection in the treatment of AML.

Sibling versus autologous versus unrelated donors

The choice of matched sibling versus autologous versus unrelated donor transplant is, of course, driven by the clinical state of the patient and the availability of hematopoietic stem cells. The only clinical situation in which there are prospective data to guide the choice is in first CR. As noted above, two large meta-analyses have been performed addressing this question and are in general agreement that allogeneic transplantation offers a survival advantage in this setting, particularly in patients with intermediate and high-risk disease, whereas with autologous transplantation, although there may be some gain in disease-free survival, no overall survival benefit was evident [33,34]. A number of single-institution and registry data support the contention that survival following matched related and matched unrelated transplants is now approximately the same [36,37]. Hence indications should not differ between the two. Also, other data suggest, at least for patients aged <50 years, that results with double cord blood transplants are similar to those seen with matched related and matched unrelated transplants [39].

Lack of prospective randomized trials make the choice of donor for patients with more advanced disease less clear. General practice is to use a matched sibling whenever one is available. Retrospective comparisons of autologous transplantation with unrelated donor transplants for patients with AML in second remission have demonstrated similar outcomes [43,44], but such comparisons are inherently limited by possible treatment selection bias, and most experts would favor matched unrelated transplants, particularly for younger patients, reserving autologous transplantation for those who are older, have significant comorbidities, or have incompletely matched donors.

Stem-cell source for autologous transplantation

Purging

Initial studies of autologous transplantation for AML utilized unmanipulated bone marrow. An obvious concern with autologous transplantation is that clonogenic leukemia cells might be reinfused with the remission marrow, a possibility formally demonstrated by Brenner et al. using gene marking of remission marrow [65]. Accordingly, various techniques were developed that in animal models are capable of eliminating clonogenic tumor cells from marrow mixtures without causing obvious harm to the marrow itself (see Chapter 41 for a thorough review). Subsequently, numerous phase I/II trials exploring these techniques were conducted. The most widely used approach was treatment of marrow in vitro with the potent alkylating agent 4-hydroperoxycyclophosphamide (4-HC). A retrospective analysis from the Autologous Blood and Marrow Transplant Registry (ABMTR) reviewed 294 patients with AML who underwent autologous HCT in first remission. In a multivariate analysis, use of 4HC was associated with improved disease-free survival at 3 years (56% with purging vs. 31% without) [66]. Despite encouraging retrospective data, no prospective randomized results have been published, hence this approach is not approved by the US Food and Drug Administration (FDA), and the drug is not currently commercially available.

Peripheral blood versus bone marrow

Autologous peripheral blood hematopoietic cells (PBHCs) collected following chemotherapy and growth factor administration provide an alternative to autologous bone marrow as a source of stem cells for autografting. Use of PBHCs is clearly associated with faster engraftment and reduced early morbidity after autografting, and therefore has become popular. However, the effects of choosing peripheral blood over bone marrow on overall survival are less clear. In fact, Gorin et al., after analyzing 2165 patients who received autografts for AML in first remission, found that the relapse rates were significantly higher with peripheral blood as opposed to marrow, resulting in inferior leukemia-free survival [67]. They further showed that those patients who received the highest CD34$^+$ cell doses, although engrafting faster, had the highest relapse rates [68].

A question of interest with the use of unpurged PBHCs is whether more intensive therapy prior to collection of hematopoietic stem cells might improve the purity of the stem-cell yield, reduce tumor burden in the patient, and thereby decrease subsequent relapse rates. Tallman et al. conducted a retrospective analysis of registry data and reported that the risk of relapse and treatment failure was significantly lower among patients receiving consolidation chemotherapy prior to hematopoietic stem-cell collection than in patients receiving no consolidation [69]. They did not find a difference between groups receiving high-dose cytarabine consolidation regimens and those receiving standard dose regimens. Because of the retrospective nature of the analysis, it is difficult to know if the observed effect was at least in part due to drop-out of high-risk patients during the consolidation phase. Nonetheless, most current trials of autologous transplantation include at least one, and often several, cycles of consolidation prior to hematopoietic stem-cell harvest and transplant.

Hematopoietic stem-cell source for allografting

Bone marrow cell dose

Numerous studies have now demonstrated that bone marrow cell dose is closely linked with clinical outcome after both matched sibling and unrelated donor transplants for AML. This association was initially shown following unrelated donor transplantation for AML where a marrow cell dose of $>3.0 \times 10^8$ nucleated cells per kilogram was found to be associated with a superior outcome [70]. Subsequently, a similar association has been found following matched sibling transplantation. For example, Rocha et al. showed that among 572 patients undergoing allogeneic bone marrow transplantation for AML in CR1, both relapse and non-relapse mortality were reduced in those patients receiving a nucleated cell dose of $> 2.6 \times 10^8$ resulting in superior leukemia-free survival for this group [71]. Similar results have been published by others using the number of CD34$^+$ marrow cells as the variable [72]. The explanation for the association of higher marrow cell dose with improved clinical outcome is not clear, but the association is maintained after accounting in multivariate analysis for patient age, gender, and other obvious potentially confounding factors.

Bone marrow versus peripheral blood

The rapid engraftment seen with the use of autologous growth factor-mobilized PBHCs encouraged pilot studies of the use of allogeneic PBHCs. These pilot studies demonstrated rapid granulocyte and platelet engraftment and, despite a far higher mature T-cell content than present in marrow, no obvious increase in acute GVHD with the use of allogeneic PBHCs. Based on these encouraging pilot studies, a number of prospective randomized trials were conducted comparing marrow with growth factor-mobilized PBHCs as the source of stem cells for matched sibling transplantation following standard high-dose preparative regimens. A meta-analysis of nine randomized trials enrolling 1111 adult patients was conducted and reported [73]. Compared with marrow, use of PBHCs was associated with more rapid neutrophil ($p < 0.00001$) and platelet ($p < 0.00001$) engraftment. Whereas there was no difference in grade 2–4 acute GVHD (54% with PBHC versus 53% with marrow), there was an increase in extensive chronic GVHD (47% versus 31%) and overall chronic GVHD (68% versus 52%) with the use of PBHCs. Use of PBHCs was also associated with a decrease in relapse at 3 years, in both late- and early-stage patients (21% versus 27% overall). Non-relapse mortality was not different between the groups. When considering AML specifically, disease-free and overall survival in late-stage patients were significantly improved with the use of PBHCs (odds ratio = 0.45), whereas the results were less definitive for early-stage patients. A large retrospective registry analysis from the EBMT yielded similar findings [74]. Therefore, for patients with AML beyond first remission undergoing matched sibling transplantation following a high-dose preparative regimen, PBHCs are preferred, but for patients in first remission the choice is less clear.

Pilot studies of the use of PBHCs for matched unrelated transplantation have been conducted [75,76]. These studies were encouraged by the results seen with the use of PBHCs for matched sibling transplantation coupled with the observation that survival appears to be improved with higher CD34$^+$ cell doses following unrelated donor marrow transplantation. The results again showed rapid engraftment without intolerable GVHD. The Blood and Marrow Transplant Clinical Trials Network (BMT CTN) recently published the results of a large prospective randomized trial comparing peripheral blood with marrow for unrelated donor transplantation [77]. The overall 2-year survival was similar with the two stem-cell sources. Peripheral blood resulted in earlier engraftment and less graft failure, but was also associated with more chronic GVHD. This study was not restricted to AML, but given the results, the use of peripheral blood should probably be restricted to those patients in need of a rapid graft, or those at higher risk for graft rejection.

Umbilical cord blood

Umbilical cord blood as a source of hematopoietic stem cells for HCT is the topic of Chapter 39. The amount of information about the utility of cord blood transplants specifically as treatment for adults with AML is limited. An International Bone Marrow Transplant Registry (IBMTR) report compared the outcome of cord blood transplants in 140 adults with leukemia with that seen in recipients of unrelated HLA-matched ($n = 367$) or single antigen mismatched ($n = 83$) marrow [78]. Outcomes with cord blood and one-antigen mismatched marrow were very similar, but both were inferior to those seen with unrelated matched transplants. Results were not provided for specific leukemia subtypes or stages. The EBMT similarly compared outcomes of 98 adult recipients of cord blood transplants with acute leukemia with those seen in 584 recipients of unrelated bone marrow [79]. Multivariate analysis showed lower risks of GVHD with cord blood, but delayed engraftment. No significant differences in transplant-related mortality, relapse rates, or leukemia-free survival were seen between the groups. When the analysis was focused on subsets of patients, a trend towards improved 2-year leukemia-free survival was found for patients with AML treated with matched unrelated marrow compared with cord blood (42% versus 32%), whereas other subgroups appeared to benefit equally from cord blood and marrow. The registry nature of the IBMTR and EBMT reports makes it impossible to account for biases in patient selection, and no attempts were made to control for treatment regimen effects or differences in supportive care or other ancillary measures. Nonetheless, the reports suggest that the outcomes of unrelated bone marrow and cord blood transplants for adults with AML are sufficiently similar that, although one might prefer a matched unrelated donor, if none were available then a partially matched cord blood with sufficient cell dose is a reasonable substitute.

The majority of studies of adult cord blood transplants published so far utilized single cords as the source of stem cells and had a threshold for an acceptable cell dose (usually in the region of 2×10^7/kg), meaning that many possible candidates were not able to be transplanted because a cord of sufficient size could not be identified. The reason for the cell dose threshold is experience showing an increased incidence of graft rejection and poorer survival with lower cell doses. More recent experience combining multiple cords for a single transplant suggests that it might be possible to overcome this limitation. A study from Minnesota and Seattle showed that survival following ablative transplants for acute leukemia in remission are similar with matched sibling, matched unrelated. and double cord blood transplants (Figure 50.5) [39]. Use of cord blood was associated with a higher non-relapse mortality rate, but a lower risk of relapse.

GVHD prophylaxis

The general principles of GVHD prophylaxis are discussed in Chapters 81–84. As outlined in Chapter 81, the results of prospective randomized trials suggest that combinations of methotrexate and either cyclosporine or tacrolimus are the preferred pharmacologic approaches for prevention of GVHD in patients with AML undergoing transplants from matched siblings or matched unrelated donors following high-dose preparative regimens [80]. As discussed in Chapter 81, many alternatives to these regimens are

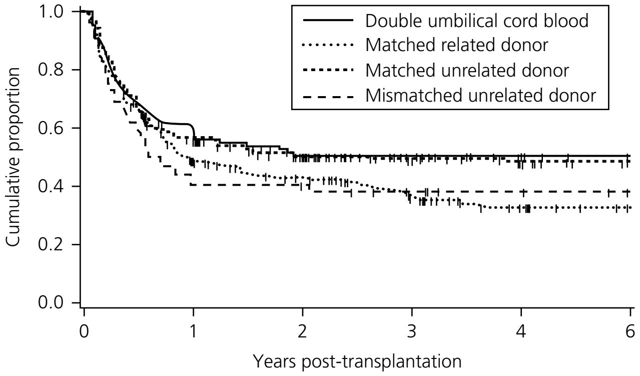


Figure 50.5 Leukemia-free survival among 536 patients by donor type. Source: Brunstein et al. 2010 [39]. Reproduced with permission of the American Society of Hematology.

currently being explored, including, for example, the substitution of sirolimus or mycophenolate mofetil for methotrexate, or the addition of antithymocyte globulin during the peri-transplant period. As yet, however, no prospective randomized trial has been reported showing superiority in terms of survival or disease-free survival of any alternative to the standard combination of methotrexate and a calcineurin inhibitor for GVHD prevention in the setting of high-dose preparative regimens.

As discussed in Chapter 82, removal of T cells from the donor stem cell inoculum as an alternative approach to GVHD prevention has been intensively studied. A variety of methods for T-cell removal have been developed and preparative regimens with increased immunosuppressive effects have been constructed that appear capable of preventing the increased incidence of graft rejection otherwise associated with the use of T-cell depletion. A single prospective randomized trial examining the effects of T-cell depletion on transplant outcome has been reported [81]. In that trial, 405 patients with a variety of hematologic malignancies undergoing matched unrelated marrow transplantation following high-dose preparative regimens were randomized to GVHD prevention with either T-cell depletion (using counterflow centrifugal elutriation or antibody T10B9 plus complement) plus cyclosporine versus pharmacologic immunosuppression with methotrexate and cyclosporine. Disease-free survival at 3 years was 27% with T-cell depletion versus 34% without ($p = 0.16$). Among the 101 patients with AML, disease-free survival at 3 years was 29% with T-cell depletion versus 33% without. This trial used marrow as the source of stem cells. More recently, availability of clinical-grade magnetic bead columns for cell separation have made it possible to accomplish a 4–5 log reduction in T cells from peripheral blood, thereby allowing a T-cell-depleted graft with a substantially higher number of $CR34^+$ cells than is possible using marrow. A recent paper compared the results of 44 patients with AML in CR1 or CR2 receiving T-cell-depleted peripheral blood grafts from matched siblings with 84 contemporaneous patients receiving matched sibling grafts followed by standard immunosuppressive therapy [82]. Overall survival at 3 years was 65% with standard immunosuppression versus 59% with T cell depletion. Chronic GVHD rates were 50% with standard immunosuppression and 19% with T cell depletion.

Measurement of minimal residual disease (MRD)

Measurement of MRD can potentially be useful to direct therapy after initial induction chemotherapy, to monitor response, or to select the best transplant approach. Two general methods have been developed to measure MRD in AML: those based on polymerase chain reaction (PCR) techniques, and those based on multidimensional flow cytometry (MFC) [83]. PCR-based approaches require that the leukemia have either a fusion gene, as in t(8;21) and inv(16) of *MLL* translocations, a mutation, as in *FLT3*, *NPM1*, or *CEBPA*, or that a gene is reliably overexpressed, such as *WT1*. The advantages of PCR-based approaches are their high sensitivity and relative ease of performance. However, only about 70% of AML populations will have fusion genes or mutations, and there are no generally agreed-upon standardized assays and cutoffs for RNA expression.

MFC takes advantage of the fact that AML blasts, in at least 90% of cases, express a combination of cell surface antigens and/or flow cytometric physical abnormalities that are absent in normal marrow cells. Depending on the number of monoclonal antibody combinations used in the assay and the skill and experience of the laboratory, this leukemic fingerprint can be used to detect as few as 0.01% leukemic cells in a remission marrow. Advantages of MFC are its wide applicability, rapid turnaround time, specificity, and ability to provide quantitative results.

Several groups have demonstrated that the level of MRD as measured by MFC after induction therapy predicts the chance of subsequent relapse, with progressively higher rates as the amount of MRD increases in cohorts from 0–0.01%, 0.01–0.1%, 0.1–1%, and above 1%. Most of these studies used marrow as the source of cells, but others suggest that peripheral blood can be used, although the cutoff levels for risk differ. How much measurement of postinduction MRD adds to information already available from pretreatment cytogenetics and molecular testing in deciding who should be transplanted in first CR is as yet uncertain. There are data suggesting that patients with favorable and intermediate risk disease by conventional standards who are MRD positive after induction have a very poor prognosis (15% relapse-free survival at 4 years). Similarly, patients with intermediate risk disease who are MRD free after induction may have an outcome almost as good as that for good-risk patients (60% relapse-free survival at 4 years). Confirmation of these results in larger studies is needed.

How these results should be used in developing a strategy for the optimal care of AML patients is uncertain. Although the presence of MRD after induction might be seen as an indication for transplantation, it also is the case that the presence of MRD at the time of transplant predicts a worse outcome. Studies at the Fred Hutchinson Cancer Research Center examined 253 consecutive patients with AML in first or second remission undergoing myeloablative HCT [84]. Among CR1 patients, the 3-year estimates of disease-free survival were 73 and 27% for patients without and with MRD respectively, and among CR2 patients, the corresponding figures were 71 and 47%. If MRD was modeled as a continuous linear variable, both the risk of death and the risk of relapse post-transplant increased as the level of MRD increased. Whether these outcomes can be improved by treating MRD-positive patients with alternative chemotherapies in the hope of rendering them MRD negative before proceeding to transplantation is unknown.

Relapse following HCT

Disease recurrence is a common cause of treatment failure following HCT, and is the topic of Chapter 70. In AML treated either with chemotherapy or with transplantation, most relapses will occur within 3 years of treatment [85]. Chemotherapy is sometimes successful in inducing a remission in patients who have relapsed after HCT. In one study of 95 patients with AML who relapsed after allogeneic HCT, the CR rate with subsequent chemotherapy was 34% [86]. Not surprisingly, CR rates were higher for those transplanted in remission (47% versus 18%) and for those who relapsed at longer intervals from transplant. Although subsequent remissions were possible, most of these were of limited duration, and without other therapies, long-term survival was seen in only 5% of patients.

By inducing an immunologic graft-versus-tumor effect, donor lymphocyte infusions (DLIs) can sometimes cause CRs in patients with a variety of malignancies who have relapsed after allogeneic HCT [87]. The EBMT retrospectively analyzed the data of 399 patients with AML in first hematologic relapse after HCT whose treatment did ($n = 171$) or did not ($n = 228$) include DLI [88]. Estimated survival at 2 years was 21 ± 3% for patients receiving DLI and 9 ± 2% for patients not receiving DLI. After adjusting for differences between the groups, better outcome was associated with younger age, relapse occurring more than 5 months after HCT, and use of DLI. Among DLI recipients, a lower tumor burden at the time of DLI (<35% marrow blasts), female gender, favorable cytogenetics, and remission at the time of DLI all were predictive of a better outcome. Survival at 2 years was 56 ± 10% for those given DLI while in chemotherapy-induced second CR.

Second allogeneic transplants have been explored as possible therapy for patients whose disease has recurred after an initial autologous transplant. Radich et al. reported on 59 patients, including 24 with AML, who were treated in this manner [89]. The 2-year disease-free survival was surprisingly good for the AML patients, at 46%. However, the majority of these patients were in the pediatric age group, received an autograft with busulfan/cyclophosphamide conditioning as the first transplant, and were able to be treated with a TBI-containing regimen with their allograft. Results in adults and those treated with TBI during their initial autograft were not as encouraging.

Second allogeneic transplantation after initial failure of allografting has also been studied. A survey conducted by the EBMT identified 170 patients with acute leukemia, including 85 with AML who underwent second allogeneic transplants after high-dose conditioning regimens for recurrent disease [90]. Both treatment-related mortality and the actuarial risk of relapse were high at 46 and 59%, respectively. Nonetheless, 25% leukemia-free survival at 5 years was reported. In multivariate analysis, a longer interval to relapse after first HCT, CR status at time of second HCT, use of TBI at second HCT, and development of acute GVHD at second HCT were associated with better outcomes. The leukemia-free survival was 52% at 3 years for those who relapsed more than 292 days after their first HCT and were in remission before receiving their second transplant. Whether one should use the same donor or switch to an alternative donor for the second transplant is unknown. Because in the above studies the development of GVHD was associated with improved tumor control, switching to an alternative donor if no GVHD developed with the first transplant is common practice.

Because of the high treatment-related mortality associated with second allogeneic transplants using high-dose regimens (46% overall, and 53% among those transplanted within 1 year of their first transplant), investigators have more recently been studying the use of reduced-intensity allogeneic transplants for patients whose disease has recurred after a first transplant. Baron et al. reported on 147 patients with hematologic malignancies, including 35 with AML or advanced MDS, who were treated with reduced-intensity conditioning and allogeneic transplantation after failing a conventional autograft or allograft [91]. The treatment-related mortality was 30%, and 44% of patients were alive at 3 years, including 29% of patients with AML or advanced MDS. Being in CR at the time of second transplant and developing chronic GVHD were both associated with improved survival.

The majority of patients included in these previous trials showed recurrence after an ablative preparative regimen. Schmid et al. examined the outcome of 263 AML patients whose disease had recurred after reduced-intensity conditioning [92]. The estimated 2-year overall survival from relapse was 14%. The only long-term survivors were those who achieved a CR with reinduction therapy and then went on to receive either DLI or a second allogeneic HCT.

HCT for APL

Because of the remarkable effectiveness of the inclusion of ATRA, and more recently arsenic trioxide, in the primary treatment of APL, there is no role of transplantation during first remission. Unfortunately, disease recurrence still occurs in approximately 10% of good-risk patients (those presenting with a WBC of <10,000/mm^3) and 25% of poor-risk patients. Second remissions can commonly be achieved with the use or arsenic trioxide, especially if patients have not previously taken the drug, with ATRA-containing regimens if the patients have relapsed off ATRA maintenance, or with the use of gemtuzumab ozogamicin. HCT is generally recommended as appropriate therapy for patients with APL who suffer an initial relapse. de Botton et al. reported on the outcome of therapy in 122 patients with APL whose disease recurred after initial treatment with an ATRA-containing regimen [93]. The 7-year event-free survival was 60.6% for those treated with an autograft, 52.2% for those treated with an allograft, and 30.4% for those treated without transplantation. Most of the patients treated with an autograft had marrow that was PCR negative for the t(15;17) at the time of hematopoietic stem-cell harvest and transplantation, a selection strategy based on previous reports that patients who are PCR positive at the time of autografting for APL have a very high chance of recurrence [94]. The report by de Botton et al., together with those of others, suggests that autologous transplantation is appropriate therapy for patients with APL who achieve a PCR-negative second remission, whereas allogeneic transplantation should be considered as appropriate salvage therapy for all others[95].

Conclusion

A major change from a decade ago is the integration of non-cytogenetic molecular abnormalities into the therapeutic decision-making process in AML. A proposed algorithm for patients with newly diagnosed disease is presented in Figure 50.6. With continued investigation into AML using gene expression arrays, mRNA patterning, and proteomics, this algorithm will likely change in the next decade, even if there are no advances in treatment modalities.

A second major change relevant to transplantation of AML over the last decade has been the continued development of reduced-intensity preparative regimens. These studies show that with appropriate pre- and post-transplant immunosuppression, engraftment can be reliably achieved and that treatment-related mortality can be substantially reduced. This is at the price of increased disease recurrence, but the development of reduced-intensity regimens offers new hope for older individuals who would otherwise not be candidates for transplantation and, importantly, provides a foundation on which to add additional therapies to the preparative regimen, such as radioimmunoconjugates specifically targeted at the tumor cell, or targeted small-molecule therapy during the early post-transplant period.

Continuing developments in the use of alternative donor transplants will further expand the potential application of allogeneic HCT for the treatment of AML. Perhaps the most interesting advance in this area over the past decade has been the emergence of cord blood transplants as a possible alternative for adults with AML. Outcomes of matched unrelated donor and cord blood transplantations for AML are approaching those seen with matched sibling donors. If this approach continues to show promise, it would address a major problem faced by the patient with high-risk AML who has achieved a first CR, does not have a suitably matched family member, and is at considerable risk for early relapse if a lengthy search for an unrelated donor is required.

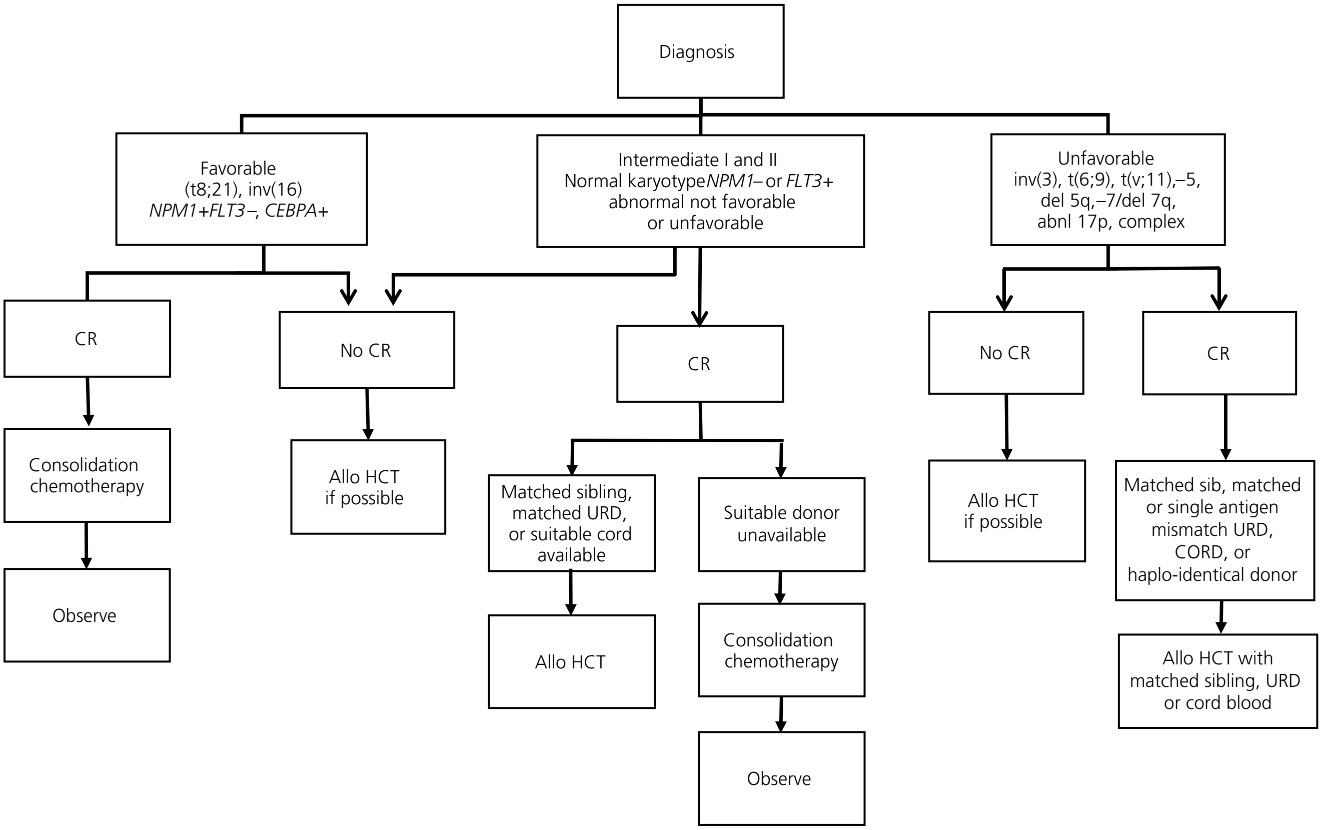

Diagnosis
Favorable
(t8;21), inv(16)
NPM1+FLT3−, CEBPA+
Intermediate I and II
Normal karyotypeNPM1– or FLT3+
abnormal not favorable
or unfavorable
Unfavorable
inv(3), t(6;9), t(v;11),–5,
del 5q,–7/del 7q,
abnl 17p, complex
CR
No CR
CR
No CR
CR
Consolidation chemotherapy
Allo HCT if possible
Matched sibling, matched URD, or suitable cord available
Suitable donor unavailable
Allo HCT if possible
Matched sib, matched or single antigen mismatch URD, CORD, or haplo-identical donor
Observe
Allo HCT
Consolidation chemotherapy
Allo HCT with matched sibling, URD or cord blood
Observe

Figure 50.6 Proposed algorithm for treatment of adults with newly diagnosed AML fit for aggressive therapy.

Although there have been important advances in HCT for AML over the last decade, there are also areas where the amount of progress has been disappointing. One such area is our inability to apply existing technologies systematically for the measurement of minimal residual disease to the decision-making process of who should be transplanted in first remission. It is also disappointing that we have been unable to develop broad clinically applicable methods to segregate the impressive power of the graft-versus-tumor effect from the toxicities of GVHD. These topics remain the focus of a great deal of research that, hopefully, will lead to real therapeutic advances in the near future.

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CHAPTER 51

Hematopoietic Cell Transplantation for Childhood Acute Myeloid Leukemia

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Introduction

Approaches used by various international study groups that include allogeneic hematopoietic cell transplantation (HCT) for childhood acute myeloid leukemia (AML) have shifted over time. Studies in the 1980s and 1990s that compared intensive chemotherapy with autologous HCT in complete remission (CR1) showed similar survival; however, recipients of matched family donor allogeneic HCT benefited from a graft-versus-leukemia (GVL) effect and achieved superior survival [1,2]. Analysis with improved risk classifiers showed that low-risk patients [i.e. t(8;21)] did well with chemotherapy alone [3], and high-risk patients did poorly with any approach [4]. A more clear survival advantage for matched related donor HCT in CR1 was shown in intermediate-risk patients [4]. This approach, however, is challenged by cooperative groups who only allow CR1 HCT in high-risk patients and others only in primary refractory or second remission patients, reasoning that salvage approaches can lead to similar overall survival (OS) [5].

These competing paradigms are being challenged due to several key advances. First, unrelated donor survival outcomes have improved dramatically, potentially suggesting that broader use of HCT could be beneficial [6]. Second, methods of minimal residual disease (MRD) measurement have been developed that more accurately predict outcome after chemotherapy, allowing better definition of low- and high-risk populations [7]. Third, specific cytogenetic abnormalities and gene mutations have been described that allow further refinement of risk, some associated with increased and others with decreased risk. Finally, small molecules and immunologic interventions targeting pathways in AML have been developed, and testing these interventions pre-/post-HCT or instead of HCT will further define indications for HCT in the coming years.

This chapter defines key AML biology and its relation to HCT outcomes, explains current approaches and indications, and introduces promising approaches that may impact outcomes in the near future.

Epidemiology/classification/molecular and cellular biology

AML is a heterogeneous and complex disease that is the culmination of interactions between genetic and epigenetic alterations in hematopoietic progenitors leading to dysregulation of multiple critical signal transduction pathways, resulting in accumulation of myeloid blasts and hematopoietic insufficiency. It is broadly accepted that AML evolves as a result of cooperation between two classes of genetic alterations. In this model, the initial transforming events are genomic alterations such as core binding factor (*CBF*) translocations and mixed lineage leukemia (*MLL*) translocations that are thought to arise in the hematopoietic stem/progenitor cells. Cells that are transformed by such mechanisms would have self-renewal properties, but lack proliferative potential and initially represent a minor clone in the hematopoietic progenitor population. These cells persist as a minor clone for extended periods until a time when a second "hit" confers proliferative properties, allowing expansion of this minor clone, leading to a leukemic phenotype. Recent technologic advances have allowed more complete interrogation of the AML transcriptome, genome, and epigenome, leading to the identification of an increasing number of disease-associated alterations, indicating that the path to AML pathogenesis may be more complex. Data suggest that additional genomic and epigenomic events in leukemic cells may lead to further alteration of the leukemic clone and introduce genomic heterogeneity and clonal evolution within the leukemic cell population.

Disease-associated alterations in AML can be grouped into several broad categories. Large structural alterations, including duplications, deletions, and translocations, are identified by standard karyotype analysis in the majority of children with AML. Karyotypic abnormalities remain the most significant tools for diagnosis and also for defining prognosis in AML. More recently, disease-associated mutations of specific genes that mediate hematopoietic development have been identified that alter multiple signal transduction pathways and contribute to AML pathogenesis. Such mutations have come to define AML, as they are prevalent, provide prognostic information, and may act as potential targets for directed therapeutics. Additional areas are emerging as major contributors to the pathogenesis of AML, including regulation of translation by non-coding RNA (miRNA) and also epigenetic alterations due to aberrant DNA methylation and altered histone modifications.

Disease-associated genomic alterations, whether translocations, numerical structural alterations (monosomies, etc.), or sequence variations (mutations, indels), define AML biology and phenotype, and have been utilized as powerful tools for prediction of response and outcomes in subsets of patients.

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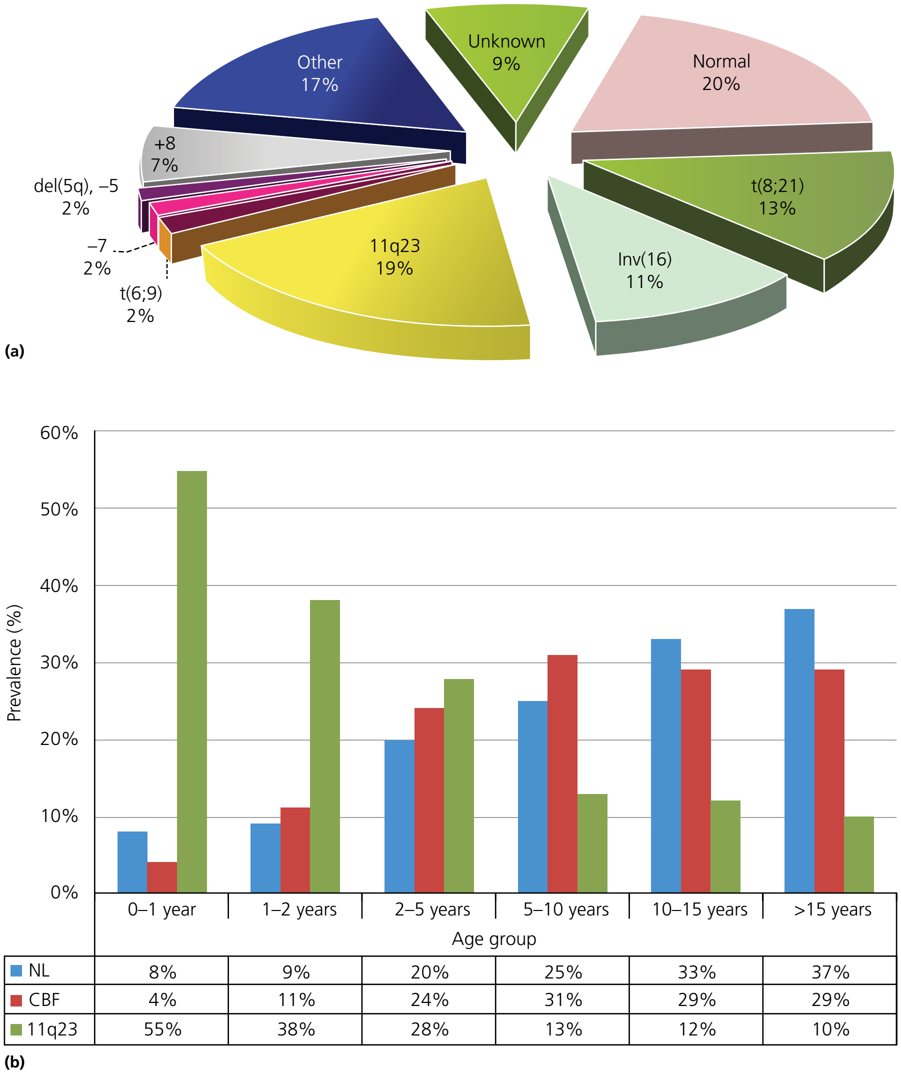
Karyotypic alterations

The number of chromosomal alterations identified in AML is in excess of 300, although >70% of pediatric AML cases fall into several specific cytogenetic categories: 25% have CBF AML [t(8;21) or inv(16)], 12% have t(15;17), 20% have rearrangements involving the *MLL* gene, and 20% do not have karyotypic abnormalities (normal karyotype, NK). In addition to karyotypic alterations, disease-associated mutations can be identified in AML, with the highest prevalence in those with NK; >90% of pediatric AML cases have at least one genomic alteration (Figures 51.1 and 51.2).

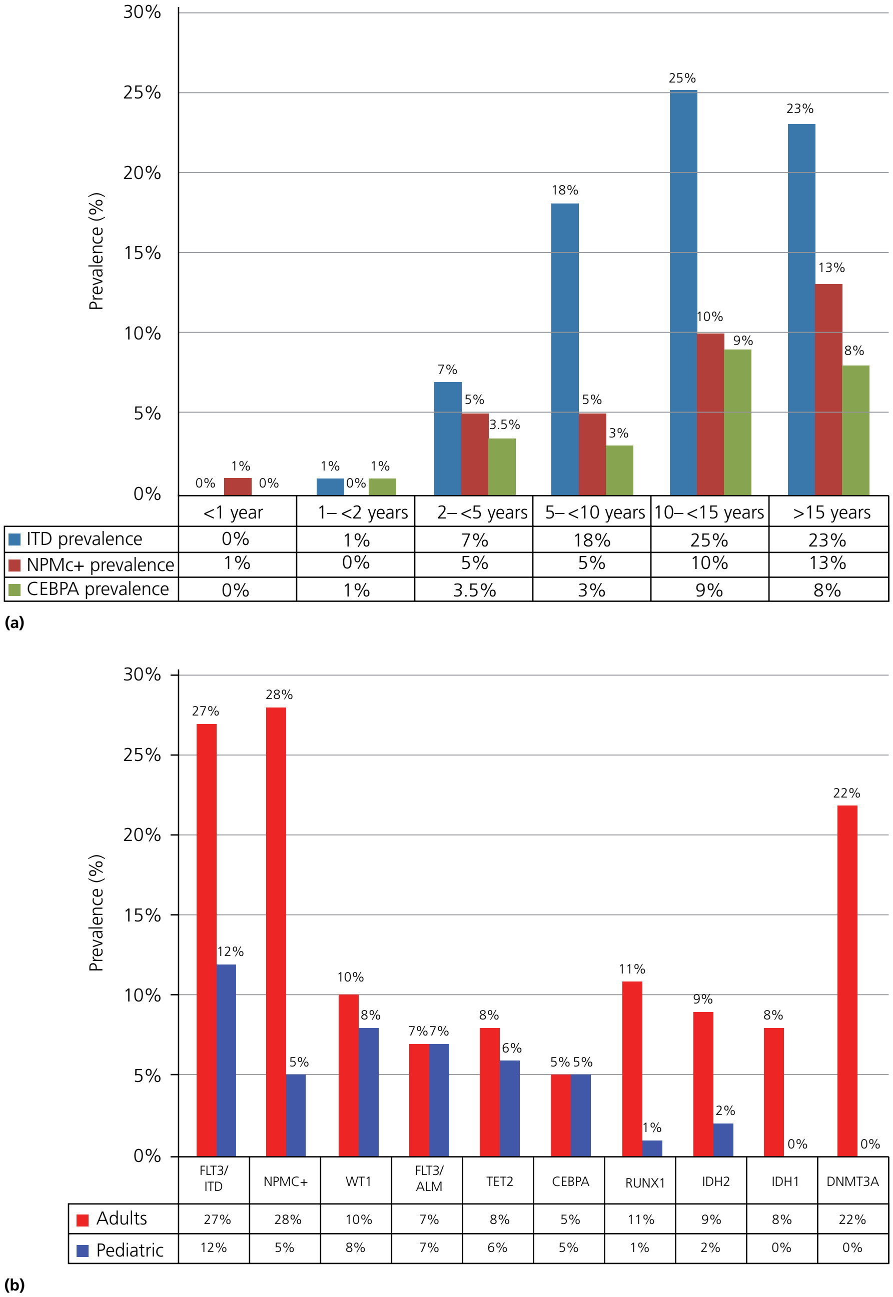
Non-random numerical and structural chromosome aberrations have been identified in approximately 80% of children with AML and are considered to be major contributors in an early stage of disease development. Karyotypic alterations are regarded as one of the most significant biologic markers that define AML biology, and impact clinical outcome. Owing to the high correlation of recurrent karyotypic alterations with AML diagnosis and prognosis, the most recent World Health Organization (WHO) AML classification is primarily based on recurrent cytogenetic alterations. The most common genomic alterations in childhood AML involve CBF transcription factors, where translocations involving genes encoding CBFα (*RUNX1*) or CBFβ (*CBFB*) are detected in those with t(8;21) and inv(16), respectively, and occur in nearly 25% of children with AML. Core binding factor AML is considered a favorable prognostic factor, and those with t(8;21) or with inv(16) have a longer duration of remission and lower relapse risk, leading to significantly improved outcomes. The mixed leukemia lymphoma gene (*MLL*, 11q23) is a methyltransferase that is involved in the regulation of *Hox* gene expression and methylation of histone residues. Translocations involving the *MLL* gene are common in children with AML and account for nearly 20% of all cases of AML. In vitro data have demonstrated that MLL fusion proteins can transform hematopoietic precursors to leukemia-initiating cells, implicating this gene in stem-cell maintenance and hematopoietic regulation [8]. In *MLL* rearrangements, the N-terminus of *MLL* can be juxtaposed to the C-terminus of over 50 different partners, resulting in the loss of the methyltransferase domain. There is an association of the translocation partner with clinical outcome; those with t(6;11) and t(10;11) have a particularly poor outcome compared with other *MLL* translocations.

In addition to the common translocations outlined above, less common structural alterations are described with variable biology and prognostic significance. Notable examples are monosomy 7 (−7),



	0–1 year	1–2 years	2–5 years	5–10 years	10–15 years	>15 years
🟦 NL	8%	9%	20%	25%	33%	37%
🟥 CBF	4%	11%	24%	31%	29%	29%
🟩 11q23	55%	38%	28%	13%	12%	10%

Figure 51.1 Key cytogenetic abnormalities in pediatric AML. (a) Major categories; (b) distribution by age.



	<1 year	1–<2 years	2–<5 years	5–<10 years	10–<15 years	>15 years
🟦 ITD prevalence	0%	1%	7%	18%	25%	23%
🟥 NPMc+ prevalence	1%	0%	5%	5%	10%	13%
🟩 CEBPA prevalence	0%	1%	3.5%	3%	9%	8%

	FLT3/ ITD	NPMC+	WT1	FLT3/ ALM	TET2	CEBPA	RUNX1	IDH2	IDH1	DNMT3A
🟥 Adults	27%	28%	10%	7%	8%	5%	11%	9%	8%	22%
🟦 Pediatric	12%	5%	8%	7%	6%	5%	1%	2%	0%	0%

Figure 51.2 Somatic mutations in childhood AML (a) by age and (b) incidence in pediatric compared with adult AML.

monosomy 5 (–5) and deletion 5q (del5q). These alterations cumulatively account for <5% of cases of childhood AML. Although specific genes in these chromosomal regions that contribute to disease pathogenesis have not been identified, the presence of these alterations is associated with high rates of induction failure and extremely poor survival [9]. Given the large age distribution within pediatric patients spanning birth to young adulthood, it is not surprising that there is significant age associated variation in cytogenetic abnormalities (Figure 51.1). There is a high prevalence of MLL in infants and young children, where nearly 50% of those younger than 2 years of age have an *MLL* translocation. In contrast, t(8;21) or inv(16) and those with NK are uncommon in infants and young children and their prevalence increases with age, approaching the prevalence observed in adult AML by age 5–10 years.

Somatic mutations

Somatic mutations in genes known to contribute to hematopoiesis have been identified in a significant proportion of children with AML and have been associated with clinical outcome. The most common somatic mutation in childhood AML is the *FLT3* internal tandem duplication (*FLT3*/*ITD*). It is detected in 15% of all children with AML with age-associated variation that increases from almost none in the first year of life increasing by age 10 years to the 20% prevalence reported in adult AML (Figure 51.2). The presence of *FLT3*/*ITD* has been associated with significant leukocytosis, increased induction failure, high rates of postremission relapse, and dismal survival [10]. In contrast, patients with mutations in *NPM* and *CEBPA* genes, have an excellent response to induction chemotherapy, a lower risk of relapse, and survival rates similar or superior to those with CBF AML. Additional somatic mutations, including *KIT*, *RAS*, *RUNX1*, and *WT1* have been identified in childhood AML. Although these groups are biologically distinct, they have not been demonstrated to have prognostic significance. Despite lack of prognostic significance, such somatic mutations may be used for directing targeted therapy. Recent application of whole genome sequencing to interrogate the AML genome in adult AML has identified a number of recurrent, clinically significant mutations [11]. Similar whole genome approaches have been performed in children have revealed novel disease-associated mutations [12] that once validated in multicenter clinical trials are likely to identify new class of biomarkers for risk stratification and therapeutic intervention.

The role of HCT in CR1 AML

Autologous versus allogeneic versus chemotherapy approaches

Early studies of HCT for CR1 AML patients sought to define whether autologous or matched family donor (MFD) allogeneic transplant approaches offered a survival advantage over chemotherapy. Table 51.1 summarizes the outcomes of autologous HCT compared with chemotherapy from studies performed in the 1980s and 1990s. All of the studies found no significant difference in OS or disease-free survival (DFS) in the two groups [1,13–17], except in the MRC-AML10 report. This study reported improved DFS with autologous HCT ($p = 0.02$), but no advantage for OS (OS 59% with chemotherapy and 70% with autologous HCT, $p = 0.2$) [17]. These studies show clear evidence that in children with newly diagnosed AML, autologous HCT offers no advantage over chemotherapy. Figure 51.3 shows the OS from the Children's Cancer Group (CCG)-2891 study comparing three different postremission regimens, where the survival difference in patients receiving chemotherapy or autologous HCT is identical and inferior to those receiving allogeneic HCT ($p = 0.006$) [1].

Table 51.2 summarizes the outcomes of studies comparing allogeneic HCT with chemotherapy for children with AML in CR1. Alonzo et al. reported the consolidation therapy results of five consecutive CCG studies (251, 213, 2861, 2891, and 2941), which included a large population of 1464 children [13]. Children in CR1 were allocated to receive an allogeneic HCT if a MFD was available. If an MFD was not available, the remaining children were assigned to consolidative chemotherapy or autologous HCT. Children assigned to receive an allogeneic HCT were found to have a significantly better OS, DFS, and relapse rate by intent-to-treat (ITT) analysis compared with those without an MFD, whether they received chemotherapy or an autologous HCT [13]. Similar results were seen in the majority of studies, where statistically significant differences were seen in outcomes for allogeneic HCT over chemotherapy alone [1,13,14,18–21]. These studies demonstrated that allogeneic HCT can offer an advantage over chemotherapy due to a GVL effect; however, as discussed below, careful definition of risk is important in defining when allogeneic HCT is most likely to result in a clinical benefit.

Table 51.1 Comparison of outcomes from studies of autologous transplantation versus chemotherapy in CR1

Ref.	Study	*n*	Chemo outcome	Auto outcome	*p*-Value
Amadori [14]	AIEOPLAM 87	Chemo 37 Auto 35	DFS 27%	DFS 21%	NS
Ravindranath [16]	POG-8821	Chemo 117 Auto 115	OS 44% EFS 36%	OS 40% EFS 38%	0.10 0.20
Stevens [17]	MRC AML10	Chemo 50 Auto 50	OS 59% DFS 46%	OS 70% DFS 68%	0.20 0.02
Woods [1]	CCG-2891	Chemo 179 Auto 177	OS 53% DFS 47%	OS 48% DFS 42%	0.21 0.31
Alonzo [13]	CCG-251, 213, 2861, 2891, 2941	Chemo 688 Auto 217	OS 42% DFS 34%	OS 49% DFS 42%	0.371 0.832
Pession [15]	AIEOP LAM 87–92	Chemo 89 Auto 110	DFS 28%	DFS 55%	Not stated

n = No. of patients; Chemo = chemotherapy; Auto = autologous; AIEOP = Associazione Italiana Ematologica Pediatrica; DFS = disease-free survival; LAM = Leucemia Acuta Mieloide; POG = Pediatric Oncology Group; OS = overall survival; EFS = event-free survival; MRC = Medical Research Council; AML = acute myeloid leukemia; CCG = Children's Cancer Group; NS = not significant.

Risk-based therapy in CR1 AML: current role of allogeneic HCT

Although it is generally accepted that patients in second remission should receive HCT from the most suitable allogeneic donor, approaches to who should be considered for HCT in CR1 have evolved over the last two decades. The studies outlined in Table 51.2 made HCT using an MFD in first CR (CR1) the standard of care for treatment of childhood AML. Patients with –7 and –5/del5q were found to have an extremely poor outcome with conventional chemotherapy; therefore, more recent Children's Oncology Group (COG) trials allocated this small cohort of high-risk patients to HCT from any suitable donor, including unrelated donors. This occurred as advances in unrelated donor HCT in children were noted to result in better survival [22]. Further, patients with favorable risk cytogenetics (CBF AML) were shown to have similar survival regardless of whether they were treated with chemotherapy or HCT, questioning the utility of HCT in patients with more favorable outcome [23]. Hence those with CBF AML were not assigned to allogeneic HCT in CR1 even if a matched donor was

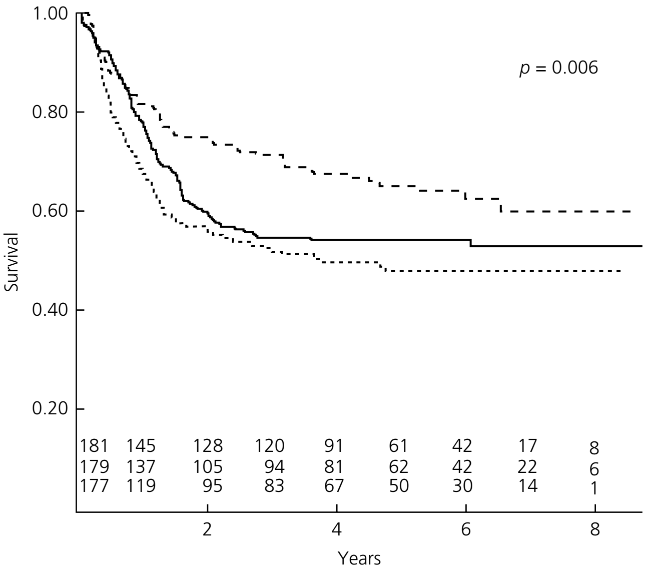


Figure 51.3 Actuarial survival from AML remission, comparing the three postremission regimens from CCG-2891. Numbers are patients at risk at yearly intervals; rows are in the same order as curves. The *p*-value is for homogeneity. Dashed line, allogeneic BMT; solid line, intensive non-marrow-ablative chemotherapy; dotted line, autologous BMT. Source: Woods et al. 2001 [1]. Reproduced with permission of the American Society of Hematology.

Table 51.2 Comparison of outcomes from studies of allogeneic transplantation versus chemotherapy in CR1

Ref.	Study	*n*	Chemo outcome	Allo outcome	*p*-Value
Dahl [101]	AML-80	Chemo 42 Allo 19	DFS 31%	DFS 43%	0.30
Amadori [14]	AIEOP LAM 87	Chemo 37 Allo 24	DFS 27%	DFS 34%	0.03 (Allo vs. Other)
Nesbit [20]	CCG-251	Chemo 252 Allo 89	OS 36% DFS 33%	OS 50% DFS 45%	<0.05 <0.05
Wells [102]	CCG-213	Chemo 298 Allo 113	OS 46% DFS 38%	OS 52% DFS 46%	0.13 0.06
Ravindranath [16]	POG-8821	Chemo 117 Allo 89	OS 44% EFS 36%	OS 46% EFS 52%	0.15 0.06
Stevens [17]	MRC AML10	Chemo 230 Allo 85	OS 60%	OS 70%	0.10
Woods [1]	CCG-2891	Chemo 179 Allo 181	OS 53% DFS 47%	OS 60% DFS 55%	0.05 0.01
Lie [19]	NOPHO-AML93	Chemo 147 Allo 53	OS 71% DFS 51%	OS 69% DFS 64%	NS 0.04
Wells [103]	CCG-2951	Chemo 13 Allo 35	OS 50% DFS 51%	OS 40% DFS 43%	NS NS
Lange [18]	CCG-2941	Chemo 43 Allo 14	OS 69% DFS 53%	OS 63% DFS 69%	0.14 0.70
Alonzo [13]	CCG-251, 213, 2861, 2891, 2941	Chemo 688 Allo 373	OS 42% DFS 34%	OS 54% DFS 47%	0.064 0.004
Entz-Werle [104]	EORTC 58921	Chemo 106 Allo 39	OS 65% DFS 57%	OS 78% DFS 63%	Not stated Not stated
Perel [21]	LAME89, 91, SP	Chemo 203 Allo 74	OS 55% DFS 52%	OS 70% DFS 57%	0.006 0.18
Pession [15]	AIEOP LAM 87–92	Chemo 89 Allo 78	DFS 28%	DFS 64%	Not stated
Liang [105]	TPOG-AML-97A	Chemo 76 Allo 29	DFS 68%	DFS 60%	0.63
Lange [23]	CCG-2961	Chemo 463 Allo 170	OS 62% DFS 50%	OS 67% DFS 60%	0.425 0.021

n = No. of patients; Chemo = chemotherapy; Allo = allogeneic; AML = acute myeloid leukemia; DFS = disease-free survival; AIEOP = Associazione Italiana Ematologica Pediatrica; LAM = Leucemia Acuta Mieloide; CCG = Children's Cancer Group; OS = overall survival; POG = Pediatric Oncology Group; EFS = event-free survival; MRC = Medical Research Council; NOPHO = Nordic Society for Paediatric Haematology and Oncology; EORTC = European Organization of Research and Treatment of Cancer; LAME = Leucémie Aiguë Myéloblastique Enfant; TPOG = Taiwan Pediatric Oncology Group; NS = not significant.

available. In this cytogenetic risk-based therapy allocation, 5% of the patients would be considered high risk, 20% would be in the favorable risk category, and the majority of patients would remain in the standard risk category and would receive HCT in CR1 if an MFD was available. More recently, incorporation of AML-associated mutations into clinical decision-making expanded the risk cohorts for therapy allocation. In this model, those with mutations in the *NPM* and *CEBPA* would be considered low risk and receive chemotherapy only [24,25], whereas those with *FLT3/ITD* would be allocated to the high-risk arm of therapeutic protocols [10]. Addition of these mutations modestly increased high- and low-risk cohorts, and the standard-risk cohort remained at nearly 65%.

Many investigators have shown that response to chemotherapy is a powerful predictor of outcome. In addition, sensitive methodologies, including multidimensional flow cytometry (MFC), have been used to define relapse risk in patients without known cytogenetic or molecular markers. COG AML protocol AAML03P1 collected comprehensive cytogenetic characteristics, mutation profiles (*FLT3/ITD*, *CEBPA*, and *NPM* mutation status), and MFC data, and demonstrated that in patients without known molecular prognostic markers [standard-risk (SR) cohort], MFC was able to identify the presence of MRD in nearly 30% of patients. In this SR cohort, those with MRD after AML induction therapy had a significantly worse survival than those who did not have evidence of MRD (26% vs. 67%, $p < 0.001$). This study also demonstrated that the presence of MRD in those with high- or low-risk disease was not associated with outcome [7]. As a result, a two-tier risk-based allocation system has been devised and implemented in the COG AML trials, where those with either high-risk cytogenetic and molecular features or those with otherwise SR disease with MRD are allocated to the high-risk arm of the study and receive HCT from the most suitable donor in CR1. The remaining patients, those with favorable cytogenetics/mutations or the MRD-negative SR cohort, are considered at favorable risk and would not receive HCT in CR1 regardless of the availability of matched family donors. Whether this revised risk-based approach to CR1 HCT is better than current approaches awaits the outcome of ongoing studies.

An alternative approach to CR1 transplantation is advocated by some study groups in Europe. A meta-analysis confirmed the findings outlined above about the efficacy of CR1 transplantation in patients with low-, intermediate-, and high-risk disease, but when only ITT studies were reviewed, as many as 10 patients were required to undergo transplantation to prevent one relapse, and salvage rates for relapse brought survival curves of donor versus non-donor patients closer together [5]. With this in mind, some European groups advocate limited up-front HCT, focusing on allogeneic HCT interventions to salvage patients who relapse. This approach has not been compared directly with up-front approaches to HCT; how such an approach compares with refined risk-based approaches being used in North America awaits further study.

The role of allogeneic HCT in CR2 + AML

Despite improvements in overall outcomes in children with AML, approximately 40% will die from relapsed disease or from toxicities related to intensive treatment. Allogeneic HCT is the best chance for cure in patients with AML who relapse, regardless of their initial risk classification (Table 51.3). Webb et al. described patients who relapsed after initial treatment in the United Kingdom Medical Research Council (MRC) AML 10 trial [26]. Not all children were treated, but of those given chemotherapy, 69% attained a CR2. The 3-year OS rate was 24%; however, the DFS was 44% for those who achieved a CR2. The most important factor affecting survival was the length of CR1. Aladjidi et al. reported on the French experience from the LAME 89/91 trial, where 71% of patients who relapsed after a CR1 were able to achieve CR2 following one to three cycles of chemotherapy [27]. HCT was performed in 78% of patients and the 5 year OS was 33%; DFS was 45% if patients achieved CR2. Similarly to the MRC AML 10 trial, this study highlighted the fact that the main prognostic factor for overall survival was the length of time to relapse from CR1.

Nemecek et al. reported the Seattle experience of patients in CR2 receiving a first HCT from a related or unrelated donor, where the estimated 5-year DFS was 58% [28]. Relapse estimates after HCT were still high at 42%, but these data were promising since DFS was higher than seen in previous studies. Bunin et al. described prognostic factors that influenced outcome after unrelated donor HCT in children with AML using NMDP data [29]. For patients in CR2, 5-year OS was 47% and the only risk factor that predicted all major outcomes was disease status at the time of transplant.

Investigators from the Berlin–Frankfurt–Münster (BFM) group analyzed the outcome of 379 children who relapsed as late as October 2007 out of 1251 children enrolled on the AML-BFM 87,

Table 51.3 Comparison of outcomes from studies of allogeneic HSCT for AML in CR2

Ref.	Study	*n*	Duration of CR1	Outcome
Gorin [106]	EBMT-EORTC	17	510 days	LFS 40% RR 52%
Webb [26]	MRC AML10	22	Not provided	OS 50%
Pession [107]	AIEOP	16	Not provided	MSD: EFS 41.6% Alt. donor: not provided
Aladjidi [27]	Relapse after LAME 89/91	28	Not provided	MSD: DFS 60% Alt. donor: DFS 44%
Nemecek [28]	Seattle	12	Not provided	DFS 58% RR 42%
Abrahamsson [108]	Relapse after NOPHO 88/93	64	<12 months (13) >12 months (24)	OS (<12) 56% OS (>12) 65%
Gassas [109]	Toronto	23	<12 months (12) >12 months (11)	EFS 49% OS 51%
Bunin [29]	NMDP	142	<12 months (96) >12 months (46)	LFS 45% OS 47%
Sander [30]	AML-BFM 87/93/98	127	Not provided	OS (<12) 33% OS (>12) 51%

n = No. of patients; CR1 = first complete remission; EBMT = European Group for Blood and Marrow Transplantation; EORTC = European Organization of Research and Treatment of Cancer; LFS = leukemia-free survival; RR = risk of relapse; MRC = Medical Research Council; AML = acute myeloid leukemia; OS = overall survival; AIEOP = Associazione Italiana Ematologica Pediatrica; MSD = matched sibling donor; EFS = event-free survival; Alt = alternative; LAME = Leucémie Aiguë Myéloblastique Enfant; DFS = disease-free survival; NOPHO = Nordic Society for Paediatric Haematology and Oncology; NMDP = National Marrow Donor Program; BFM = Berlin–Frankfurt–Münster.

Table 51.4 Comparison of outcomes from studies of autologous HSCT for AML in CR2

Ref.	Study	*n*	Duration of CR1	Outcome
Gorin [106]	EBMT-EORTC	35	334 days	LFS 40% RR 54%
Webb [26]	MRC AML10	22	Not provided	OS 27%
Pession [107]	AIEOP	53	Not provided	EFS 40.4%
Aladjidi [27]	Relapse after LAME 89/91	25	Not provided	DFS 47%
Godder [32]	ABMTR	73	<12 months (30) >12 months (29)	RR (<12) 60% RR (>12) 36% LFS (<12) 23% LFS (>12) 60%
Sander [30]	AML-BFM 87/93/98	26	Not provided	OS (<12) 29% OS (>12) 58%

n = No. of patients; CR2 = second complete remission; EBMT = European Group for Blood and Marrow Transplantation; EORTC = European Organization of Research and Treatment of Cancer; LFS = leukemia-free survival; RR = risk of relapse; MRC = Medical Research Council; AML = acute myeloid leukemia; OS = overall survival; AIEOP = Associazione Italiana Ematologica Pediatrica; EFS = event-free survival; LAME = Leucémie Aiguë Myéloblastique Enfant; DFS = disease-free survival; ABMTR = Autologous Blood and Marrow Transplant Registry.

93, and 98 trials [30]. The 5-year OS for patients treated with curative intent was 29% (86% chose intensive therapy), with an increase in survival from 18 to 34% over the period of the trial. Late relapse (>12 months from diagnosis), age <10 years, no HCT in CR1, and favorable cytogenetics were independent predictors of survival. The most important determinant of outcome, however, was whether patients achieved a complete remission with induction chemotherapy. A total of 63% achieved a second remission and OS was 44% of those achieving CR2 compared with 3% of those who did not ($p < 0.0001$). Outcomes with chemotherapy were similar to those receiving HCT (35 vs. 46%, $p = 0.11$), but those treated with chemotherapy had a greater likelihood of having experienced a late relapse or a relapse isolated to the extramedullary space. Because these groups have very different risks, directly comparing the chemotherapy with the allogeneic HCT outcomes in this group is not meaningful.

Finally, investigators from the Therapeutic Advances in Childhood Leukemia (TACL) Consortium reviewed 99 patients with relapsed or refractory non-APL AML treated at nine institutions between 1995 and 2004 [31]. It was reported that 56 ± 5% of patients achieved remission with the first reinduction attempt, with 25 ± 8% succeeding after a follow-up induction attempt and 17 ± 7% after any subsequent attempts, illustrating that if early remission is not achieved, multiple attempts are less likely to result in remission. Early relapses (<12 months from diagnosis) were less likely to achieve remission with first reinduction (83% vs. 51%). In multivariate analysis, 5-year DFS was improved in patients whose CR1 duration was ≥12 months (65 ± 11%) compared with a CR1 duration of <12 months (38 ± 11%) versus not achieving CR1 (0%, $p = 0.002$). Receiving HCT as consolidation therapy was associated with a marked improvement in survival in multivariate analysis [hazard ratio (HR) 0.29, $p < 0.001$], whereas factors such as age, cytogenetic risk group, and white blood cell (WBC) count at diagnosis were not associated with event-free survival (EFS) or OS [31]. These studies define a clear role for allogeneic transplantation in the treatment of relapsed, non-APL AML, and show that early relapse and delayed or inadequate remission prior to HCT are key risk factors for poor prognosis.

A limited role for autologous HCT in CR2 AML

In CR1, there is a clear advantage to allogeneic HCT over autologous HCT; however, in CR2, some reports hint that in highly selected lower risk patients, autologous approaches yield survival rates that approach those of allogeneic HCT (Table 51.4). In a review of CIBMTR (Center for International Blood and Marrow Transplant Research) data, Godder et al. showed that that the probability of leukemia-free survival (LFS) at 3 years was 60% versus 23% in children undergoing autologous HCT in CR2 who relapsed <12 months from diagnosis compared with those who relapsed later, mainly due to high relapse (60%) in the <12 months from diagnosis group [32]. Other reports show reasonable survival using autologous approaches for patients with low-risk cytogenetics or APL who relapse late, especially if those patients achieve an MRD-negative CR and the autologous product harvested for the procedure is polymerase chain reaction (PCR) negative for a molecular marker [33,34]. Although many clinicians favor sibling HCT for AML patients in CR2 not transplanted in CR1, consideration of autologous approaches is reasonable for patients with relapses ≥12 months from diagnosis, especially if a deep remission can be achieved and if the patient has favorable prognostic factors or poor donor choices.

Outcomes of children undergoing HCT with AML not in remission

Chemotherapy-refractory AML, either at diagnosis or after relapse, has been treated with HCT by some investigators hoping either (1) to overcome chemotherapy resistance with high-dose chemo/radiotherapy or (2) to use the GVL effect immunologically to cure patients. Survival rates have been poor (range 2–32%) and concern about selection and reporting bias has made understanding the relevance of the published literature difficult [28,35,36]. An early IBMTR study offered insight into some characteristics that may help define subgroups of patients who could benefit from HCT [37]. The study reviewed 88 patients with non-remission AML undergoing HCT from 1982 to 1989 at multiple centers. The three-year

LFS was 21%. Independent associations with improved survival included (1) fewer than 25% blasts on bone marrow (BM) biopsy, and (2) fewer rounds of induction chemotherapy (2 or 3 versus >4). In addition, relapse was decreased in patients without circulating blasts. Duval et al. performed a more contemporary analysis (myeloablative HCT between 1995 and 2004) of 1673 patients reported to the CIBMTR with AML refractory to induction or reinduction therapy at the time of HCT [38]. The 3-year OS of the cohort was 19%, and the five pre-transplantation variables that independently correlated with worse survival on multivariate analysis were (1) CR1 <6 months, (2) presence of circulating blasts at HCT, (3) use of donors other than matched siblings [matched unrelated donor better than mismatched related donors (including haploidentical donors)], (4) Karnofsky/Lansky score <90, and (5) poor-risk cytogenetics. Recipients with none of these risk factors had a 3-year OS of 42% (95% CI 34–50%), whereas those with one, two, or three of these factors had survivals of 28% (95% CI 23–33%), 15% (95% CI 11–19%), and 6% (95% CI 3–9%), respectively. This and other studies point to survival in the 20–40% range of a selected cohort of children with active AML at HCT. The optimal transplant approach has not been defined, as published responses have been observed using myeloablative and reduced-intensity regimens.

Pre-HCT MRD positivity: effect on survival

The complexity of gene rearrangements has made PCR-based detection of MRD for AML challenging; however, over time, PCR-based methods, MFC, and assays of *WT1* gene expression have been developed and are now being routinely used pre- and post-HCT for prognostic indications and screening [39]. Flow cytometric approaches developed by the COG and St. Jude's AML groups have shown that the persistence of MRD in newly diagnosed patients after the first induction regimen defines a group at very high risk for relapse [7,40], validating the use of flow cytometry in a multicenter pediatric setting. In a mixed adult and pediatric cohort, investigators in Seattle reported a marked decrease in survival for patients with >0.01% MRD detected by flow cytometry pre-HCT compared with those who were negative or had lower levels [2-year OS 30% (95% CI 13–49%) vs. 77% (95% CI 64–85%), MRD positive vs. negative], with the difference being explained by a marked increase in relapse in MRD-positive patients [2-year relapse estimate 65% (95% CI 42–86%) vs. 18% (95% CI 10–28%)] [41]. Multivariate analysis adjusting for cytogenetic risk and other key AML risk factors confirmed that the presence of detectable pre-HCT MRD was associated with a markedly increased risk of overall mortality (HR 4.05, $p < 0.001$) and relapse (HR 8.49, $p < 0.001$).

Large pediatric-specific studies to establish better the role of pre- and post-HCT MRD in defining risks of poor outcome are under way in the COG and the Pediatric Blood and Marrow Transplant Consortium (PBMTC). Of note, two small pediatric studies offer some insight into this issue. Jacobsohn et al. [42] compared the outcomes of children with high MRD as measured by *WT1* expression pre-HCT with those with low *WT1* expression. Patients with high *WT1* expression had a lower 5-year EFS than those with low expression (18% vs. 68%, $p = 0.007$). Leung et al. [43] reported a single-center study of the predictive effect of the presence of pre-HCT MRD by flow cytometry in 122 children [AML, $n = 58$; acute lymphoblastic leukemia (ALL), $n = 64$]. They noted a strong effect of pre-HCT level on survival, with MRD-negative, low-positive, and high-positive patients surviving at rates of 68, 52, and 29%, respectively ($p = 0.0019$) (Figure 51.4).

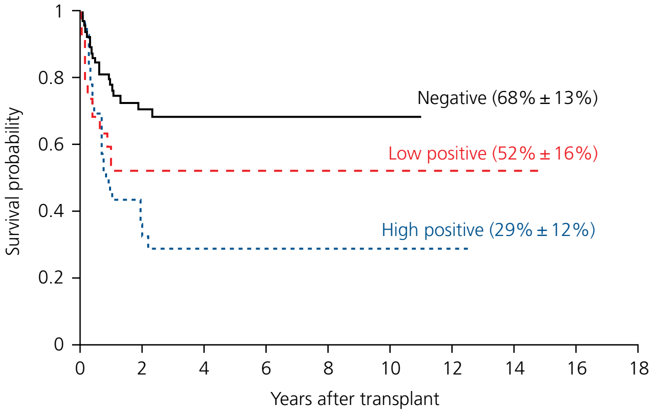


Figure 51.4 Effect of pre-HCT MRD level on survival in children with AML and ALL [2].

Although pre- and post-HCT measurement of MRD clearly predict poor outcomes, survival estimates of MRD-positive patients with AML after HCT range from 30 to 60%; therefore, transplantation is still indicated for MRD-positive patients if multiple attempts to achieve MRD-negative remissions have failed. It is not clear whether sequential rounds of chemotherapy or other strategies to reduce MRD pre-HCT will improve survival, but studies looking at this question are under way.

The role of HCT in acute promyelocytic leukemia

Acute promyelocytic leukemia (APL) has distinct biology [characteristic translocation of the *PML-RARA* genes, t(15;17)], and presentation compared with other AML subtypes. The addition of all-*trans*-retinoic acid (ATRA) to anthracycline-based regimens has dramatically improved outcomes, making early remission HCT unnecessary [44]. More recent development of arsenic trioxide monotherapy, initially for salvage therapy [45] and later for combined therapy with ATRA [46], has further limited the need for HCT, as only rare patients are refractory or relapse.

In cases where patients are (1) unable to receive these key medications (reached anthracycline limits, intolerant of ATRA or arsenic trioxide), (2) resistant or refractory, or (3) otherwise at high risk for poor outcome with chemotherapy approaches (mainly patients suffering one or more relapses), both autologous and allogeneic approaches have resulted in successful long-term survival. Retrospective pediatric studies have demonstrated long-term survival of APL patients undergoing allogeneic or autologous HCT in remission exceeding 70% [33,47]. The choice of allogeneic versus autologous stem-cell source should be made based on the quality of remission and degree of match of the related or unrelated donor. Patients who achieve a PCR-negative remission and then have a hematopoietic cell product harvested that is also PCR negative have achieved long-term survival approaching 80%, compared with 50% survival when PCR negativity has not been achieved [48]. A separate report documented relapse in all seven patients receiving autologous HCT with PCR-positive products, but in only one of eight patients who received PCR-negative products [49]. A further study demonstrated a superior 5-year survival in patients with relapsed APL who underwent autologous HCT in second molecular remission compared with maintenance therapy with arsenic trioxide or arsenic trioxide–ATRA combination regimens (83.3% vs. 34.5%, $p = 0.001$)

[50]. Although no studies randomly comparing autologous versus allogeneic approaches or consolidation with transplant versus chemotherapy have been performed in CR2, the data above support a role for autologous transplantation when products are PCR negative and patients can tolerate a myeloablative regimen in relapsed and other patients at high risk for failure with chemotherapy approaches.

The role of HCT in therapy-related AML

Studies addressing HCT for adult patients with therapy-related AML (t-AML) showed poor rates of survival due to high non-relapse mortality (NRM) and high relapse rates [51]. Some improvement in survival has been noted with targeted busulfan/cyclophosphamide and other non-total body irradiation (TBI)-based regimens [52], but poor prognosis after transplant has been associated high levels of adverse cytogenetics [53]. There are only a few reports describing the outcome of children undergoing HCT for therapy-related AML. Woodard et al. [54] described 27 children with t-AML receiving related and unrelated donor HCT. Three-year OS and EFS were 18.5 ± 7.5% and 18.7 ± 7.5%, respectively. Poor survival was mainly due to very high treatment-related mortality (TRM) (59.6 ± 8.4%). Aguilera et al. [55] reported a second retrospective single-center experience of 14 patients transplanted for t-AML/therapy-related myelodysplastic syndrome (t-MDS) between 1975 and 2007. Survival was 29%, but in this review only 63% of patients diagnosed with tMDS/tAML underwent HCT. A multicenter study from the CCG (2891) looked at outcomes of 24 children with t-AML/t-MDS compared with other children enrolled on the study with de novo AML ($n = 898$) or MDS ($n = 62$) [56]. Children with t-AML/t-MDS were older and low-risk cytogenetics rarely occurred. Although rates of achieving CR and OS at 3 years were worse in the t-AML/t-MDS group (CR rate 50% vs. 72%, $p = 0.016$; OS 26% vs. 47%, $p = 0.007$), if patients achieved a CR, survival was similar (OS 45% vs. 53%, $p = 0.87$). The importance of remission to survival in these patients is further illustrated by another single-center experience. Kobos et al. [57] described their studies of 21 children undergoing HCT for t-AML/t-MDS performed between 1994 and 2009. Eleven of 12 children with t-AML were in remission at the time of HCT, seven had refractory anemia (RA), and two had refractory anemia with excess blasts (RAEB). Survival of the entire cohort was 61%; those in remission or with RA had a DFS of 66%, and those with >5% blasts at the time of HCT had 0% survival ($p = 0.015$).

Because t-AML is rare in children, it is not known whether the significant decrease in TRM after unrelated donor HCT noted over the past several years will translate to improved survival in this population. Patients should be carefully assessed for pre-HCT morbidities caused by earlier therapies and approaches should be adapted to give adequate intensity while minimizing TRM.

Effect of hematopoietic cell source on outcomes

Related versus unrelated donors for children with AML

The use of HLA-matched related donors as the first choice for hematopoietic cell source has long been recognized. Since at most 20–30% of patients who need an HCT will have a matched related donor, the use of unrelated donors (URDs) has increased, and most data suggest similar outcomes to related donors. One of the early pediatric-specific URD experiences was published by investigators at the Medical College of Wisconsin, who reported outcomes of 50 children who received a URD HCT at their center between 1986 and 1991. The EFS for all patients was 44% and OS was 50% with a median follow-up was 49 months. These data were similar to published reports for children transplanted with HLA-matched sibling donors at the time and led to URDs being considered a favorable option for children requiring HCT [58]. The Seattle group reported their experience with 87 children who were transplanted with a URD and showed similar outcomes to the Wisconsin group [59]. They later reported their experience of URD HCT for AML, which included 26 pediatric patients (16% of the cohort). LFS was 50% for transplants in CR1, but only 28% for those transplanted in CR2. Favorable prognostic factors included receiving HCT during remission, a marrow cell dose above 3.5 × 10^8/kg and cytomegalovirus (CMV) seronegative status in both the donor and recipient before HCT [60].

Since 2000, significant improvements in survival after URD HCT have occurred in children with AML. Children transplanted between 2003 and 2006 had 2-year EFS of 69, 66, and 36% compared with 42, 30, and 28% a decade earlier for HCT in CR1, CR2, or >CR2/relapse, respectively. The most important improvement over time was a marked decrease in TRM (1-year TRM 40% in 1987–1995 vs. 15% in 2003–2006, $p < 0.001$) (Figure 51.5) [6]. It is not clear which elements of the HCT process have led to improved survival, but better HLA typing/matching, improved supportive care, and possibly better patient selection, with reduced-intensity approaches given to patients with comorbidities, may have contributed.

With these more modern data in mind, most agree that current survival outcomes after URD HCT are comparable to those for matched sibling HCT. A Korean study showed identical survival with matched URD recipients undergoing HCT in CR1 versus sibling donor recipients [61], and a large CIBMTR study showed only a slight advantage in survival of matched sibling donor recipients compared with fully matched URD or mismatched/or matched non-sibling related donors recipients [62]. It is important to note, however, that the CIBMTR analysis clearly illustrated that although survival after matched URD HCT or mismatched related donor HCT is similar to that in sibling donor procedures, the non-survival outcomes are very different. URD and mismatched or non-sibling

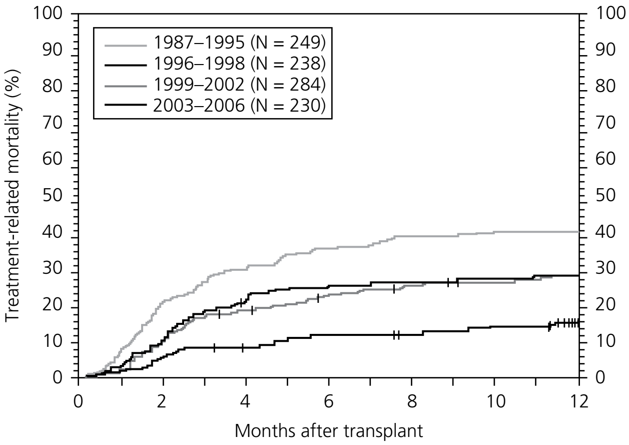


Figure 51.5 Decreasing transplant-related mortality after unrelated donor bone marrow transplantation. Source: MacMillan et al. 2008 [6]. Reproduced with permission of Elsevier.

donor procedures result in higher rates of significant acute and chronic graft-versus-host disease (GVHD) and increased TRM. With these risks in mind, some cooperative groups distinguish between indications for sibling donor HCT versus URD HCT, reserving URD HCT for higher risk clinical scenarios.

T-cell depletion of matched related or unrelated donors in AML

T-cell depletion techniques have varied through the years, but have almost always been associated with higher rates of graft rejection and relapse. Because of this, the approach has generally been practiced at a small number of centers with a special research emphasis in this area. In the past several years, however, with the advent of high-quality magnetic bead separation techniques, multicenter phase II trials have been successfully performed in younger adult patients with CR1 and CR2 AML undergoing myeloablative procedures [63]. Results from a recent phase II Blood and Marrow Transplant Clinical Trials Network (BMT CTN) trial of young adult patients undergoing T-cell-depleted matched sibling HCT for AML in CR1 and CR2 were compared with a matched cohort of CR1 and CR2 patients with AML enrolled on other contemporaneous BMT CTN trials who received standard approaches to GVHD prophylaxis (calcineurin inhibitor plus methotrexate) [64]. Engraftment rates were similar between the T-cell-depleted patients and matched controls, day 100 rates of acute GVHD were marginally improved (23% vs. 39%, $p = 0.07$), and 2 year cumulative incidence of chronic GVHD was markedly improved (19% vs. 50%, $p < 0.001$). Rates of graft rejection, relapse, TRM, DFS, and OS were the same in two groups. Of note, all of the T-cell-depleted cohort underwent a TBI-based regimen, whereas half of the matched cohort used chemotherapy-based regimens. There are limited publications demonstrating the successful use of this approach in pediatric patients [57,65], including related approaches such as in vivo depletion using alemtuzumab [66]. No prospective trials comparing T-cell depletion with standard approaches that include pediatric patients have been performed. Therefore, although the data for adults are intriguing, further study is required in pediatrics before this approach could be generally adopted. The data for T-cell depletion of haploidentical donors, especially those with favorable killer immunoglobulin receptor (KIR) donor–recipient combinations, are more abundant and are reviewed in Chapter 11, which is focused on natural killer cell- and KIR-related approaches.

Cord blood outcomes in children with AML

Although acceptable unrelated donors (currently 7/8 or 8/8 HLA allele matched) can be found in the majority of Caucasian patients, Black, Asian-Pacific islanders, and Hispanic recipients are much less likely to have acceptable matches in the registry [67]. Because cord blood matching requirements are less stringent (currently 4/6 HLA antigen/allele matched with adequate cell dose), studies have shown that >95% of recipients can find acceptable units [68]. Early studies established the safety and efficacy of unrelated umbilical cord blood transplants [69]. Investigators from Minnesota performed a comparison of pediatric patients transplanted with either HLA-mismatched URD cord blood grafts or HLA-matched BM and showed that neutrophil recovery was slower after cord blood transplant, but platelet recovery, rates of acute and chronic GVHD, and OS were similar in the groups [70]. To evaluate multiple donor strategies, Rocha et al. compared the outcomes of children receiving cord blood unit transplant with those receiving either a URD BM transplant or a T-cell-depleted URD BM transplant in a retrospective multicenter study [71]. Patients who received a cord blood transplant were more likely to have HLA mismatches, delayed hematopoietic recovery, decreased acute GVHD, and increased TRM at 100 days. EFS was similar in all three groups, suggesting that cord blood transplantation is a reasonable option for children with acute leukemia without a suitable matched unrelated BM donor. The Eurocord Group later published results of URD cord blood transplantation specifically in children with AML [72]. They found acute GVHD in 35%, 100-day TRM in 20%, a 2-year cumulative incidence of relapse of 29%, and a 2-year LFS of 42%. LFS was highest for children in CR1 (59%) than in CR2 or not in a CR (50 and 21%, respectively). These and other studies show that matched or mismatched URD cord blood units can be used as an alternative donor source for children with AML who are undergoing an HCT and survival is comparable to that with other hematopoietic cell sources.

Haploidentical approaches to children with AML

A final alternative hematopoietic cell source for children with AML is the use of partially matched or haploidentical family donors. These donors are readily available because parents or siblings can be used and offer an advantage for patients who need to go to transplant quickly. Godder et al. described the use of partially mismatched related donors as hematopoietic stem cell sources in pediatric patients with acute leukemia [73]. They used partial T-cell depletion along with immune suppression. They found that 96% of patients engrafted, 24% developed acute GVHD, and the 3-year relapse rate was 41%. This study showed that the use of partially mismatched related-donor HCT is feasible in pediatric patients with acute leukemia. Marks et al. reported on 34 children who underwent a haploidentical HCT for acute leukemia [74]. They received cyclophosphamide and TBI for conditioning, followed by infusion of T-cell-depleted peripheral blood hematopoietic cell grafts. The OS at 2 years was 26% and the major determinant of outcome was the disease state at the time of HCT. No child transplanted with refractory AML survived, suggesting that this approach is unlikely to be beneficial for children if their disease is not in remission. The major barriers to OS were infection and relapse. More contemporary experiences with haploidentical transplantation for children with AML are likely impacted by the selection of KIR favorable donor–recipient combinations (see Chapter 11 for a discussion of natral killer cell and KIR approaches), but have also benefited from significant decreases in TRM, especially as a result of decreased infection-related mortality due to better viral screening and antimicrobials. Researchers at St. Jude demonstrated significant improvements in recent outcomes compared with those a decade earlier [5-year OS for AML patients 77% (95% CI 35–94%) versus 22% (95% CI 3.4–51%)] using haploidentical approaches [75]. These results make haploidentical approaches a viable alternative when performed by centers experienced in the procedure.

Preparative regimens for pediatric AML patients

Most published pediatric approaches to AML have utilized myeloablative regimens, mainly busulfan/cyclophosphamide (BU/CY)- or TBI-based approaches. A small retrospective study published in 1994 of 74 children in CR1 undergoing HCT using matched family donors compared BU/CY with cyclophosphamide dosed at either 120 or 200 mg/kg with TBI-based conditioning [76]. A significant decrease in relapse was noted in the BU/CY 200 mg/kg–cyclophosphamide dose cohort compared with the 120 mg/kg dose group ($p = 0.02$);

however, the resultant improvement in survival did not reach statistical significance ($p = 0.07$). Children undergoing TBI-based approaches had survival identical with that for patients receiving BU/CY 200 mg/kg. Because of equivalent outcomes compared with TBI-based regimens and less relapse compared with BU/CY 120 mg/kg, the BU/CY 200 mg/kg regimen was adopted by the COG and other groups as a standard preparative approach for patients with CR1 AML undergoing MFD HCT.

Although BU-based regimens have been widely used for matched related donor AML in CR1, most pediatric groups have used TBI-based approaches for URD HCT for AML through the 1990s and first decade of the 2000s [29]. Recent data have questioned the need for TBI for patients undergoing URD HCT. A retrospective study of the PBMTC compared TBI-based with BU-based regimens in 151 children undergoing unrelated (99) or related (52) donor HCT for AML beyond CR1 [77]. At 6 years after HCT, no difference was noted in relapse [31% (95% CI 17–45%) BU/CY vs. 24% (95% CI 15–34%) TBI; $p = 0.74$], TRM [20% (95% CI 7–30%] vs. 32% (95% CI 17–43%); $p = 0.289$], EFS [37% (95% CI 19–70%) vs. 45% (95% CI 35–58%); $p = 0.293$], and OS [56% (95% CI 43–72%) vs. 45% (95% CI 35–58%); $p = 0.117$]. The CIBMTR retrospectively compared outcomes in 1593 patients (192 aged <20 years) undergoing HCT for AML, chronic myelogenous leukemia (CML), and MDS who received BU/CY, compared with TBI at standard doses (>1000 and <1260 cGy) or high doses (>1320 and <1500 cGy) [78]. Although high-dose TBI led to more grade III–IV acute GVHD ($p = 0.011$), relapse, TRM, DFS, and OS were comparable between the three groups. With these data in mind, BU/CY has become an acceptable approach for children at any stage of response with either related or unrelated donors.

Studies in adults attempting to decrease hepatic toxicities associated with BU/CY have substituted fludarabine for cyclophosphamide with dramatic decreases in TRM. Andersson et al. compared busulfan/fludarabine (BU/FLU) with a retrospective cohort of patients treated with BU/CY 120 mg/kg and, after adjusting for treatment period differences, the BU/FLU group had a 20% increase in 3-year survival, mainly due to decreased TRM [79]. Prospective randomized comparisons of this approach with BU/CY have not been performed. Another promising preparative regimen using treosulfan (Treo), a conjugate of BU with less liver toxicity, has been described. Nemecek et al. described remarkably low levels of TRM (8% at 2 years) with 2-year relapse free survival (RFS) of 88% in patients without high-risk cytogenetics [80]. The COG is currently exploring the role of BU/FLU in children with high-risk CR1 AML and the PBMTC is studying a combination of treosulfan, fludarabine, and mini-dose TBI in pediatric patients with AML or MDS. These trials and possible future direct comparative trials will determine whether "reduced-toxicity" myeloablative approaches such as BU/FLU or Treo/FLU result in similar or improved survival compared with BU/CY.

Reduced-intensity conditioning (RIC) regimens in pediatric AML

The regimens described above are considered intense, myeloablative approaches. In the past decade, however, a variety of approaches have been developed that utilize the GVL effect and focus on preparative agents that substitute potent immunosuppression for more toxic myelosuppression. The intensity of these approaches varies, and has been grouped into three major categories: non-myeloablative (NMA) (just enough to establish donor engraftment), reduced-intensity (RIC) (establishes engraftment and gives some intensity for disease control), and myeloablative (MA) (significant intensity). A large CIBMTR study comparing the three approaches in AML showed equivalent 5-year survival between MA and RIC, but a slight disadvantage for NMA regimens [81]. The patients receiving NMA approaches were older, however, and may have had unmeasured comorbidities that contributed to the slightly inferior outcome.

A number of individual pediatric centers have used RIC approaches for children with AML [82–84]. Outcomes in centers using RIC regimens as preferred approaches seem to be similar to those with traditional MA approaches, but selection bias is a concern. Because pediatric patients can generally tolerate intensive MA regimens, many of the early studies of RIC included only patients ineligible for standard MA HCT because of organ toxicity, history of infection, or other reasons. With this in mind, the published survival outcomes in the 40–60% range are encouraging. A multicenter PBMTC study of patients ineligible for MA regimens due to infection, organ toxicity, or previous MA HCT used a preparative approach of low-dose BU, FLU, and antithymocyte globulin (ATG). OS at 2 years was 45%, and was the similar between AML and ALL and between different stem-cell sources. Patients who were MRD negative pre-HCT had improved survival (2-year OS 63%) [85]. These studies show that RIC approaches can be used for pediatric patients in remission (especially MRD-negative remission) if they are ineligible for MA approaches. Studies randomizing MA with RIC or NMA regimens in pediatric patients with AML have not been performed and, until they are, MA approaches are still considered standard for children who are medically eligible.

The graft-versus-leukemia (GVL) effect in pediatric AML

Multiple studies have suggested that the GVL effect may contribute to the overall cure rate in patients with leukemia, although there is a delicate balance between GVL and GVHD. One of the first studies of this effect in children was reported by a Swedish group, where GVL and its relation to chronic GVHD were associated with improved survival and a reduced risk of relapse [86]. In patients with AML, the probability of relapse was reduced from 40 to 29% if patients developed chronic GVHD. Chronic GVHD also had a favorable effect on survival in the AML group, although this effect was greater in children with ALL. Acute GVHD was not found to be associated with a lower risk of relapse in this study. Another early report establishing the effect of GVL on survival in children with AML was the CCG 2891 study [87]. In this study, patients who developed grade III–IV acute GVHD had an inferior DFS of 15% at 6 years, compared with patients without acute GVHD or with grade I–II acute GVHD ($p < 0.001$). In the patients with grade I or II acute GVHD, the RFS was higher at 76 and 87%, respectively, compared with patients without acute GVHD, whose RFS was 62%. Although patients with grade III–IV acute GVHD had an inferior DFS, those patients who survived did not relapse from their disease. Chronic GVHD was also associated with improved RFS ($p = 0.05$) but the decreased rate of relapse did not correlate with an improved OS or DFS.

In an analysis by the CIBMTR describing the incidence and risk factors for GVHD in children receiving URD HCTs, the risk of relapse was significantly decreased in children with ALL who developed grade III–IV acute GVHD ($p = 0.0052$) but not in children with AML ($p = 0.26$) [88]. The reason why children with AML who developed acute GVHD and did not have a reduced risk of relapse was unclear. The authors postulated that competing mortality from severe GVHD might have overcome any benefit from GVHD, although the small sample size made it difficult to see statistically significant differences.

Some investigators have posited that early immune recovery after transplant leads to a GVL effect in the form of immune surveillance that decreases relapse with or without GVHD. Powles et al. described a multivariable analysis of 201 adult patients transplanted with HLA-matched sibling donors for AML [89]. Patients with absolute lymphocyte counts ≥0.2 × 10^9/L at day 29 after HCT had 3-year relapse probabilities of 16% versus 42% in patients with lower ALC at that point ($p = 0.004$), translating into improved survival (66% vs. 35%, $p = 0.003$). This observation remained significant on multivariate analysis (relative risk for relapse 2.9, $p = 0.02$), and was independent of GVHD. Although a pediatric group showed that this observation was valid in children with ALL after HCT [90], a follow-up study of children with AML after HCT was not able to demonstrate an association with early lymphocyte recovery and GVL [91]. It is not clear why children would be different from adults with AML, and further study regarding the importance of immune reconstitution to relapse in pediatric AML is required.

Relapse after HCT

The major determinant of outcome with relapse after transplant is whether families and physicians decide to treat patients with curative intent. Approaches vary, but generally only immunologic approaches [withdrawal of immune suppression, donor lymphocyte infusion (DLI), or second transplant after CR is achieved] result in long-term cure. Lee et al. reviewed the clinical course of 49 children at a single institution who relapsed with AML after HCT between 1993 and 2011 [92]. Of the 49 patients, 36 (73%) underwent intensive chemotherapy and 26 achieved CR (53%). The probability of OS at 5 years was 31.6 ± 8.7% for those who underwent intensive chemotherapy and 23% of the entire population. Bajwa et al. reviewed the outcomes of 160 consecutive relapses out of 532 patients transplanted between 1992 and 2010 at five pediatric centers [93]. ALL, AML, CML, juvenile myelomonocytic leukemia, secondary AML, acute biphenotypic leukemia, acute undifferentiated leukemia, and MDS were included. Relapsed patients received either palliative therapy (no curative intent, $n = 43$, 27%), salvage chemotherapy without second HCT ($n = 55$, 34%), or chemotherapy followed by a second HCT ($n = 62$, 39%). DFS at 2 years was 35% versus 2% in those who underwent a second HCT versus those who did not ($p < 0.0001$) (Figure 51.6). Multivariate analysis showed that improved survival was associated with (1) a longer time to relapse and (2) undergoing a second HCT. Withdrawal of immune suppression followed by chemotherapy and second HCT in those who achieve remission was recommended.

Immunologic interventions to treat AML relapsed after HCT

An approach taken by many transplant physicians if patients relapse while on immune suppression and GVHD is absent or controlled is withdrawal of immune suppression. Anecdotal reports of development of GVHD with resultant, sometimes long term, response have been published, but this approach rarely controls overt relapse effectively. Attempts to modulate immune suppression based upon detection of increasing recipient chimerism, a strong risk factor for relapse, have been pursued in pediatric AML. Bader et al. described the achievement of complete chimerism and continuous complete remission in two of three AML patients after withdrawal of cyclosporine [94]. Six other patients with ALL or MDS showed complete chimerism and continuous complete remission after withdrawal of cyclosporine and the addition of DLI. This study showed that treatment strategies guided by mixed chimerism can prevent relapse in children with AML after transplant, but this and other studies published were relatively small.

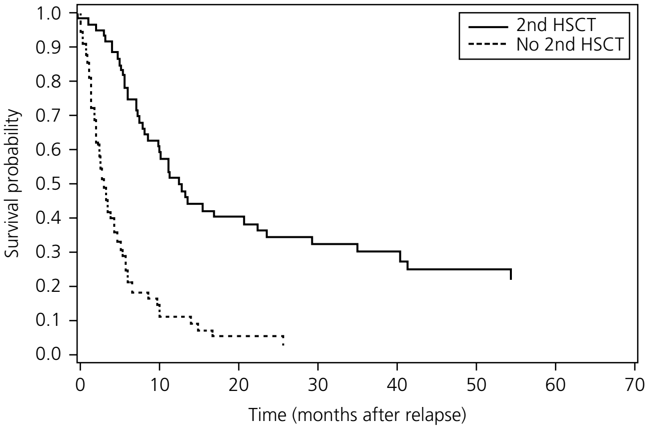


Figure 51.6 Outcome of treatment with curative intent followed by second HCT in children relapsing after first HCT. Source: Bajwa et al. 2012 [93]. Reproduced with permission of Nature Publishing Group.

There is greater experience using DLI to treat relapse after transplant for AML. An early report by the European Group for Blood and Marrow Transplantation (EBMT) evaluated the risks and benefits of DLI in recurrent leukemia in adults, including AML. Transfusion of donor lymphocytes induced complete remissions in five of the 17 patients with AML, but these remissions were not always durable [95]. Levine et al. reported a prospective trial in which patients with myeloid leukemia relapse were treated first with cytoreductive chemotherapy, followed by granulocyte colony-stimulating factor (G-CSF)-primed DLI [96]. The majority of patients had AML, ages ranged from 2 to 59 years, and the 2-year OS was 19%. It was concluded that salvage treatment with this approach may benefit some patients with myeloid relapse after HCT, especially those who relapsed >6 months after HCT. A CIBMTR study of DLI in pediatric patients showed very poor survival in children who relapsed <6 months after transplant (0/14) [97]. Of 16 patients with AML receiving DLI, 11 were not in remission. One was a long-term survivor and the other 10 relapsed. The remaining five received chemotherapy and achieved a CR prior to DLI, and 3/5 remained alive at 3, 13, and 55 months. DLI for early relapse and patients with uncontrolled relapse leads to poor outcomes. Extramedullary relapse following chemotherapy and DLI approaches has been reported. Choi et al. treated patients with chemotherapy followed by DLI and reported a 2-year OS of 31% [98]. All five patients who relapsed after achieving a CR did so at extramedullary sites without BM relapse. This study and other data suggest that the central nervous system (CNS) is immunologically protected from DLI, and clinicians using DLI may need to use other approaches to CNS control.

Second HCT

If remission can be achieved with chemotherapy after a post-HCT relapse, a second HCT can lead to long-term survival in a portion of patients. Michallet et al. reported the French experience of a second

HCT after relapse of acute and chronic leukemia in pediatric and adult patients [99]. Pediatric patients achieved a 2-year OS of 50% with a DFS of 47% and TRM of 20%. Factors associated with a better outcome included age <16 years, relapse >12 months after the first HCT, and the occurrence of chronic GVHD. Meshinchi et al. described 25 pediatric patients who previously underwent an autologous (n = 11) or allogeneic (n = 14) HCT and subsequently relapsed [100]. These patients then underwent a TBI-based preparative regimen followed by a second transplant using related or unrelated donors. All patients engrafted and DFS at 10 years was 44%.

Although the majority of reports of successful second HCT in children describe myeloablative approaches, both the adult and pediatric literature suggests that RIC approaches can also be performed successfully. Pulsipher et al. reported a PBMTC study using a RIC regimen of low-dose busulfan/cyclophosphamide/ATG for children ineligible for myeloablative procedures, mostly due to relapse after previous HCT [85]. Children with a history of a previous transplant with a non-TBI regimen achieved a 2-year survival of 63% compared with 23% when the previous HCT involved TBI ($p = 0.02$). Very low survival was achieved when disease was present by flow cytometry, emphasizing that deep remission is required for success with RIC approaches. These approaches show reasonable outcomes with second HCT after relapse using either myeloablative or RIC approaches if remission is achieved and the interval after first HCT is at least 6–12 months [100].

Conclusions – targeted chemotherapeutic, HCT, and cellular approaches to pediatric AML

Tremendous progress over the past two decades has fundamentally changed approaches to HCT for AML in children. After early data showed an advantage of sibling donor allogeneic approaches over autologous HCT or chemotherapy, refinements in risk classification have defined patients who do well with chemotherapy alone, and do not need HCT in CR1. Improvements in unrelated donor, cord, and haploidentical approaches have led to survival outcomes similar to those with sibling donor procedures, with hopes of further improvement in the near future when “targeted” donors will be chosen for immunologic advantage, decreasing relapse and toxicity risk.

Further refinements in the risk–benefit ratio of HCT compared with chemotherapy are likely, especially with a myriad of new agents coming forward that target pathways important in AML tumorigenesis and propagation. Concomitant with the development of targeted chemotherapeutic approaches to AML, both general and targeted immunologic approaches are being developed that show promise of improving outcomes. With this is mind, over the next decade we should anticipate an expanding repertoire of agents and approaches to deal with an increasingly subdivided group of diseases that are called AML. With still more than one-third of children eventually succumbing to this disease, there is room for new agents and approaches ultimately to improve the survival and quality of life of children with this challenging disease.

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CHAPTER 52

Hematopoietic Cell Transplantation for Acute Lymphoblastic Leukemia in Adults

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This chapter is dedicated to the memory of my friend, colleague, and mentor, Karl G. Blume, MD.

Introduction

Acute lymphoblastic leukemia (ALL) is a hematologic malignancy of the bone marrow characterized by the rapid proliferation and subsequent accumulation of immature lymphocytes. ALL accounts for 20% of all acute leukemias in adults over the age of 20 years and affects approximately two persons per 100,000 in the United States annually. Over the past two decades, there has been substantial improvement in the understanding of the molecular biology of the disease and management of adult patients with this disorder. The success of therapy in children with ALL has continued to fuel the quest for similar success in adults utilizing the same principles that guide therapy in children, namely intensive induction and consolidation, maintenance therapy, and prevention of disease in extramedullary sites such as the central nervous system (CNS). Laboratory and clinical studies, including assessment of minimal residual disease (MRD) before transplant, can refine the decisions concerning the indication and timing of allogeneic hematopoietic cell transplantation (HCT). This chapter reviews the biology and treatment of adult ALL, the relationship of specific disease characteristics to the natural history of the disease, and the role of allogeneic and autologous HCT in the management of adult ALL patients.

Etiology

The cause of ALL in adults remains unknown and the associations are less distinct than they are with acute myeloid leukemia (AML) and are more closely identified with pediatric leukemia. For instance, in children with Down syndrome, there is an 18-fold higher incidence of childhood ALL than in the general population. In addition, there is an increased risk of ALL associated with inherited disorders such as Klinefelter syndrome, Fanconi anemia, Bloom syndrome, ataxia telangiectasia, and neurofibromatosis.

The incidence of acute leukemia, mainly AML, but also ALL, was increased almost 20-fold in survivors of atomic bomb explosions who were exposed to more than 1Gy of radiation. ALL can also occur after exposure to alkylating agents, epipodophyllotoxins, and other topoisomerase 2 inhibitors.

Signs and symptoms of disease

Patients with ALL generally present with signs and symptoms of uncontrolled growth of leukemic cells in the bone marrow, lymphoid organs, and other sites of extramedullary disease. The resulting bone marrow involvement results in varying degrees of anemia, thrombocytopenia, and granulocytopenia with manifestations of pallor, fatigue, petechiae, bleeding, and fever. Liver, spleen, and lymph node enlargement is common. ALL frequently involves organs other than the bone marrow and this spread is an important consideration in the treatment strategy. Many patients have extramedullary disease at diagnosis and extramedullary relapse is a known complication. The most common sites of extramedullary disease include the CNS, testes, lymph nodes, liver, spleen, and kidney, with the CNS and testes having the most clinical significance. The occurrence of extramedullary relapse after achievement of remission frequently heralds the development of a bone marrow relapse and requires systemic chemotherapy in addition to local treatment such as radiation (testes) or intrathecal treatment (CNS).

Clonal origin of leukemic lymphoid cells

Human ALL arises from a single progenitor cell that has undergone genetic damage leading to dysregulated growth and arrested differentiation. There is considerable evidence that each leukemic cell has descended from a single transformed progenitor as cytogenetic studies show common numeric and structural chromosome abnormalities within discreet leukemic cell populations. Uniform rearrangements of immunoglobulin (*Ig*) or T-cell receptor (*TCR*) genes, as compared with a more diverse pattern of rearrangements observed in populations of normal T- and B-lymphocytes, are further evidence of the clonal development. Additional evidence of clonality comes from studies by X chromosome-linked genes that are inactivated during embryogenesis and can be used to demonstrate the unicellular development of a leukemic cell population by detecting a single type of glucose-6-phosphate dehydrogenase in female patients. In addition, the methylation patterns of restriction fragment length polymorphisms in X-linked genes detected by Southern blot analysis can also be used to show the clonal evolution from a single transformed progenitor.

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Lineage-specific features of leukemic lymphoblasts

Malignant lymphoblasts share many features of normal lymphoid progenitor development with rearrangements of the *Ig* or *TCR* genes. In many cases, the leukemic cells appear to represent a clonal expansion of a lymphoid progenitor cell that is blocked at an early stage of either B- or T-cell differentiation. Leukemic cells can be classified according to their normal developmental stage.

Mature B-cell ALL

The diagnosis of mature B-cell ALL depends on the detection of surface immunoglobulin on leukemic blasts. This rare phenotype accounts for only 2–3% of ALL cells and has distinct morphology (L3). It is important to diagnose this form of ALL accurately because most investigators believe that mature B-cell ALL is a disseminated form of Burkitt lymphoma, sharing cytogenetic, molecular genetic, immunologic, cytologic, and clinical features. For this type of ALL, responses and outcomes are better when treatment regimens are based on the treatment of Burkitt lymphoma that include rituximab, cyclophosphamide, and rapid rotation of antimetabolite drugs in high doses.

Pre-B- and early pre-B-cell ALL

Approximately 75% of ALL patients have lymphoblastic phenotype corresponding to those of B-cell progenitors. Within the B-cell lineage, the profiles of cell surface markers differ according to the stage of B-cell maturation. Pro-B-cell ALL accounts for about 10% of ALLs and is characterized by the presence of TdT, CD19, CD22, and CD79a, but negative for CD10 and other more mature B-cell markers. Approximately 50% of ALLs are positive for CD10 (the common ALL antigen or CALLA) and are termed CALLA-positive ALL. These cases frequently express CD19 and at least one other recognized B-lineage-associated antigen which includes CD20, CD24, CD22, CD21, and CD79 and also nuclear terminal deoxynucleotidyl transferase (TDT) or CD34. Approximately 10% of ALL express cytoplasmic Ig μ heavy-chain proteins but do not express surface Ig and are designated pre-B-cell ALL.

T-cell acute lymphoblastic leukemia

Leukemias of T-cell precursors are identified according to sequence of expression of T-cell-associated surface antigens during normal thymic ontogeny. The earliest T-cell precursors are characterized by the lack of expression of CD4 and CD8 surface markers, but these double-negative thymocytes express CD7, TDT, and cytoplasmic CD3 and proceed through a well-defined development, defined by the expression of CD44 and CD25. After this time, the *TCR* gene becomes rearranged and proceeds through a phenotype that is double negative to one that is double positive. This process is followed by the gain of CD3 expression and then the separation into mature CD4 and CD8 single positive cells. Similarly, T-cell ALL is frequently classified into early T-precursor ALL, expressing CD7 but not CD1a or CD3, thymic (or cortical) T-ALL, expressing CD1a but not cell-surface CD3, and mature T-cell ALL, expressing CD3. Thymic T-ALL makes up about 50% of T-ALLs and has the most favorable prognosis. In contrast to pre-B-cell ALL, the clinical features of T-cell ALL include high blood leukocyte counts, predominance in males, a higher incidence of CNS involvement, and radiographic evidence of a thymic mass in approximately half of the patients.

Mixed-lineage leukemia (ambiguous lineage)

Hematologic malignancies related to ALL include acute leukemias with ambiguous lineage, such as the mixed phenotype acute leukemias (MPALs). MPALs include bilineage leukemias in which two distinct populations of lymphoblasts are identified, with one meeting the criteria for AML, and biphenotypic leukemia in which a single population of lymphoblasts express markers consistent with B- or T-cell ALL, in addition to expressing myeloid or monocytic markers. To fulfill WHO criteria for an MPAL, the leukemic blasts must express myeloperoxidase and a strictly specific lymphoid marker. Many of these patients will have rearrangements of *BCR/ABL* or *MLL*. Treatment is guided by these findings [1].

Cytogenetic and molecular genetic analyses

In ALL, the multiple acquired genetic abnormalities are likely related to the malignant transformation and distorted cell growth and differentiation seen in ALL. These can include structural abnormalities detected by standard chromosomal analysis and molecular analysis of the DNA. Chromosomal translocations are found in many cases of ALL and are broadly classified as being recurring lineage-restricted abnormalities, accounting for two-thirds of the translocations, or random translocations or abnormalities of chromosome number. Figure 52.1 shows the frequency and distribution of major chromosomal abnormalities in patients with adult ALL [1]. In addition, the genes affected by chromosomal translocations are shown in bold face in the caption.

Cytogenetic abnormalities represent the strongest independent prognostic variables for predicting the outcome of treatment. In several multicenter studies, clonal chromosomal abnormalities could be detected in approximately 62–85% of patients, with the major abnormalities being clonal translocations (9;22, 4;11, 8;14, 1;19, 10;14) and also other structural abnormalities, including 9p, 6q, and 12p [2–4]. When there are no structural abnormalities

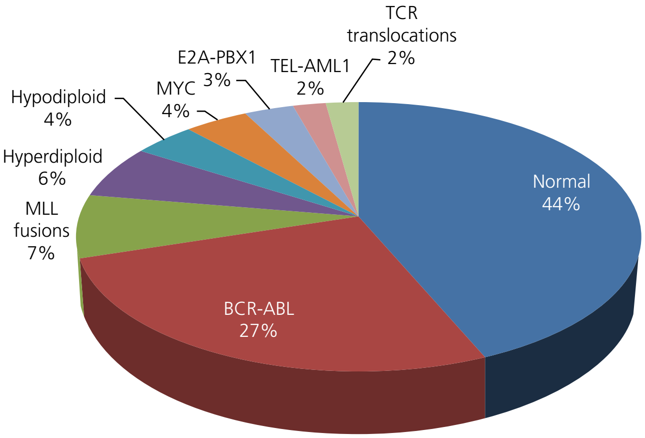

Figure 52.1 Frequency and distribution of cytogenetic abnormalities in adult patients with ALL. Dark blue includes normal cytogenetics (no recurrent chromosomal abnormalities), rust is **BCR-ABL** [t(9:22)], olive green is **MLL fusions** [t(4;11), t (1;11). T (11;19)], purple is hyperdiploid (>50 chromosomes), teal is hypodiploid (<45 chromosomes), orange is **MYC** [Ig translocations t(8;14), t(2;8), t(8;22)], light blue is **E2A-PBX1** [t(1;19)], pink is **TEL-AML1** [t(12;21)], and light green is **TCR translocations** (7q35/TCRβ, 14q1/TCRαδ), where the genes affected by chromosomal translocations are shown in bold face. Source: adapted and redrawn from Gutierrez et al. 2009 [1]. (For a color version of this figure, please see the color plate section.)

Table 52.1 Characteristics of immunologic subtypes of acute lymphoblastic leukemia (ALL)

Subgroup (incidence) (synonyms)	Disease characteristics	Cytogenetic/ molecular marker	Specific poor prognostic factors
Pro-B-ALL (11%) (CD10negALL)	High WBC (>100,000/μL in 70%) CD13/CD33 coexpression (>50%)	70% t(4;11)/ ALL1-AF4 20% Flt3 in ALL1-AF4$^+$	High risk
c-ALL (49%) Pre-B-ALL (12%)	Incidence increasing with age (75% if >55 years) Partly CD20$^+$ (45%)	4% t(1;19)/ PBX-E2A (pre-B only)	WBC >30,000–50,000/μL t(9;22)/BCR-ABL t(1;19)/PBX-E2A
Mature B-ALL (4%) (L3-ALL, Burkitt Lymphoma)	Large tumor mass (LDH increased in >90%) Organ involvement (32%) CNS involvement (13%) CD20$^+$ (>80%)	t(8;14)/c-myc-IgH	
T-ALL (25%)	Mediastinal tumor (60%) CNS involvement (8%) High WBC (>50,000/μL) (46%) Subtypes: Early T (6%) Thymic T (12%) Mature T (6%)	20% t(10;14)/ HOX11-TCR <20% t(11;14)/ LMO/TCR 8% SIE-TAL1 4% NUP213-ABL1 33% HOX11 5% HOX11L2 50% Notch-1	Early, mature T-ALL WBC >100,000/μL HOX11L2

Source: adapted from Gökbuget and Hoelzer 2006 [12].

present, the abnormalities can be classified according to the modal number of chromosomes.

Molecular analyses are used to detect gene rearrangements in ALL by polymerase chain reaction (PCR), Southern blot analysis, or fluorescence in situ hybridization (FISH) utilizing chromosome-specific probes. The most frequent molecular markers in ALL are *BCR*/*AB*L and *ALL1*/*AF4*. The Philadelphia chromosome results from a translocation involving the breakpoint cluster region of the *BCR* gene on chromosome 22 and the *ABL* gene on chromosome 9 and can be demonstrated by molecular techniques. It occurs in about 25% of patients with adult ALL and increases with age. Approximately one-third of adult ALL with Philadelphia chromosome-positive (Ph$^+$) ALL showed a *BCR* rearrangement, resulting in a 210 kDa protein, similar to patients with chronic myelogenous leukemia (CML). Two-thirds, however, have an arrangement resulting in a 190 kDa protein. The most frequent form of 11q23 abnormality in ALL is the t(4;11) translocation. One partner gene is located on chromosome 11 and is named *MLL* for mixed-lineage leukemia. This gene is fused to a gene located on chromosome 4 that is named the *AF-4* and is frequently detected in infant leukemia and in patients with early pre-B subtype (CD10$^-$). The overall incidence of this chromosomal translocation in adults is 5%. Table 52.1 summarizes molecular aberrations in ALL with associated immunophenotypic, cytogenetic translocations, and clinical features seen in adults.

Treatment of ALL in adults

The treatment of ALL begins with a proper diagnosis and immunologic and cytogenetic characterization of the disease. Bone marrow aspiration and biopsy, and also lumbar puncture, are required for all patients. These are used both for evaluating prognostic features and disease monitoring and, in some circumstances, to determine specifics of therapy including the timing and indication for HCT. For patients with mature B-cell ALL, combination chemotherapy with rituximab has resulted in a high rate of remission and cure of the disease [5]. For patients with Ph$^-$ B- and T-cell ALL, chemotherapy is usually divided into several phases, beginning with remission induction, consolidation therapy, CNS prophylaxis, and maintenance treatment. Standard induction for therapy for B- and T-cell ALL includes prednisone, vincristine, and anthracyclines, mostly daunorubicin but also L-asparaginase. Other drugs such as cyclophosphamide and cytarabine (cytosine arabinoside, Ara-C) are added in many protocols. Prednisone and prednisolone have been the most frequently administered, although dexamethasone has a higher antileukemic activity in vitro and possibly better penetration into the CNS. Although the anthracycline dose intensity and schedule may play a role, many trials include a dose intensification with a 2–3-day schedule at the onset. Table 52.2 shows chemotherapy regimens commonly used in the treatment of adult ALL and the published response [6–10].

Table 52.2 Chemotherapy regimens for adult ALL

Study	Induction	Consolidation	CR (%)	Overall survival (%)
CALGB 8811 [6]	D, Pred, V, C, L-Asp	C, SC Ara-C, 6-MP, V, L-Asp, Dox, MTX, 6-TG, Dex	84%	50% (3-year)
Linker [7]	D, Pred, V, L-Asp	V, Pred, D, L-Asp, 6-MP, Ara-C, E, MTX	93	47 (5-year)
Hyper-CVAD [8]	C, V, Dox, Dex alternating with MTX, Ara-C	See "Induction"	92	38 (5-year)
UKALL/ECOG 2993 [9]	D, Pred, V, C, Ara-C, 6-MP	Ara-C, E, V, Dex, D, C, 6-TG	91	38 (5-year)
BFN [10]	D, V, Pred, L-Asp (phase I) C, Ara-C, 6-MP (phase II)	V, L-Asp, Dex (phase I) C, Ara-C, 6-TG (phase II)	83	35 (5-year)

ALL, acute lymphoblastic leukemia; Ara-C, cytarabine; BFN, Berlin–Frankfurt–Münster; C, cyclophosphamide; CALGB, Cancer and Leukemia Group B; CR, complete remission; D, daunorubicin; Dex, dexamethasone; Dox, doxorubicin; E, etoposide; ECOG, Eastern Cooperative Oncology Group; L-Asp, L-asparaginase; 6-MP, 6-mercaptopurine; MTX, methotrexate; Pred, prednisone; SC, subcutaneous; 6-TG, 6-thioguanine; UKALL, United Kingdom ALL; V, vincristine.

In addition, studies derived from childhood ALL, where the upfront therapy is curative in most patients, have also been explored in adults, with a suggestion of improved outcome. These and other studies that utilize pediatric-based regimens and schedules may provide a more effective treatment for patients up to age 35–50 years who are otherwise in reasonable health [2,11].

About 15–20% of adult patients do not achieve a complete remission (CR) after induction therapy compared with <3% of patients with childhood ALL. Although <10% of adult patients die during induction, the mortality is related to age, with the major cause of death being infection. Patients who fail to achieve a remission, analogous to those patients who are slow to achieve a remission and require two or more cycles of remission induction therapy, have a very poor prognosis and are candidates for early HCT [12]. Several studies have demonstrated that allogeneic HCT performed after failure to achieve CR can be curative in approximately 20% of patients [13–15]. Therefore, for adult patients, human leukocyte antigen (HLA) typing of the patient and family members is an important test to be performed early after diagnosis.

CNS prophylaxis

CNS leukemia occurs in about 6% of patients, with a higher incidence in T-cell ALL (8%) and mature B-cell ALL (13%). Treatment and prophylaxis of CNS leukemia usually consist of intrathecal methotrexate

(MTX) alone or in combination with Ara-C. Adult ALL patients who do not receive specific CNS treatment have a CNS relapse rate of 30%, similar to that observed in children. Therefore, all patients should receive some form of CNS therapy. In the past, prophylaxis often included CNS radiation to 24 Gy, which reduced the CNS relapse rate to about 9%. Most programs no longer include cranial radiation and utilize early systemic high-dose MTX and intrathecal therapy. The risk of CNS relapse is associated with other prognostic factors, including T- and B-cell ALL, extreme leukocytosis, elevated lactate dehydrogenase (LDH), and extramedullary organ involvement.

Management of CNS prior to HCT

In general, the development of a CNS relapse in patients with ALL portends a poor prognosis both for the effect it has on the CNS and because it is a harbinger of systemic relapse. Most patients with ALL have had some form of CNS prophylaxis with radiation or chemotherapy prior to their first systemic relapse. Adults with an isolated CNS relapse fare poorly and such an event is an indication for HCT [16]. Patients who relapse in the CNS require additional therapy to control the disease prior to HCT. Previous prophylactic intrathecal therapy and CNS radiation do not necessarily preclude the use of total body irradiation (TBI) in the preparation for HCT. However, if a patient has had CNS irradiation and relapses, a second course of irradiation prior to a TBI-containing preparative regimen is not recommended as it may lead to more neurotoxicity following transplantation. In general, these patients can be managed with either intrathecal MTX or the combination of MTX, Ara-C, and hydrocortisone until there is clearing of leukemic cells from the spinal fluid. Following HCT, such patients should receive five intrathecal MTX injections during the first 100 days followed by once-monthly injection for 12–18 months unless toxicity precludes additional treatments. This approach, designed in Seattle, can result in control of the leukemia without substantially increasing the risk of leukoencephalopathy [17]. The risk of CNS damage caused by cranial radiation, intrathecal chemotherapy, and high-dose chemotherapy has been documented and is related to the amount of intrathecal chemotherapy following the HCT procedure [17].

Currently, a common approach for patients with ALL who will be undergoing an HCT, without any evidence of prior CNS disease, is to administer intrathecal MTX as prophylaxis for a total of five times prior to the HCT procedure. Cranial radiation and post-transplant intrathecal therapy is not necessary if the patient then receives a preparatory regimen containing fractionated TBI.

Maintenance therapy

The optimal duration and form of maintenance therapy in adult ALL is unknown. The goal of maintenance treatment is to eliminate MRD that may have persisted after induction and consolidation treatment. Standard maintenance is usually based on a combination of 6-mercaptopurine and MTX. Attempts in previous trials to omit maintenance after induction and consolidation therapy resulted in inferior results.

In general, the cure rates with chemotherapy for adults with ALL are 35–40%. These results may be better in adolescents and younger adults utilizing more pediatric chemotherapy-based regimens and are poorer in older adults, especially over the age of 60 years [18,19].

Treatment of Philadelphia chromosome ALL

As noted above, t(9;22) is the most frequent genetic alteration in adult ALL, found in 20–30% of patients, and the incidence increases with age. The phenotype of this leukemia is almost exclusively CD10$^+$ precursor B-ALL and there are only rare reports of its presence in T-lineage ALL. Clinically, patients present with a variable white blood cell (WBC) count, surface expression of CD19, CD10, and CD34, and the co-expression of myeloid markers, especially CD13 and CD33. Cytogenetic and molecular genetic analyses are required to establish the diagnosis of Ph$^+$ ALL and can be obtained within 1 week of diagnosis. Historically, the prognosis of adult patients with Ph$^+$ ALL treated only with chemotherapy has been poor, with less than a 10% probability of long-term disease-free survival (DFS). Complete remission rates after induction therapy range from 60 to 90%; however, the median remission duration was inferior, between 9 and 16 months. The disease has been even more difficult to treat in older patients with ALL, where this cytogenetic abnormality actually is more common.

The understanding of the leukemogenic role of the *BCR/ABL* oncogene and its dependence on the constitutive activation of the *ABL* tyrosine kinase led to the development of selective *ABL* inhibitors, with imatinib being the first to gain clinical approval. Its initial activity was demonstrated in phase I and II trials in CML, but also in ALL. When treated with imatinib as a single agent, many patients with Ph$^+$ ALL achieved a remission, but there were no long-term survivors. Only the subset of patients who then went to allogeneic HCT while in remission had a favorable outcome, with 50% of the patients disease free at 1 year [20]. The limitations of single-agent imatinib led to various investigations to explore the efficacy of imatinib as front-line therapy in combination with chemotherapy. Phase II trials combining imatinib or dasatinib (days 1–14 of each cycle), with hyper-CVAD (fractionated cyclophosphamide, vincristine, adriamycin, and dexamethasone) chemotherapy had a very high remission rate after 21 days of treatment, suggesting synergy between imatinib and concurrent chemotherapy, with some patients achieving a molecular remission [21,22]. Similarly, encouraging results have been achieved in other centers in which imatinib was started after 1 week of induction therapy and then coadministered with chemotherapy during the remaining induction. In these trials, the 1-year event-free survival (EFS) and overall survival (OS) were 78–88% with tolerability comparable to that seen with chemotherapy alone. Both alternating and concurrent imatinib chemotherapy combinations have been studied, with a clear advantage of the simultaneous over the alternating schedule in terms of the achievement of a negative PCR for the *BCR/ABL* gene [23]. Those patients who undergo allogeneic HCT for ALL do not seem to have any detrimental effect of prior imatinib treatment.

Given the increased incidence of Ph$^+$ ALL in older patients with ALL and the difficulty of achieving a remission in these patients, imatinib has also been studied as front-line treatment in older patients with ALL, with an improved outcome compared with chemotherapy and with better tolerability [24]. Several studies now suggest that imatinib combined chemotherapy in newly diagnosed Ph$^+$ ALL with subsequent treatment with allogeneic HCT has improved the results of treatment. With regard to the CNS, patients with Ph$^+$ ALL are at significant risk for developing CNS leukemia [25]. Imatinib concentrations in the CSF reach about 1–2% of serum levels and are not therapeutic. hence all patients with ALL, independent of their chromosome abnormality, require CNS prophylaxis. Additional novel tyrosine kinase inhibitors with significantly more potent activity against the *BCR/ABL* gene are now available. Nilotinib has recently been tested in combination with chemotherapy in newly diagnosed patients with encouraging results [3,4]. As discussed below, the use of these drugs may have an impact on the pre-transplant disease burden of the patient and reduce the risk of relapse following HCT.

Prognostic factors in ALL

The major prognostic factors for achieving a CR are advanced age and, historically, Ph^+ ALL, although this has now changed with the use of tyrosine kinase inhibitor (TKI) drugs as part of induction therapy. Prognostic factors are of even greater importance in predicting the durability of remission and survival and are utilized to assess the need for allogeneic HCT. Table 52.3 identifies some of the adverse prognostic factors for remission duration in adult ALL that have been identified in previous clinical trials and are utilized in determining a patient's risk of relapse.

Role of minimal residual disease

In addition to age and cytogenetic analysis at the time of diagnosis, the most important prognostic factor, and a direct reflection of sensitivity to chemotherapy, is the rapid achievement of CR. Thus, a slower time to achieving a remission is an indicator of relative chemoresistance, similar to what has been observed in pediatric patients. Those patients who take more than one cycle of induction chemotherapy have a poor long-term prognosis and shorter remission duration [6,9,10].

A more quantitative approach to assess the response of an individual patient to chemotherapy is the measurement of minimal residual disease at various time points after therapy. Quantitative assessment of tumor cell kill has emerged as an independent prognostic factor that reflects the resistance of the cells to chemotherapy and may allow individualization of treatment [16,26,27]. The assessment allows the identification of potential patients at high risk for relapse despite achieving a morphologic remission and who may benefit from early HCT. At present, there are ongoing efforts to standardize MRD quantitation using real-time quantitative PCR of clonal immunoglobulin and *TCR* gene rearrangements, fusion gene transcripts or breakpoints, and multiparameter flow cytometric immunophenotyping (see Chapter 25 for a more complete discussion of the MRD measurement of utility). It remains to be determined what the benefit of HCT may be in patients who are in first remission, but have evidence of this new factor defining high-risk disease, that is, high MRD, compared with those patients who are MRD negative at the time of transplant. Hence the future of treatment of adult patients with ALL in first remission will be refined to determine those patients who are unlikely to benefit from further chemotherapy and should be considered for transplantation during first remission and those likely to do well with continued chemotherapy [28]. The detection of MRD at the completion of therapy also has implications for the success of transplant when patients with detectable MRD have a greater chance of relapse than those patients who are MRD negative [14]. An additional important question is whether MRD-positive patients should receive additional therapy pre-transplant in order to reduce the disease burden. Monitoring MRD in patients after transplantation in order to treat prior to overt relapse may also become a useful practice.

Table 52.3 Clinical and laboratory risk factors for adult ALL

Risk factor	Prognosis association
At presentation	
Age	Continuous variable; adverse outcome with advancing age
CNS involvement	Adverse outcome
Presenting WBC count	Adverse for B-cell phenotype: >30 × 10^9/L Adverse for T-cell phenotype: >100 × 10^9/L
Immunophenotype	Adverse with CD20 expression
Cytogenetics	Adverse: t(9;22); t(4;11); complex (>5 abnormalities); low hypodiploidy, near tetraploidy Favorable: high hyperdiploid; del 9q
Molecular abnormalities	Adverse: JAK2; IKFZ1; PAX5; TLX3; ERG; BAALC Favorable: TLX1
In response to therapy	
Time to initial response	Adverse: failure to attain CR within 4 weeks of induction (variably demonstrated among studies)
Detection of MRD	Adverse: detection at various time-specific points in several studies

Allogeneic HCT in first complete remission of ALL

Allogeneic HCT in first CR has generally been reserved for those patients who present with poor risk features, such as those described earlier. In several phase II studies, patients with high-risk disease treated with allogeneic HCT had a DFS longer than would have been predicted, especially those with Ph^+ ALL. Studies have been conducted that indicate that HCT offers some groups of high-risk patients long-term disease survival rates of between 40 and 60%. At Stanford University, a series of patients with high-risk features underwent full-intensity allogeneic HCT in first CR included patients with WBC >25,000/μL, chromosomal translocations t(9;22), t(4;11), and t(8;14), age >30 years, extramedullary disease at the time of diagnosis, and/or requiring more than 4 weeks to achieve a CR. Two-thirds of the patients had at least one risk factor and the remaining patients had two or more high-risk features at presentation. The majority of these patients underwent allogeneic HCT within the first 4 months after achieving a CR. At a median follow-up of >5 years, the probability of EFS was 64% with a relapse rate of 15% [29] (Figure 52.2).

Postremission therapies: matched-sibling HCT versus chemotherapy or autologous HCT

There have been several prospective studies that have reported patient outcomes after a variety of postremission therapies, with or without a family donor, who could then proceed to transplant (Table 52.4). Some of these studies also compared matched-sibling HCT versus chemotherapy or autologous HCT. Ribera et al. analyzed 83 Ph^- ALL patients in CR1 (eligibility age, 15–50 years) and failed to demonstrate that having a family donor available for allogeneic HCT provided superior outcomes compared with chemotherapy or autologous HCT [30]. The reported relapse rate of 62% at 5 years in the donor group, however, seemed surprisingly high in patients transplanted in first remission. The MRC UKALLXII/ECOG 2993 study was designed to determine the impact of transplant in first remission on relapse and survival [31,32]. The trial enrolled patients aged 15–64 years, but 59 years was the upper age limit for allogeneic HCT recipients. Using a donor versus no-donor analysis, they showed that Ph^- ALL patients with a donor had an improved 5-year OS (53% vs. 45%, $p = 0.01$) and an associated significantly lower relapse rate ($p < 0.001$) (Figure 52.3). The survival difference was significant in standard-risk patients, whereas in high-risk (especially older) patients, the reduced relapse risk was offset by an increase in non-relapse mortality (NRM). In this large study, subjects randomized to chemotherapy had a better 5-year OS (46%) than those randomized to autologous HCT (37%, $p = 0.03$), demonstrating no benefit for a single autologous HCT. Cornelissen et al., for Hemato-Oncologie voor Volwassenen Nederland (HOVON), reported on two consecutive, prospective studies of myeloablative (MA) HCT in ALL CR1 patients aged 15–55 years, similar to the MRC UKALLXII/ECOG

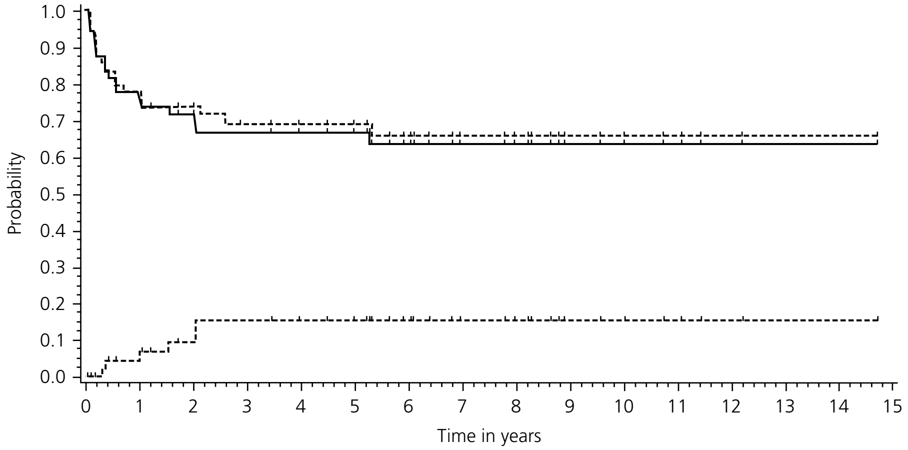

Figure 52.2 Probability of event-free survival (top curve), overall survival (middle curve), and relapse (bottom curve) for 55 adult patients with high-risk acute lymphoblastic leukemia transplanted in first remission. Source: Jamieson et al. 2003 [29]. Reproduced with permission of Elsevier.

Table 52.4 Randomized trials in adult ALL comparing allogeneic transplant to non-transplant therapies

Study	Patients	TRM/NRM	Relapse	Survival
Ribera et al., PETHEMA [30]	*n* = 156 Ph$^-$; age 15–50 yr; 72 donor; 84 no donor	10% donor; 2% no donor	@5 yr: 62% and 51%	@5 yr: DFS 37% donor, 46% no donor; OS 40% donor, 49% no donor
Goldstone et al., MRC-ECOG [31,32]	*n* = 1031; age 15–64 yr (allo 15–59 yr); 443 donor; 588 no donor	@2 yr: high-risk 36% donor, 14% no donor; standard-risk 20% donor, 7% no donor	@10 yr: high-risk 37% donor, 63% no donor; standard risk 49% donor, 24% no donor	@5 yr: OS: high-risk 41% donor, 35% no donor; standard-risk 62% donor, 52% no donor
Cornelisson et al., HOVON [33]	*n* = 257, age 15–55 yr; 96 donor; 161 no donor	@5 yr: 16% donor, 3% no donor	@5 yr: 24% donor, 55% no donor	@5 yr: DFS: 60% donor, 42% no donor (p = 0.01)
Kako et al., JALSG [34]	*n* = 649; age 15–54 yr; 241 chemotherapy; 408 HCT	NR	NR	@10 yr: OS: standard-risk: chemotherapy 40%, HCT 54%; high-risk: chemotherapy 25%, HCT, 38%

Source: adapted from Lazarus and Advani 2012 [76].

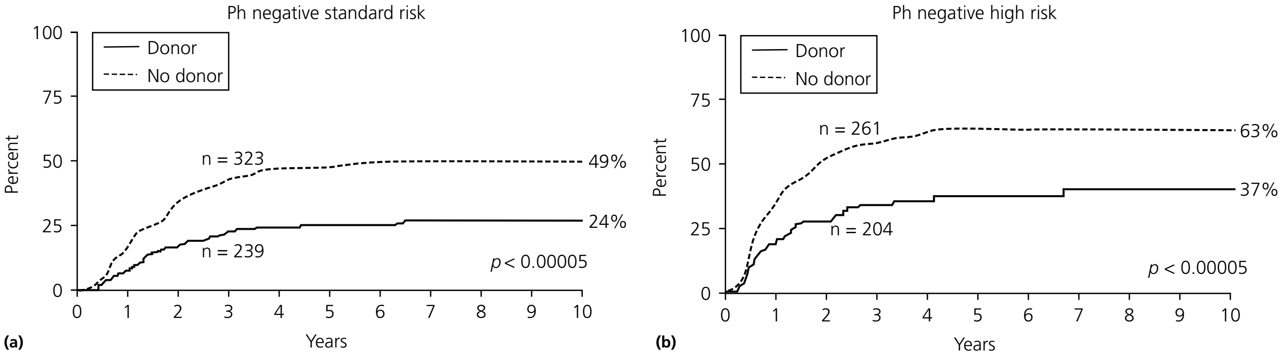

Figure 52.3 Comparison of relapse rates between allogeneic transplant and chemotherapy for (a) Ph$^-$ patients at standard risk and (b) Ph$^-$ patients at high risk. Source: Goldstone et al. 2008 [32]. Reproduced with permission of the American Society of Hematology.

trial described above [33]. They showed significantly better 5-year DFS in the donor group (60% vs. 42%, $p = 0.01$). As expected, NRM was significantly higher in the donor group (16% vs. 3%, $p = 0.002$), but those patients with a donor had a markedly lower incidence of relapse at 5 years (24% vs. 55%, $p < 0.001$), confirming the improved disease control for patients undergoing transplant. As in the MRC/ECOG study, the benefit was most pronounced in the standard-risk group, although in this study poor-risk patients in the donor group also had a significantly improved outcome. Kako et al., for the Japan Adult Leukemia Study Group (JALSG), analyzed two JALSG studies (ALL93 and ALL97) in order to identify the optimal strategy of postremission therapy in Ph$^-$ ALL CR1 patients who had an HLA-matched sibling in patients aged 15–54 years [34]. In their analysis, allogeneic HCT was superior to other therapies, with a 10-year survival probability of 48.3% versus 32.6%, respectively.

Overall, these studies suggest that allogeneic HCT is more effective than chemotherapy or autologous HCT for control of leukemia in Ph$^-$ ALL CR1 patients. The statistically significant survival advantage, however, appears to be greater for standard-risk than high-risk ALL patients, especially if MRD positive after induction [5] mostly related to the impact of age on outcome, a finding that likely reflects the increased treatment-related mortality (TRM) in the high-risk group that negated the graft-versus-leukemia (GVL) effect in these patients.

HCT for Philadelphia chromosome-positive ALL

Historically, the dismal outcome with chemotherapy has led to trials focusing on the use of allogeneic transplantation for treatment of adult Ph$^+$ ALL. Most have been single-institution studies utilizing a variety of regimens and the cure rate varies from 30 to 65%, dependent upon age and remission status [35,36]. Investigators from City of Hope and Stanford University analyzed their experience in 79 patients with Ph$^+$ ALL transplanted from HLA-identical siblings while in first CR between 1984 and 1997 to determine long-term survival and disease control [37]. All patients but one were conditioned with fractionated total body irradiation (FTBI) (1320 cGy) and high-dose etoposide (60 mg/kg). The 3-year probability of DFS and relapse was 55% and 18%, respectively ,with the latest relapse at 27 months. Beyond first remission, HCT is curative in a much smaller minority of patients but remains the treatment of choice (Figure 52.4).

The development of imatinib and dasatinib for the treatment of *BCR/ABL*-positive hematopoietic malignancy has changed the upfront treatment strategy and outcome and may also affect the outcome after HCT. Dasatinib is a second-generation TKI that has enhanced potency against the BCR/ABL protein and the ability to block the SRC family of kinases. In a clinical trial of Ph$^+$ ALL, newly diagnosed patients with Ph$^+$ ALL were treated with hyper CVAD and dasatinib 100 mg/day; 94% achieved a CR and the 2-year survival was 64%. The feasibility of performing allogeneic HCT after first-line treatment with imatinib plus chemotherapy has also been reported [38,39]. In one series, 29 adult patients who completed induction therapy were treated with allogeneic HCT and the authors compared their results with those for 31 patients who had received transplantation in their unit prior to the availability of imatinib treatment. The data suggest that the risk of relapse was significantly less in the imatinib group (3.5% vs. 47.3%, ($p = 0.002$), potentially reflecting a lower burden of residual disease at the time of HCT and allowing a higher percentage of patients to come to transplantation in a low MRD first remission. The results also indicated

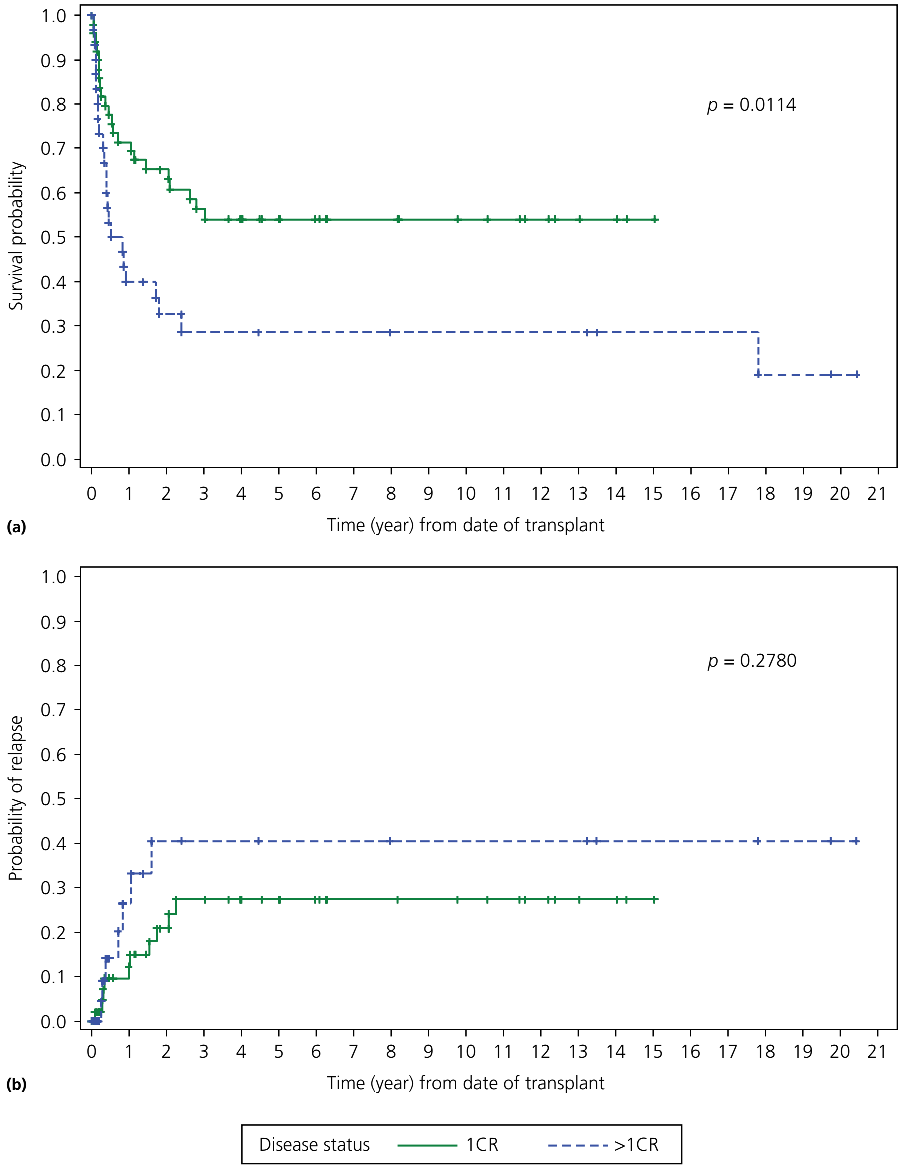

Figure 52.4 Probability of long-term (a) disease-free survival and (b) risk of relapse in 79 patients with Ph$^+$ ALL following allogeneic HCT in first remission and beyond first remission Source: Laport et al. 2008 [37]. Reproduced with permission of the American Society of Hematology.

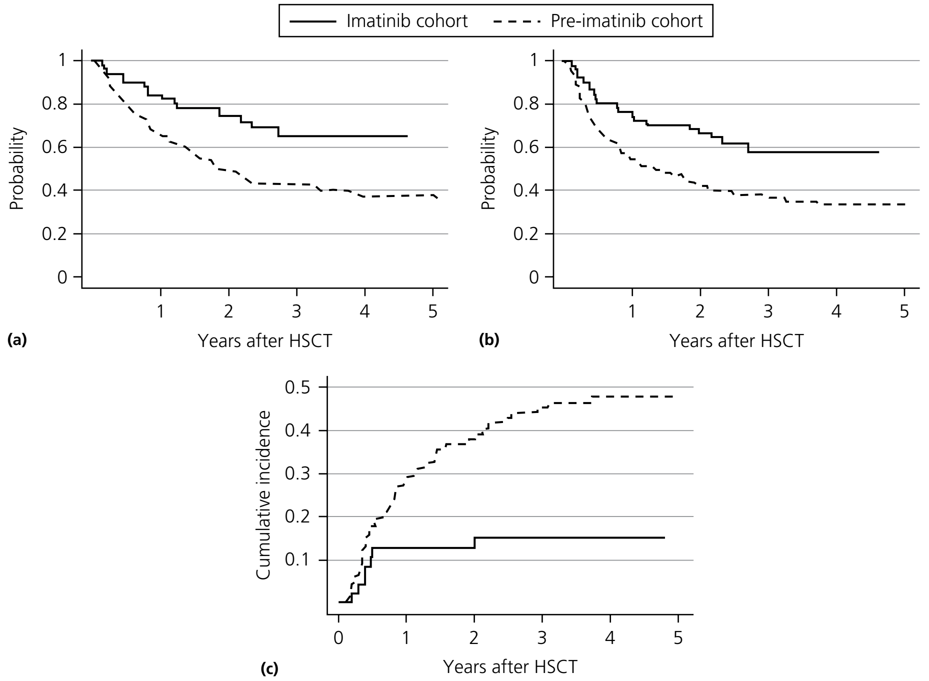

Figure 52.5 Transplantation outcomes of 51 patients who received imatinib-based therapy and 122 historical patients. (a) Overall survival, (b) disease-free survival, and (c) cumulative incidence of relapse. Source: Mizuta et al. 2011 [39]. Reproduced with permission of Nature Publishing Group.

superiority in DFS (76% vs. 38%, $p < 0.001$) without much difference in the transplant-related toxicities. In the second series of patients, the probability of OS, DFS, and relapse were improved in these patients who received pre-transplant imatinib (Figure 52.5) [39]. Thus, in the same way that imatinib may improve the upfront success of induction therapy and potential long-term outcome of patients with Ph^+ ALL, entering transplantation with lower burden of disease may improve the cure rate for such patients [38,39]. Currently, a national trial comparing hyper-CVAD and dasatinib with allogeneic transplant followed by post-transplant dasatinib to determine the best upfront approach for patients with Ph^+ ALL has been completed.

Relapsed or primary refractory ALL

ALL is refractory to primary chemotherapy in approximately 10–15% of patients and allogeneic HCT can be successfully used to achieve both remission and long-term control in approximately 20% of such patients and should be considered when patients fail to achieve a CR after the first course of intensive induction therapy treatment. Of all those patients who do achieve a first CR to primary therapy, approximately 50–70% will relapse. Relapsed ALL in an adult is not curable with standard chemotherapy, but remissions are sometimes achieved with reinduction using either a standard vincristine, prednisone, and anthracycline regimen or with a cytarabine-based regimen, particularly high-dose Ara-C (HDAC) combined with an anthracycline or clofarabine, especially in patients with a long first remission [40]. Many studies have confirmed that in the absence of HCT, adult patients with relapsed ALL have an extremely poor prognosis regardless of initial remission duration, and that transplantation, when feasible, is the only possible curative therapy [41]. Available data from the Center for International Blood and Marrow Transplant Research (CIBMTR) show that patients transplanted using an HLA-identical sibling donor for ALL in CR2 have approximately a 35–40% chance of long-term DFS, whereas those transplanted with disease not in remission have a DFS of only 10–20%. Figure 52.6 shows the overall DFS for patients with ALL who were able to achieve a second remission, had a donor, had no major contraindications, and underwent allogeneic HCT. As in all series, these data do not account for those patients who suffer a relapse and do not undergo transplantation, which represents the majority of patients [42]. When accounting for all patients with relapsed ALL, the overall cure rate for such patients is much less than 20%. There is an urgent need for new medicines that can be used as a bridge to transplant, the most promising of which are bispecific engaging antibodies to CD19 (blinatumomab) drug immunoconjugates such as inotuzumab, and SAR 3419, CD19-specific chimeric antigen receptor (CAR) transduced T cells and ponatinib for patients with refractory Ph^+ ALL. Nevertheless, for an

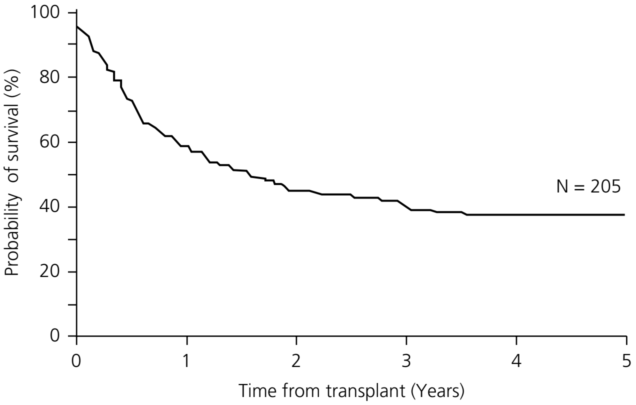

Figure 52.6 Disease-free survival of patients undergoing allogeneic transplant while in second complete remission. Source: Forman and Rowe 2013 [42]. Reproduced with permission of the American Society of Hematology.

adult patient with ALL after a first relapse, therapy that facilitates treatment with an allogeneic HCT is the best curative option.

Unrelated donor HCT for ALL

Historically, the outcome after transplantation from unrelated donors has been inferior to that observed after matched-sibling transplantation because of increased rates of graft rejection and graft-versus-host disease (GVHD) resulting from increased alloreactivity in this setting. Over the past few years, improved results have been reported from several single-center studies, reflecting improvements in donor–recipient allele level molecular matching in both class I and II histocompatibility genes, GVHD prophylaxis, and supportive care, especially prevention of viral and fungal infections. Published reports suggest equivalent overall survival results for patients with Ph^- ALL undergoing transplant in first or subsequent remission with the risk of relapse being lower in patients receiving transplant from an unrelated donor [43–45], making this a very reasonable option for patients who need an allogeneic HCT for treatment of their disease, but do not have a sibling donor.

Role of GVL effect in patients with ALL

The low response rate in patients with ALL following donor lymphocyte infusion (DLI) has led to questions about the significance of the GVL effect in preventing relapse in this disease. The GVL effect is derived from observations of a higher relapse rate after autologous or syngeneic HCT compared with allogeneic HCT, lower incidence of relapse in patients who had GVHD, and increased relapse rates in recipients of T-cell-depleted marrow grafts. The most compelling argument for a strong GVL effect in ALL comes from both single-institution and registry data [46–48]. These studies show a consistent decrease in relapse rates in patients who develop GVHD compared with those who do not. Table 52.5 shows the rate of relapse after HCT for ALL in CR1 and the correlation with GVHD. The occurrence of acute, chronic, or both forms of GVHD correlated with the best DFS. A subsequent study in 182 patients suggested a potential GVL effect in patients who were not in remission at the time of transplant [49]. Studies have also demonstrated the beneficial impact of chronic GVHD on reducing relapse in patients undergoing allogeneic HCT for ALL, including Ph^+ ALL [50,51].

Although the data support the importance of a GVL effect in mediating a clinically useful antileukemic response in patients with ALL, the reasons for the limited beneficial effect for patients with relapsed ALL treated with DLI are not clear. Nonetheless, cytoreduction with chemotherapy prior to infusion of DLI is a better strategy for patients with relapsed ALL. It is for these reasons that HCT after reduced-intensity conditioning (RIC) is of limited effectiveness in patients with ALL who are not in remission. Studies focused on developing CD19-specific CAR transduced T-cell immunotherapy for ALL may help augment the GVL activity of donor T cells (see Chapter 69).

Table 52.5 Relapse after transplantation for ALL in CR1 [47]

Group	Relapse probability at 3 years (%)
Allogeneic, non-T-depleted	
No GVHD	44 ± 17
Acute only	17 ± 9
Chronic only	20 ± 19
Both	15 ± 10
Syngeneic	41 ± 32
Allogeneic, T-depleted	34 ± 13

HCT after reduced-intensity conditioning for treatment of ALL

There have been only a limited number of studies of RIC in patients with ALL. In general, the consensus has been that for patients with ALL, high dose chemoradiotherapy was required for an improved cure rate, but this approach is of limited use in patients over the age of 50 years. In addition, an evaluation of outcomes suggests that the graft-versus-tumor effect is more effective against myeloid malignancies such as AML and CML and B-cell malignancies of mature B cells such as low-grade non-Hodgkin lymphoma, chronic lymphoid leukemia (CLL), and multiple myeloma, but less so with a more undifferentiated B-cell disease such as pre-B-cell ALL, especially if not in remission [52,53] (see Chapter 16). Nevertheless, an increasing number of small studies have been conducted that suggest that there is a role for reduced-intensity allogeneic transplant even in this disease, particularly in older patients, with 34% achieving long-term remission in a report from the European Group for Blood and Marrow Transplantation (EBMT) [52]. A report of patients undergoing HCT utilizing either a related, unrelated ,or cord blood donor and a fludarabine/melphalan or clofarabine/busulfan-based regimen showed optimistic outcomes in a group of patients either at high risk during first remission or who were transplanted after achieving a second or subsequent remission [54]. Table 52.6 shows the outcome for several series of patients with ALL undergoing reduced-intensity transplantation with regimens of varying intensity with DFS ranging from 38 to 63% with only 50% of patients receiving this therapy in CR1 [16,55–62]. Patients with Ph^+ ALL who received post-transplant imatinib showed a DFS of 62% [62]. The results of the recent UK ALL XII ECOG 2993 study of adult ALL show high toxicity and limited improvement in DFS despite better disease control in older adult patients. Hence there is increased interest in the development of clinical trials exploring these reduced-intensity approaches in older patients with ALL in remission who would otherwise be candidates for transplantation based on age, cytogenetics, MRD, and response to initial treatment. The poor outcome of older patients

Table 52.6 Reduced-intensity transplant outcomes for ALL

Study	Patients	TRM/NRM	Relapse	Survival
Stein et al., COH [55]	n = 24 (11 CR1)	22% @ 2 yr	21% @2 yr	DFS/OS: 62% @ 2 yr
Bachanova et al., UMN [56]	n = 22 (12 CR1; 8 Ph-)	27% @ 3 yr (8% CR1)	36% @ 3 yr	OS: 50% @ 3 yr (81% CR1)
Cho et al., Korea [57]	n = 37 (30 CR1)	18% @ 3 yr	20% @ 3 yr	DFS: 63% @ 3 yr; OS: 64% @ 3 yr
Nishiwaki et al., JMDP/JSHCT [58]	n = 81 MA; n = 26 RIC (21 CR1)	40% @ 2 yr 36% @ 2 yr	18% @ 2 yr 26% @ 2 yr	DFS/OS: 58% @ 2 yr DFS/OS: 63% @ 2 yr
Mohty et al., EBMT [59]	n = 449 MA (391 CR1); n = 127 RIC (105 CR1)	29% @ 2 yr 21% @ 2 yr	31% @ 2 yr 47% @ 2 yr	LFS: 38% @ 2 yr LFS: 32% @ 2 yr
Marks et al., CIBMTR [60]	n = 1428 MA (747 CR1); n = 93 RIC (55 CR1)	33% @ 3 yr 32% @ 3 yr	26% @ 3 yr 35% @ 3 yr	OS: 51% @ 3 yr OS: 45% @ 3 yr
Kebriaei et al., MDA [61]	n = 51 (30 CR1, 13 CR2)	32% @ 1 yr	16% @ 1 yr 37% @ 2 yr	OS: 61% LFS: 54%
Ram et al., FHCRC [62]	n = 51	28% @ 3 yr	40% @ 3 yr	34% (3 yr) 62% (Ph^+ ALL)

COH, City of Hope; EBMT, European Group for Blood and Marrow Transplantation; JMDP, Japan Marrow Donor Program; JSHCT, Japan Society for Hematopoietic Cell Transplantation; MDA, M.D. Anderson, Fred Hutchinson Cancer Research Center; UMN, University of Minnesota.
Source: adapted from Lazarus and Advani 2012 [76].

with ALL using standard chemotherapy makes this an important consideration for treatment in those patients.

Regimen development for allogeneic HCT for ALL

Historically, the most commonly used ablative regimen for transplantation of patients with ALL is cyclophosphamide (CY) combined with FTBI. Several other preparative regimens have been developed, each based on substituting a different chemotherapeutic agent for CY in combination with TBI for patients with ALL.

Investigators at Johns Hopkins University approached the problem by substituting busulfan (BU) for TBI in order to decrease the long-term side-effects of TBI [63,64]. These non-radiation-dependent regimens have shown activity in the treatment of advanced ALL, demonstrating that TBI is not an absolute requirement for successful treatment of ALL by HCT. A retrospective analysis from the CIBMTR found that a conventional CY/TBI regimen was superior to a non-TBI-containing regimen of BU plus CY in children, with a 3-year survival of 55% versus 40% for BU/CY [65]. However, despite these differences in survival, the risk of relapse was similar. Studies of BU, fludarabine and 400 cGy of TBI showed a low transplant-related mortality of 3% and a projected DFS of 65% [66].

The group at the City of Hope studied the substitution of etoposide (VP16) for CY in combination with FTBI (13.2 Gy) followed by allogeneic HCT [67]. A phase I/II trial indicated that a VP16 dose of 60 mg/kg is the maximum tolerated dose when combined with a TBI dose of 1320 rad. In that study, 36 patients with ALL were treated, 20 of whom were in relapse. The actual DFS was 57% with a 32% relapse rate, suggesting that the regimen had significant activity in patients with advanced ALL, a result confirmed in a subsequent trial from the Southwest Oncology Group [68]. A subsequent study from City of Hope/Stanford showed a 64% DFS for adult patients undergoing transplantation with this regimen in CR1 (see the section on allogeneic transplant for ALL in first remission). The UK ALL XII/ECOG 2993 trial, a comparative study of chemotherapy and autologous and allogeneic HCT, utilized this regimen for patients in CR1.

A comparative analysis of TBI combined with either cyclophosphamide or etoposide chemotherapy was conducted to determine the relative efficacy of chemotherapy in the radiation-based transplant regimen and suggested that when the TBI dose was >13 Gy, the results are the same for TBI/CY and FTBI/VP16 [69]. Transplant-related mortality did not differ by conditioning regimen.

Management of relapse after allogeneic transplant for ALL

If patients with ALL relapse after HCT, the prognosis is very poor. Similarly to the approach for patients with AML and CML, manipulation of the antitumor effect mediated by the donor graft is often employed as a treatment strategy. Unfortunately, in patients with ALL, this therapy has not been as effective as it has been for patients with other hematologic malignancies. In a report from Europe, 40 patients with ALL received DLI as treatment for relapse and of 29 evaluable patients, only one achieved CR [70]. Therefore, DLI as a sole therapy appears to have low potential for contributing to remission and long-term control of disease in patients with relapsed ALL and, if considered, should be a component of a chemotherapy-based treatment program. For those patients with Ph^+ ALL, imatinib or dasatinib, either alone or with chemotherapy, is effective in helping some patients achieve another remission, although the duration of the remission is often fairly short and DLI should be performed before another relapse occurs. The role of donor-derived CAR transduced CD19-specific T cells is also being explored as a treatment for relapse after transplant [6]. Remissions have also been achieved following relapse utilizing a CD19 BiTE antibody without inducing a flare of GVHD [7].

Autologous HCT for adult ALL

There is much less experience with autologous transplantation for ALL and studies have been focused primarily on those patients in either first or second remission who lacked a sibling or unrelated allogeneic donor. Several groups have reported outcomes for large series of adults with ALL undergoing autologous HCT in first remission [71]. The most important prognostic factor was the interval between achieving a CR and proceeding to transplant, with those patients being transplanted later having the better DFS, but no real improvement over the results achieved with chemotherapy. This effect may represent the dropout of high-risk patients who relapse before transplantation or possibly the effect of consolidation therapy in reducing tumor burden administered prior to HCT. The European Cooperative Group/MRC report on over 1000 patients indicated a leukemia-free survival (LFS) of 36% and the CIBMTR reported a similar plateau at 40%.

One randomized trial evaluated outcomes of adults with ALL in first remission treated with chemotherapy versus autologous transplantation. The French LALA 87 trial allocated patients under 40 years of age with HLA-matched siblings to allogeneic transplantation whereas the remaining patients received consolidation treatment with modest dose chemotherapy or an autologous transplant. There was a significant dropout rate in the autologous arm due to early relapse and the long-term follow-up showed no significant difference in OS between the two groups: 34% for autologous HCT and 29% for chemotherapy. This lack of difference applied to both the standard-risk and high-risk groups. A large trial involving collaboration between the Eastern Cooperative Oncology Group (ECOG) and the MRC reported the outcome comparing allogeneic transplant, autologous transplant or chemotherapy in all adult patients with ALL in first remission and showed an inferior result for autologous transplantation versus continued chemotherapy.

Cell sources for HCT

As is true for most patients undergoing allogeneic transplantation, peripheral blood has replaced bone marrow as the hematopoietic cell graft preference. A report from the EBMT retrospectively compared the outcome of 858 patients, 513 who were in first remission given marrow, with 345 patients who were given blood. Peripheral blood, as noted in many studies, resulted in faster engraftment but also in an increased risk for chronic GVHD. Recent reports from the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) trial showed that in patients undergoing an ablative transplant, relapse was equivalent between patients receiving blood or marrow, but chronic GVHD was higher in those patients receiving blood [72]. In this series, there was no obvious difference in survival or relapse, but the increased incidence of chronic GVHD in patients who received peripheral blood as the stem-cell source should be factored into the decision regarding which source of cells to utilize.

Umbilical cord transplantation

There has been considerable interest in exploring the potential of umbilical cord transplantation, but there are less specific data in patients with ALL. In a CIBMTR study of 1525 patients, a comparison was made between hematopoietic cells derived from umbilical cord transplant and an unrelated donor following high-dose conditioning, which resulted in comparable outcomes [73]. Several studies have now reported the results of unrelated cord blood grafts, with outcomes similar to those with unrelated adult donor-derived stem cells, but with different patterns of treatment failure [74,75]. In one study of reduced-intensity transplantation, there were only 16 adult patients with ALL and the diagnosis of ALL predicted a high risk for relapse [56]. At present, the data suggest that this cell source is acceptable for transplant where an unrelated donor cannot be identified and there is an adequate CD34$^+$ cell dose in the cord blood [8,9].

Haploidentical transplantation

Haploidentical-related donor transplantation is being evaluated in patients with a variety of hematologic malignancies and has shown promise, especially with the use of post-transplant cyclophosphamide to reduce the alloreactivity of donor T cells. To date, there are limited data about the outcomes in patients undergoing haploidentical transplantation for treatment of ALL [10,11]. In the context of a clinical trial, this would be an option in a patient lacking a suitable matched related, unrelated, or cord blood donor source of stem cells.

Treatment strategy for ALL

As discussed in this chapter, advances in molecular genetics and clinical treatment outcomes have resulted in more refined recommendations concerning the timing of transplantation for patients with ALL. Most studies indicate that allogeneic transplantation in first remission provides a better chance of DFS, especially in younger patients with high-risk disease where the risk is related to the leukemia cell and its response to treatment (MRD positive). For patients who are younger than 50 years without other high-risk features, intensive pediatric-type chemotherapy programs may offer an option for successful treatment, reserving transplantation for those patients who do not achieve a molecular CR. For patients who relapse, transplantation is the only treatment with the potential for cure, especially if a second remission can be achieved. Therefore, it is important to perform HLA typing at the time of diagnosis to determine what the transplant possibilities might be either from related donors or through the unrelated donor registries, especially for patients with high-risk features such as high WBC count, older age, slow rate of achieving remission, or failure to achieve a remission, or when there is detection of MRD either early or emerging late in treatment. In addition, this information would be useful in planning for treatment in patients who suffer a relapse after achieving a remission. Identification of the Philadelphia chromosome early after diagnosis gives patients an additional beneficial therapeutic option with the inclusion of TKIs as part of the induction, consolidation, and maintenance regimen. Trials are ongoing that will compare the outcome of this modern approach to ALL along with a more modern approach to ALL following transplantation. Figure 52.7 provides a suggested algorithm for the use of transplantation in adult patients with ALL.

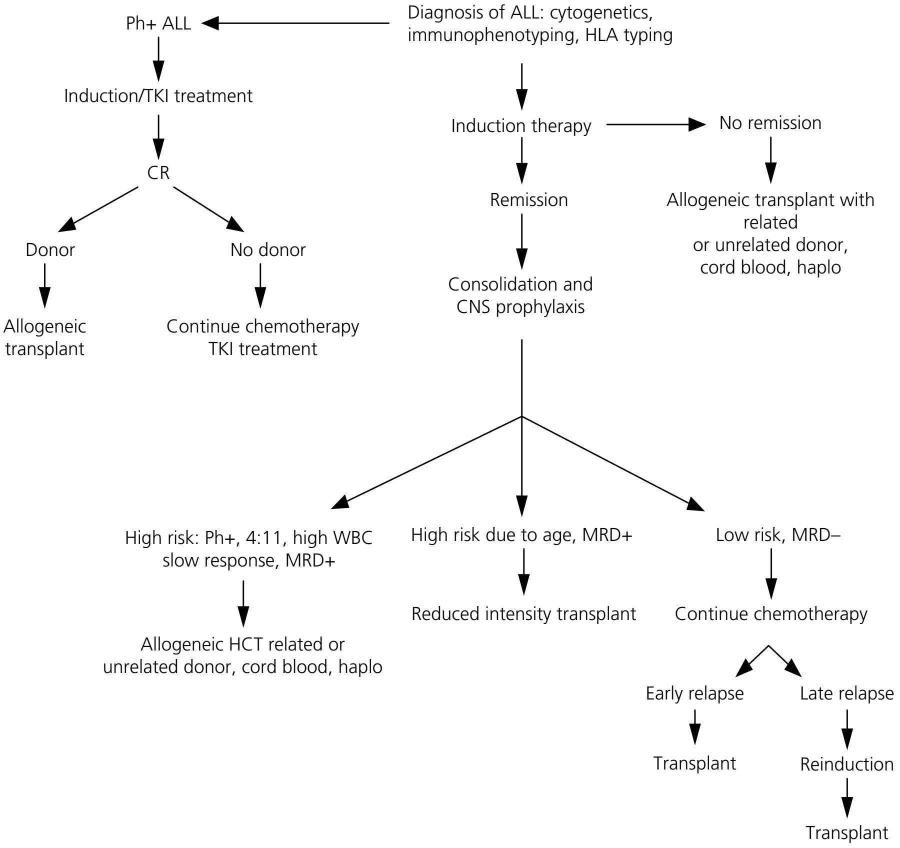
Ph+ ALL
Diagnosis of ALL: cytogenetics, immunophenotyping, HLA typing
Induction/TKI treatment
CR
Donor
No donor
Allogeneic transplant
Continue chemotherapy TKI treatment
Induction therapy
No remission
Remission
Allogeneic transplant with related or unrelated donor, cord blood, haplo
Consolidation and CNS prophylaxis
High risk: Ph+, 4:11, high WBC slow response, MRD+
High risk due to age, MRD+
Low risk, MRD–
Allogeneic HCT related or unrelated donor, cord blood, haplo
Reduced intensity transplant
Continue chemotherapy
Early relapse
Late relapse
Transplant
Reinduction
Transplant

Figure 52.7 Suggested treatment and transplant algorithm for adult patients with ALL.

Future considerations

For a number of years, there were few, if any, innovations in the treatment of ALL in adults, especially in the development of new agents. However, over the last few years, a number of agents, including monoclonal antibodies to CD20, CD52, and CD22 and new drugs for T-cell ALL such as nelarabine and TKI drugs imatinib, dasatinib, and nilotinib for the treatment of Ph^+ ALL may improve the treatment outcome for patients with ALL. In addition, the development of novel immune-based approaches, including CD19-specific CAR T cells to treat relapse, is now being tested as part of upfront therapy in the setting of allogeneic or autologous transplant. Blinatumomab and drug immunoconjugates are being developed that may serve as a more effective bridge to transplant and lower MRD at the time of transplant and are being explored as part of the upfront management.

The most important contribution may come from studies defining which patients are most likely to benefit from transplantation early in the course of their disease. Thus, the monitoring of MRD may have a predictive value for relapse, as a reflection of sensitivity to chemotherapy. This would be an important development for all patients. Those patients, even those with higher risk disease, who achieve a CR with lack of detection of MRD may be spared the risk of transplantation whereas other patients, even those with low-risk disease, who have not achieved an adequate depth of remission would be better served by early transplantation, even if there were no overt high-risk features at the time of diagnosis. Thus, introduction of MRD monitoring over the course of treatment might provide a new strategy for how to advise patients about the timing of transplantation. Some investigators believe that MRD measurement, as a reflection of sensitivity to therapy, may substitute for all other prognostic features once a patient achieves a remission [27].

In addition, transplantation regimens are being developed to address patients with high-risk disease that could include radioimmunotherapy targeted to the CD45 antigen and the use of marrow-based radiation therapy techniques to augment the radiation dose that can be delivered to the marrow. Studies are also being conducted to determine the efficacy of utilizing antigen-specific T cells to augment a graft-versus-tumor effect. These cells can be derived from normal donors and could be transduced to recognize the CD19 antigen present on nearly all patients with pre-B-cell ALL. Finally, given the results of chemotherapy treatment of older patients with ALL, studies of RIC should be pursued for such patients in order to explore the potential therapeutic role of GVL effects in this high-risk patient population.

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CHAPTER 53

Hematopoietic Cell Transplantation for Acute Lymphoblastic Leukemia in Children

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Introduction

Acute lymphoblastic leukemia (ALL) of childhood includes a heterogeneous group of disorders, each with differing molecular genetic abnormalities and clinical behavior. Significant advances in chemotherapy regimens have been made over the last 50 years, such that more than 80% of children with ALL are now cured with chemotherapy [1–7].

The remarkable progress achieved in the treatment of childhood ALL is the result of series of large-scale clinical studies conducted by cooperative clinical trials groups. In addition, biologic investigations have led to improvements in risk group identification and allowed the administration of risk-adapted therapy, improving the results of clinical trials. These advances in chemotherapy treatment have occurred in parallel with important changes in transplantation techniques, donor availability, and supportive care in hematopoietic cell transplantation (HCT). Together, these events have led to changes over time in indications for, and outcome of, HCT for childhood ALL. The indications for HCT in childhood ALL are likely to continue to change in future years as advances are made in chemotherapy, HCT, and understanding of biology.

This chapter reviews the genetic characteristics of childhood ALL, which are central to accurate risk assessment, and discusses the biologic differences between childhood and adult ALL. Outcomes of sibling donor HCT are reviewed, followed by a discussion of the selection of conditioning regimens. For the majority of patients who have no available sibling hematopoietic cell donor, the use of alternative hematopoietic cell sources is an important therapeutic option, and outcomes of mismatched related marrow, unrelated marrow, and umbilical cord blood (UCB) donor transplants are reviewed. Two particular pediatric populations, infants with ALL and children with Down syndrome and ALL, require special consideration, and the appropriate use of HCT in these cases is discussed. Finally, data regarding the effectiveness of management of relapse after HCT with a second transplant are presented.

Diagnosis and classification of ALL in childhood

The last 25 years have seen an explosion in our knowledge of the phenotypic and genotypic characteristics of childhood ALL. The advent of monoclonal antibodies allowed the characterization of cell surface phenotypes of childhood ALL, and now categorization of leukemia according to cell of origin is routine. Immunophenotyping demonstrates that most cases of childhood ALL are B lineage, while the remainder are T lineage (Table 53.1) [8]. B-lineage leukemias generally arise early in B-lymphocyte development before the maturation of surface immunoglobulin, and are termed "B precursor ALL." Cases with cytoplasmic immunoglobulin are termed "pre-B ALL." Cases with similar pre-B surface markers that do not express cytoplasmic immunoglobulin are termed "early pre-B ALL" [9]. Some mixed lineage phenotypes (expressing lymphoid and myeloid markers) have been observed. The association of B-cell and monocyte markers is especially important in childhood leukemia because it is seen frequently in the high-risk leukemias that arise in infants [10].

While immunophenotyping allows the categorization of ALL by cell of origin, the development of molecular genetics has allowed for a finer classification of ALL into prognostically important subgroups on the basis of acquired genetic abnormalities. A large number of specific genetic alterations, commonly but not always associated with specific chromosomal translocations, have been described (summarized in Table 53.1), and are important predictors of outcome of chemotherapy. Some genetic abnormalities appear to be the consequence of mistakes in the normal process of DNA rearrangement used to generate immunologic diversity in lymphoid progenitor cells, as shown in Table 53.1. Examples include leukemias that involve immunoglobulin or T-cell receptor rearrangements. The functional result of such rearrangements is usually a fusion between the immunoglobulin or T-cell receptor gene and a "caretaker gene" or proto-oncogene, such as *MYC*, resulting in deregulation of cellular proliferation.

The most frequent genetic abnormalities in childhood ALL are translocations that result in fusion of two genes that are important in the regulation of gene transcription in hematopoietic cells, such as *TEL*, *AMLI*, *MLL* or *AF4*. The fused genes produce a chimeric or fusion gene protein product that alters normal cellular function, frequently through regulation of signal transduction or transcriptional pathways [11,12].

It is important to note that the major genotypic forms of childhood ALL cannot be distinguished by morphology in most cases, and even by cytogenetics in some cases. An excellent example is the *TEL-AMLI* fusion that is generally cryptic in cytogenetic analysis and is important to identify by molecular analysis because of the excellent prognosis of these leukemias when treated with chemotherapy [13]. In contrast, rearrangements of the *MLL* gene in infants are associated with markedly inferior outcomes with chemotherapy [14]. Rapid identification of molecular abnormalities that can

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Table 53.1 Cellular genotype defines major forms of childhood acute lymphoblastic leukemia

Molecular genetic abnormality	Translocation	Biochemical defect	Associated features	Recommended timing for HCT
B-cell lineage leukemia				
ETV6-RUNX1 fusion	t(12;21) cryptic	Transcription	Good prognosis with chemotherapy	Following relapse
BCR-ABL fusion	t(9;22)(q34;q11)	Signal transduction	Outcomes improving with TKIs + chemotherapy	Newer data now suggest transplant – likely following relapse
E2A-PBX fusion	t(1;19)(q23;p13)	Transcription	Pre-B phenotype, intermediate response to intensive chemotherapy	Following relapse
MLL-AF4 fusion	t(4;11)(q21;q23)	Transcription	Younger infants have poor prognosis	Controversial; early in very young – if using research protocol
MLL-ENL fusion	t(11;19)(q23;p13)	Transcription	Hyperleukocytosis	Following relapse
IGH-MYC fusion	t(8;14)(q24;q32)	Transcription	Extramedullary disease	Following relapse
IGκ-MYC fusion	t(2;8)(p12;q24)	Transcription	Extramedullary disease	Following relapse
IGλ-MYC fusion	t(8;22)(q24;q11)	Transcription	Extramedullary disease	Following relapse
Hyperdiploidy	None	Unknown	Good prognosis with chemotherapy	Following relapse
Hypodiploidy (<45 chromosomes)	None	Unknown	Poor prognosis with chemotherapy	Early in disease
T-cell lineage leukemia				
TAL1 (SCL) deletion	None	Transcription	Extramedullary disease, CD2$^+$, CD10$^-$	Following relapse
TCRδ-TAL1 (SCL) fusion	t(1;14)(p32;q11)	Transcription	Extramedullary disease, CD2$^+$, CD10$^-$	Following relapse
TCRβ-TAL1 (SCL) fusion	t(1;7)(p32;q35)	Transcription	Extramedullary disease, CD2$^+$, CD10$^-$	Following relapse
TCRα-MYC fusion	t(13;14)(q24;q11)	Transcription	Extramedullary disease	Following relapse
TCRδ-RBTN1 fusion	t(11;14)(p15;q11)	Transcription	Extramedullary disease	Following relapse
TCRδ-RBTN2 fusion	t(11;14)(p13;q11)	Transcription	Extramedullary disease	Following relapse
TCRδ-HOX11 fusion	t(10;14)(q24;q11)	Transcription	Extramedullary disease	Following relapse
TCRβ-LCK fusion	t(1;7)(p32;q35)	Signal transduction	Extramedullary disease	Following relapse

importantly affect the prognosis of ALL has allowed risk-adjusted therapy assignment to optimize outcomes. Despite advances in molecular diagnosis, cytogenetic studies, including fluorescence in situ hybridization (FISH), remain important for identifying leukemias with hyperdiploidy, confirming the results of molecular analyses, and guiding the development of molecular assays to detect specific rearrangements.

Biologic differences between pediatric and adult ALL

Chemotherapy outcomes in adults with ALL are notably inferior to results achieved in children. With intensive combination chemotherapy, more than 80% of children with ALL will be cured of their disease, whereas results in adult studies indicate survival rates of 40–50%, despite increasing adoption of similar treatment strategies [1–7,15,16]. Biological differences between adult and pediatric ALL likely influence response to chemotherapy and include age-dependent differences in immunophenotype, cytogenetic, and molecular genetic characteristics [17].

While the incidence of ALL increases in the elderly, there is also a marked peak in incidence in children between the ages of 2 and 5 years. The leukemias occurring within this peak are generally of B-precursor origin, express CD10 surface antigen, and are hyperdiploid, all features associated with an excellent response to therapy [18]. Interestingly, occurrence of this peak appears to be related to improvements in socio-economic status, as it did not occur in the early years of this century and does not currently occur in developing countries [19]. Worldwide, the lowest rates of childhood ALL are reported in black African children (4 per 10^6 annually), with rates approximately 10-fold higher in white Caucasian children [20–23]. These epidemiologic characteristics may indicate that this subset of childhood ALL has a unique etiology. Experimental studies indicate that at least two molecular events are required for leukemogenesis, and the age of the children in this peak suggests that the first of these events take place in utero [24,25]. The nature of the later events that trigger overt leukemia have been the subject of speculation centering around possible infectious etiologies, perhaps related to delayed exposure to common viruses leading to an abnormal immune response [19–21,26,27]. Genetic studies of stored blood samples collected at birth have confirmed the presence of an abnormal clone at that time in at least a proportion of children with ALL, some of whom develop overt leukemia as late as their teenage years [28,29].

The majority of both adult and childhood ALL cases (70–75% of adult and 85% of pediatric cases) are derived from B-lineage cells. Approximately two-thirds of childhood ALL cases express early pre-B-cell markers, in contrast to 50% of adult cases. This phenotype is associated with the best response to chemotherapy, regardless of patient age, perhaps because lymphocytes in this developmental stage are particularly susceptible to apoptosis, and so to killing by glucocorticoids and antimetabolites. Approximately 15% of pediatric and 25% of adult ALL cases express T-lineage markers [30–32]. Although some early studies indicated that T-lineage ALL was associated with poor outcome, treatment of these patients with intensive multidrug regimens has improved prognosis such that the T-cell phenotype in general is no longer an adverse risk factor in adults or children [33–35]. An exception to this is a newly recognized early T-cell precursor ALL (ETP-ALL), a distinct, previously unrecognized, pathobiologic entity that confers a dire prognosis with use of standard intensive chemotherapy. ETP-ALL is characterized by immunologic markers and a gene expression profile reminiscent of double-negative stage 1 thymocytes that retain the ability to differentiate into both T-cell and myeloid, but not B-cell, lineages. Whole genome sequencing shows that the mutational spectrum of this subtype shares characteristics of acute myeloid leukemia (AML) and the transcriptional profile of the blasts is similar to that of normal hematopoietic stem cells and granulocyte macrophage precursors, suggesting that this subtype of leukemia is a stem-cell disease [36,37].

Approximately one-third of childhood early pre-B leukemia cases are hyperdiploid (>50 chromosomes), in contrast to less than 5% of adults. Hyperdiploidy is associated with a particularly favorable response to chemotherapy, perhaps because of the ability to accumulate high levels of methotrexate polyglutamates [38–40]. The most frequent structural rearrangement in childhood ALL, present in 20–30% of cases, is the translocation t(12;21)(p13;q22), which encodes a chimeric fusion product of the *ETV6* and *RUNX1* genes [41–43]. In addition to the formation of the chimeric protein, the non-translocated *ETV6* allele is deleted in many cases. Loss of *ETV6* activity is clearly a secondary event in some cases, occurring in a subclone of leukemia. This observation suggests that loss of ETV6 expression gives an additional proliferative advantage to the malignant cells, possibly because the normal ETV6 gene product can interact with the ETV6-RUNX1 fusion protein in a dominant negative fashion [42]. The *ETV6-RUNX1* translocation seems to define a subgroup of childhood ALL characterized by age 1–10 years, a non-hyperdiploid karyotype, and an excellent prognosis [41]. The *ETV6-RUNX1* translocation occurs at a low frequency (<5%) in adult leukemia, which likely contributes to the poorer overall prognosis of adult ALL [44–47].

The Philadelphia chromosome t(9;22)(q34;q11) results in the production of a chimeric fusion protein derived from fusion of the *BCR* and *ABL* genes. In contrast to *ETV6-RUNX1*, the Philadelphia chromosome is frequent in adult leukemias (25–30%), infrequent in childhood leukemias (3–5%), and used to be associated with a reduced prognosis regardless of age [48,49]. Incorporation of tyrosine kinase inhibitors (TKIs) with chemotherapy has improved survival in the children with Philadelphia-positive ALL, and pending confirmation in follow-up studies, transplant is likely no longer routinely indicated for Philadelphia-positive ALL in first remission [50].

The biologic characteristics described here indicate that the majority of children with leukemia have disease likely to be responsive to current chemotherapy regimens with acceptable toxicity, for example, early pre- B disease with either hyperdiploidy or the cryptic ETV6-RUNX1 fusion transcript. In contrast, adult patients commonly have biologic features associated with drug-resistant disease, and less favorable immunophenotypes. The biologic differences between adult and pediatric ALL lead to important differences in the appropriate indications for transplantation for children with ALL compared with adults.

Outcome of sibling donor transplantation in children with ALL

Transplantation in first remission

Children with ALL with a limited number of very adverse biologic features, such as hypodiploidy, have sufficiently poor outcomes with chemotherapy that they may benefit from HCT in first remission. Results of HCT for childhood ALL in first complete remission (CR1) generally show a reduction in relapse rate with transplantation in CR1, and an increased treatment-related mortality (TRM) compared with chemotherapy. However, it is a continuing challenge for such analyses that study data no longer reflect contemporary outcomes with chemotherapy by the time they are sufficiently mature for publication. It is important to note that many of the criteria used to define high risk in each of these studies, for example. T-cell ALL and mediastinal mass, high white blood cell (WBC) count, and t(4;11), are no longer associated with markedly inferior outcomes with modern chemotherapy and would not currently be considered an indication for HCT in first remission.

Cooperative children's cancer therapy groups worldwide have successfully investigated the role of chemotherapy in the treatment of childhood ALL for over 30 years [1–7,51]. Considerable effort has been directed towards identifying subsets of children with inferior prognoses so that additional or alternative therapies can be targeted to these children, and the use of risk-adapted therapies has been one of the major achievements in the management of childhood ALL. Over time, approaches to risk classification have become more complex and more effective in predicting prognosis [52]. The simplest level of ALL risk classification is the use of National Cancer Institute (NCI)/Rome standard criteria (age and WBC count at diagnosis) to broadly categorize leukemias as high risk (WBC count ≥50,000/μL or age ≥10 years) or standard risk (WBC count <50,000/μL and age 1–9.99 years). These criteria are widely used to assign therapy for children with B-lineage ALL, but are less effective in categorization of children with T-lineage disease. The molecular genetics of the ALL blasts and the response of the disease to initial therapy (rapid or slow early response) are now routinely also used in risk classification, and intensified chemotherapy is given to children in poor prognostic groups [1]. In addition, the International Berlin–Frankfurt–Münster Study Group (I-BFM-SG) has pioneered the evaluation of minimal residual disease (MRD) by polymerase chain reaction in childhood ALL. MRD assessment at days 33 and 78 of treatment by two clonal Ig/TCR markers, with sensitivity of at least 10^{-4}, discriminates outcome within risk groups defined by traditional features. MRD-based stratification was found to be superior to that based on other clinically relevant risk factors, including age, blast count at diagnosis, and immunophenotype.

Typically, only a minority of children with ALL are considered for HCT in first remission. In an effort to identify children with very-high risk ALL who should be considered for HCT in first remission, Schultz et al. [52] proposed a new classification system and treatment algorithm based on a combined analysis of clinical, biologic, and early response data predictive of event-free survival (EFS) from previous ALL studies conducted by the Children's Cancer Group (CCG) and the Pediatric Oncology group (POG). A total of 11,779 children (aged 1–21.99 years) with newly diagnosed B-precursor ALL were consecutively enrolled by the CCG (December 1988 to August 1995, $n = 4986$) and POG (January 1986 to November 1999, $n = 6793$). A total of 6238 patients had informative cytogenetic data and were included in the retrospective analysis. The very high-risk group was defined in this analysis as those with extreme hypodiploidy (fewer than 44 chromosomes), t(9;22), and/or *BCR-ABL* fusion, and induction failure. These data are in agreement with reports from other cooperative study groups in the United States and Europe that reported significantly inferior outcomes in childhood ALL with t(9:22), with hypodiploidy and in infants [3,7].

At the time these data were published, outcomes reported with chemotherapy supported the use of HCT in CR1 in children with ALL falling into these categories, as outcomes with chemotherapy were clearly unsatisfactory. However, it is always important to continue to follow chemotherapy outcomes carefully over time, as newer drugs or other improvements in chemotherapy may improve survival sufficiently to allow the use of transplantation only in CR2, as TRM and late consequences of therapy are greater with transplantation than chemotherapy. This concept is best exemplified by the changing trends in transplantation for Philadelphia-positive ALL. The small number of Philadelphia-positive ALL cases has limited our ability to determine the effectiveness of early HCT in increasing survival. Pooled data from multiple clinical trials groups in general suggested an advantage for transplant in CR1 when a matched sibling donor was available, but not when alternative donor transplants were used. These data all preceded the availability of TKIs, however, which have had a major impact on the treatment of Philadelphia-positive ALL in children.

The TKI imatinib has been shown to have important activity in childhood ALL. Building upon work describing improved outcomes with use of imatinib together with intensive chemotherapy [53–58], a Children's Oncology Group (COG) study incorporated an escalating dose of imatinib with chemotherapy before maintenance therapy, whereas those with a matched sibling donor received a transplant. Continuous imatinib exposure improved survival in the children receiving the highest dose of imatinib, with a 3-year EFS of 80%, more than twice that of historical controls (35%). Importantly, outcomes in those receiving chemotherapy plus imatinib (88% 3-year EFS) were not statistically different from those see in children receiving imatinib and a matched sibling donor transplant (57% 3-year EFS), or an alternative donor transplant (71% EFS) (Figure 53.1). Imatinib appeared to improve survival by improving the outcome of children with a higher burden of MRD, and the highest risk group, the small number with induction failure. The follow-up of this study is not yet complete, but no relapses after completion of therapy have yet been reported. Although it remains possible that the addition of imatinib could have delayed but not prevented relapse, these data encourage the hope that the leukemia has been eradicated without the need for transplant, and that remissions will be sustained. Pending confirmation with longer follow-up, these data suggest that transplant is now not routinely needed for Philadelphia-positive ALL in CR1 [50]. In agreement with these findings, a small number of patients treated in Spain with a high-risk chemotherapy protocol (SHOP-2005) plus imatinib followed by transplant, showed improved outcome compared with historical controls [59].

Results of the European intergroup study of post-induction treatment of Philadelphia-positive ALL (EsPhALL), which is contemporary to the COG study, showed the safety and long-term efficacy of post-induction imatinib plus chemotherapy compared with the standard BFM backbone intensive treatment, using a risk-stratified approach for the treatment of patients on the basis of early response to therapy. The role of allogeneic stem-cell transplantation in first complete remission could not be addressed despite the large number of patients, since most patients in the study received a transplant [60]. Administration of imatinib has been explored in the post-transplant setting. Investigators have shown that imatinib is well tolerated when given prophylactically after transplantation, although current data are too limited to determine whether outcomes are improved [61]. Studies addressing these gaps and investigating the role of newer TKI inhibitors are in progress and will help define better the role of transplant in patients with Philadelphia-positive ALL.

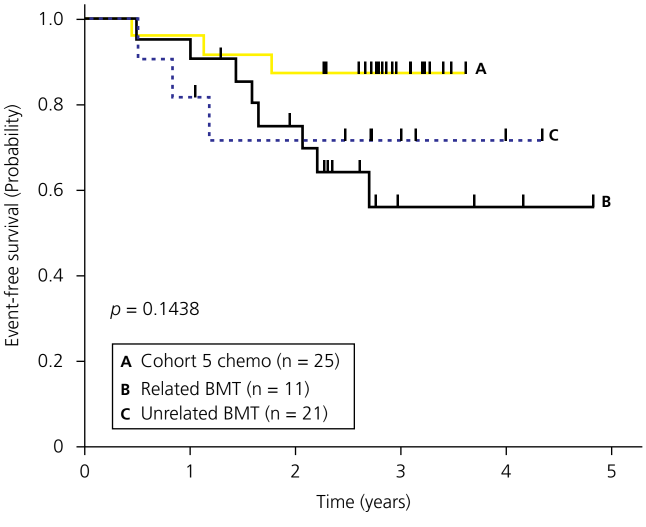


Figure 53.1 Comparison of event-free survival (EFS) for chemotherapy-only versus related donor HCT versus unrelated donor HCT in Philadelphia-positive ALL.

Children with severe hypodiploidy (<45 chromosomes) have poor outcomes with chemotherapy. In a report from the Children's Cancer Group (CCG), 6-year estimates of EFS for patients with 45 chromosomes, 33–44 chromosomes, or 24–28 chromosomes were 65, 40, and 25%, respectively (log rank, $p < 0.002$) [62]. In the largest study of hypodiploid ALL to date, Nachman et al. [63] reported on 139 patients with ALL and hypodiploidy (<45 chromosomes) collected from 10 different national ALL study groups and single institutions. In contrast to previous studies suggesting that near-haploid cases with 24–29 chromosomes have particularly poor outcome, this report found no difference in outcome between patients with 24–29, 33–39, or 40–43 chromosomes, with outcomes poor in all of these groups. However, compared with patients with fewer than 44 chromosomes, patients with 44 chromosomes had a significantly better EFS (52.2% vs. 30.1%, $p = 0.01$) and overall survival (OS) (69% vs. 37.5%, $p = 0.017$). Seventy-eight children with hypodiploid ALL transplanted in first or second remission with a matched sibling or unrelated donor (URD) between 1990 and 2010 have been reported to the Center for International Blood and Marrow Transplant Research (CIBMTR), and transplant outcomes are being analyzed. Based on an earlier analysis, 3-year survival was 65% [95% confidence interval (CI) 45–80%] in 29 patients undergoing matched sibling donor transplant, supporting the use of early HCT in this small subgroup (M. Eapen, personal communication).

Cooperative group studies have contributed the important observation that initial response of childhood ALL to therapy is a strong indicator of long-term outcome, with better outcomes in rapid early responders [64–66]. Additionally, it has been shown that a proportion of slow responders (whether measured by clearance of peripheral blasts after 7 days of prednisone or marrow examination after 7 or 14 days of induction chemotherapy) can be subsequently rescued by intensification of therapy [67,68]. Intensification of chemotherapy improves survival to around 70% in poor responders, so these children do not generally require transplantation in first remission. However, a small subset of slow responders fail to achieve remission after 28 days of chemotherapy, and for those children, if they do achieve remission, only 40% will survive, suggesting that early transplantation once remission is achieved might be of benefit [64].

The detection of MRD using immunophenotyping or molecular detection of residual cells is increasingly being applied to clinical trials of chemotherapy for ALL [69–72]. These studies have the potential to identify the small subset of children with primary induction failure and a poor prognosis based on residual MRD who may be candidates for transplantation in CR1. MRD measurements can also serve as a guide to the effectiveness of chemotherapy prior to transplantation in children in CR1 or beyond, as outcomes of transplantation are clearly improved in those with the lowest disease burden at the time of transplant [73,74]. MRD after transplantation is clearly undesirable and predicts relapse, and can be used as a trigger for interventions such as reduction or withdrawal of immune suppression, or infusion of donor lymphocyte infusion (DLI), although the efficacy of these strategies remains suboptimal.

Gene expression studies

Increased precision in risk assignment at diagnosis might be achieved in the future using array-based analyses. Current data are of great biologic interest, but interpretation is limited by the use of different and evolving platforms for analysis in different studies, and it is unclear whether these strategies augment MRD as a prognostic tool, rather than identifying the same subset of children with reduced prognosis using a different technology, although it may be hoped that in the future these studies will identify novel subsets with a poor prognosis [75–79].

A small number of studies have directly compared outcomes of children with very high-risk ALL transplanted in first remission with matched chemotherapy-treated controls. Some of these studies have shown superior outcomes in transplanted cases [80–82]. In a retrospective analysis of the ALL-BFM-90 and -95 trials, Schrauder et al. reported significantly improved 5-year disease-free survival (DFS) and OS with allogeneic HCT versus chemotherapy in pediatric patients with very high-risk T-cell ALL in CR1 [83]. Similarly, results from a prospective, collaborative group study reported a significant improvement in 5-year DFS, but not OS, for very high-risk pediatric patients with ALL in CR1 who had a donor versus those who did not [intent-to-treat (ITT) analysis], including those who had primary induction failure, and also those who underwent an MRD or URD allogeneic stem-cell transplant (SCT) versus those who received chemotherapy only (as treated analysis) [84]. In contrast, two consecutive MRC protocols identified cases considered to be very high risk for relapse (survival expected to be <40%), and offered sibling donor HCT to those with available donors [85]. The data showed a small benefit of 4.6% improvement in 10-year EFS in transplanted patients. However, when patients with a human leukocyte antigen (HLA)-matched donor were compared with those without, regardless of treatment received (ITT analysis), patients with a donor had an adjusted EFS 10.7% lower than those without a donor. Similarly, results of the Programa para el Tratamiento de Hemopatías Malignas (PETHEMA) ALL-93 trial [86] failed to show any advantage of HCT over chemotherapy in CR1.

These studies illustrate some of the challenges of transferring excellent single-center outcomes to cooperative group studies. Difficulties include heterogeneous populations, non-compliance with assigned therapy leading to selection bias, and transplantation at multiple smaller centers. Transplantation at centers with higher annual numbers of transplants and longer experience has been associated with improved outcomes [87,88]. Continuing investigation of the role of HCT in first remission will require careful attention to the optimization of statistical and clinical trial design, and the evaluation of newer transplantation strategies, including newer hematopoietic cell sources and perhaps reduced-intensity conditioning regimens.

Outcomes of transplantation in second or subsequent remission

Many single- and multiple-institution studies describe outcomes of HCT for patients with relapsed ALL, and often combine data for children and adults. In this chapter, where possible, the data from children are highlighted. Overall, results of sibling donor HCT for children with ALL in second or subsequent remission in single- and multi-institution studies have reported DFS ranging from 23% for patients with advanced disease to 35–64% for cases transplanted in second remission [89–94].

The length of first remission is key in determining the outcome of relapsed ALL in children. Survival rates are typically poor with chemotherapy when relapse has occurred early (commonly defined in US studies as duration of first remission less than 3 years) [95,96]. A proportion of children with later relapses can be long-term survivors after retreatment with chemotherapy [49,97–99]. The appropriate use of HCT or chemotherapy alone for children with relapsed ALL remains a controversial topic, and there have been no successful randomized studies to compare transplantation in second remission with further chemotherapy. A number of cooperative groups, including the COG, have attempted to compare outcomes of transplant and chemotherapy in randomized studies and have been unsuccessful, largely owing to lack of acceptance of randomization by either or both of physicians and parents.

In the absence of randomized data, the best available comparison comes from registry analyses. Eapen et al. compared 188 patients enrolled in POG chemotherapy trials and 186 children who received HLA-matched sibling transplants, treated between 1991 and 1997 (see above) [100]. For children with early first relapse (<36 months from diagnosis), risk of a second relapse was significantly lower after total body irradiation (TBI)-containing transplant regimens [relative risk (RR) 0.49, 95% CI 0.33–0.71%, $p < 0.001$] than after regimens based on chemotherapy alone. Patients receiving radiation-based regimens also had higher leukemia-free survival (LFS) and OS rates. In contrast, transplantation in CR2 did not offer advantage over chemotherapy for children with a late (36 months) relapse ($p = 0.78$). Transplant recipients who received a non-TBI-containing regimen fared poorly; recurrence rates were higher than those in children treated with chemotherapy alone, with correspondingly lower LFS and OS rates (Figure 53.2).

The Italian Bone Marrow Transplant group reported on 57 children who received allogeneic transplantation for ALL in second remission and compared them with 230 patients who received chemotherapy following their relapse [96]. They demonstrated that patients who had an early (<30 months) first relapse had significantly longer DFS following allogeneic transplantation than treatment with

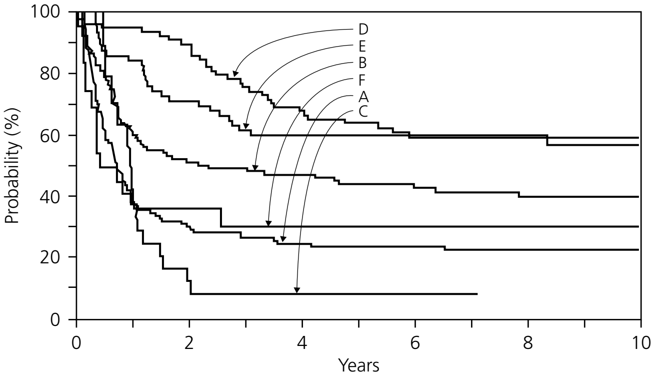


Figure 53.2 Probability of leukemia-free survival in CR2. A, Chemotherapy recipients after an early first relapse; B, transplant recipients with TBI regimens after an early first relapse; C, transplant recipients with non-TBI regimens after an early first relapse; D, chemotherapy recipients after late first relapse; E, transplant recipients with TBI regimens after late first relapse; and F, transplant recipients with non-TBI regimens after late first relapse.

chemotherapy. However, similarly to the IBMTR analysis, this advantage was lost in patients with a later relapse (in this study >30 months following diagnosis).

In a long term follow-up, a multicenter trial in Germany and Austria reported a significant improvement in 15-year EFS in pediatric patients with ALL who experienced a bone marrow (BM) relapse after treatment in the ALL-REZ BFM 87 trial and who subsequently underwent an MRD allogeneic HCT (n = 27) versus chemotherapy (n = 156) in CR2. The probability of EFS after allogeneic HCT was superior (0.59 ± 0.09) to that for the patients who received chemotherapy and radiotherapy as postremission treatment (0.30 ± 0.04, p = 0.026) [101].

Outcomes of 56 children with relapsed ALL were reported in a single-institution study in Australia [90]. All patients with a matched family donor (n = 20] received transplantation, and the other patients received chemotherapy (n = 32) or alternative donor transplants (n = 2). EFS at 8 years was 55% in children with an available family donor compared with 9.2% in patients without a matched family donor (p = 0.002).

In contrast, a US study reported no significant difference in 5-year DFS among pediatric patients with ALL in CR2 in the COG/CCG-1941 trial who underwent an MRD allogeneic HCT or were randomized to receive either an alternative (URD allogeneic or autologous) HCT or chemotherapy, although compliance with study recommendations was limited in this study [102]. Similarly, Harrison et al. found no significant difference in 8-year EFS in a MRC trial [103].

In a large multicenter population-based trial (ALL-REZ BFM 90), Tallen et al. reported that patients in high-risk groups did better with HCT than with chemotherapy (probability of EFS = 0.33 ± 0.05 vs. 0.20 ± 0.05, p < 0.005), suggesting that allogeneic HCT should be recommended in these high-risk patients achieving second complete remission. In this study, high-risk patients were defined as patients with very early BM relapse (<18 months from diagnosis) or any relapse of T-lineage ALL, early isolated BM relapse, or combined BM and extramedullary disease relapses (relapse >18 months from initial diagnosis and <6 months after cessation of frontline therapy) [104].

Taken together, these data indicate that generally HCT from a matched family donor is the best option for a child with an early marrow relapse of ALL or relapse of T-cell disease.

Some data suggest that similar benefit can be achieved with alternative donor HCT, especially a well-matched unrelated donor. Sarrinen-Pihkala et al. reported similar outcomes in children transplanted for ALL in CR2 using URD compared with MSD [105]. Similarly, advances in donor selection and transplantation using cord blood have led to similar outcomes to those obtained with geno-identical donors. This is discussed further under donor selection. Although survival may be equivalent, it is important to appreciate that risk of graft-versus-host disease (GVHD) is increased with the use of alternative donors, and is likely to have an impact on quality of life of survivors [106–108].

The majority of studies indicate that children with later relapse can achieve similar survival with chemotherapy to that with transplantation, so treatment options should be discussed carefully with the family.

Transplants performed beyond CR2 usually involve unrelated donors, as children with matched sibling donors are commonly transplanted in CR2. CIBMTR data on outcomes of 155 URD transplants, performed between 1990 and 2005 for children with ALL in CR3, showed 5-year LFS of 33% for the children with a second relapse 26 months beyond the first, and 26% for those with a second relapse less than 26 months after the first [107]. Although the outcomes are less than optimal, these data are perhaps better than might be expected, and indicate that in a proportion of children with multiply relapsed ALL, later retrieval is possible. Further analysis of predictors of survival in this group will be instructive in guiding future practice.

A careful reassessment of the morphologic, cytogenetic, and molecular genetic characteristics of the leukemia is essential at the time of relapse to confirm recurrence of the original disease and not development of a new secondary leukemia, either ALL or AML, perhaps related to the use of chemotherapy [109,110]. It is also important to rule out a second leukemia in a susceptible person, who has perhaps retained a premalignant population of cells, for example, with an ETV6-RUNX1 translocation, leaving them at risk for a new malignancy with similarities to the initial leukemia [111]. These children with a second primary leukemia, and not a relapse, can do well with further chemotherapy [112].

Reinduction chemotherapy is successful in achieving remission in 70–90% of children with relapsed ALL. The problem of how to manage a patient when attempts at chemotherapy reinduction fail has not been resolved. In some centers, such patients will be considered for allogeneic transplantation even in relapse. There are currently insufficient published data to predict outcomes accurately in these patients, although experience suggests extremely high TRM and a high post-transplant relapse rate because of resistant leukemic cells, limiting enthusiasm for such an approach.

When considering the role of HCT in children with ALL, it is important to recognize that there has been continuous improvement in chemotherapy outcomes, and that HCT can be associated with significant late morbidity [113]. Comparisons of HCT and chemotherapy outcomes must be contemporary and ongoing to allow appropriate therapeutic choices, and the impact of late complications such as GVHD on quality of life must be considered. Table 53.2 summarizes current general approaches to indications for HCT for children with ALL.

Selection of conditioning regimens

The conditioning regimen used to prepare a patient for an HCT for childhood ALL is expected to provide sufficient immunosuppression to facilitate donor cell engraftment, and cytotoxicity to assist in the eradication of residual leukemic cells. In the early years of transplantation, most regimens included TBI given in a single fraction. More recently, TBI has typically been administered in multiple fractions to reduce toxicity. Hyperfractionated TBI combined with CY, now considered a standard regimen, was first introduced for the treatment of children with leukemia by investigators at MSKCC [114]. After the original reports of the use of CY and TBI, several investigators added other chemotherapeutic agents to the regimen, or substituted other drugs for CY, in an attempt to improve outcomes. Although studies with some of these other combinations showed initial promise, additional toxicity often occurred and the long-term outcome was often not appreciably changed.

A large CIBMTR study compared 298 patients (adults and children) with ALL in CR1 or CR2 receiving HLA-matched sibling allografts after CY/TBI with 204 patients receiving etoposide/TBI [115]. Four groups were compared: CY/TBI <13 Gy (n = 217), CY/TBI of ≥13 Gy (n = 81), etoposide/TBI <13 Gy (n = 53), and etoposide/TBI of ≥13 Gy (n = 151). TRM did not differ by conditioning regimen, and in CR1 there were no significant differences in relapse,

Table 53.2 Indications for transplantation for childhood acute lymphoblastic leukemia

Stage of leukemia at time of BMT	Donor type	
	Matched sibling or one-antigen-mismatched family member	**Unrelated marrow or cord blood**
CR1, Philadelphia chromosome positive	Likely not indicated; improved results with TKIs + chemotherapy [50,59]	Likely not indicated; improved results with TKIs + chemotherapy [50,59]
CR1, t(4;11) or other MLL rearrangement in infant <1 year old at diagnosis	Transplant indicated in some but not all patients. Reported data suggest mixed results with HCT [167–169] – involve small numbers and careful prospective evaluation needed. Note: some infants have biologic characteristics of higher risk disease (infants with MLL translocations, aged <6 months at diagnosis and either a poor response to steroids at day 8 or a leukocyte count ≥300 g/L) may have improved outcome with HCT	Poor results with chemotherapy, mixed results for outcome with URD HCT [167–169]. Should be considered for patients with high-risk features. Data should be contributed to registries/group studies to allow evaluation of outcomes
CR1, >28 days to achieve CR	Transplant may be offered; limited data available comparing outcome with outcome of chemotherapy [52,84]	Transplant may be offered, particularly in cases with other high-risk features; data not available comparing outcome with outcome of chemotherapy
CR2, relapse with CR1 <36 months	Indicated. Outcome improved in transplant recipients in case–control comparison [96,100,101,104]	Indicated. Good outcomes reported in significant series of cases [105]; however, careful case-control comparison still needed to confirm improved outcome. Single and multicenter studies suggest outcomes can be equivalent to related donor BMT using URD matched at HLA -A, -B, -C, and -DRB1 [146,147]
CR2, relapse with CR1>36 months	Transplant typically not offered; chemotherapy offers an equivalent outcome [54,115–118]	Generally not offered to those with very late relapses, those with relapse just beyond 36 months may be considered. Single- and multicenter studies suggest outcomes can be equivalent to related donor BMT using URD matched at HLA-A, -B, -C, and -DRB1 [146,147]
CR3 and higher	Transplant indicated, cure with chemotherapy unlikely	Transplant indicated, cure with chemotherapy unlikely [107]
Relapse	Results poor; transplant not generally offered unless in context of investigation of new HCT strategies	Results poor; transplant not generally offered unless in context of investigation of new HCT strategies
Isolated extramedullary relapse	Registry data suggest no benefit from transplant [191]. Transplant not generally offered unless associated with marrow relapse	Transplant not generally offered unless associated with marrow relapse

LFS, or survival by conditioning regimen. In CR2, important differences in outcome among conditioning groups were identified. The risk of relapse, treatment failure, and mortality were lower in recipients of etoposide (regardless of TBI dose) or with TBI doses of ≥13 Gy in patients receiving CY. A striking reduction in relapse risk was observed in patients receiving ≥13 Gy TBI with CY compared with CY/TBI using <13 Gy of radiation ($p = 0.0016$). These data are further evidence of the contribution of the conditioning regimen to disease control in transplantation for ALL.

Concerns about the late effects of TBI in children have led to a number of studies of conditioning regimens using only chemotherapy. Early studies of busulfan (BU) and CY as a conditioning regimen focused on the treatment of myeloid malignancies, and data indicated that the regimen was adequate to achieve donor cell engraftment [116]. The use of a radiation-free conditioning regimen is an attractive option for young children if equivalent survival can be demonstrated as fewer late adverse effects on growth, endocrine, and cognitive function might be expected.

An IBMTR analysis of children with ALL receiving HLA-identical sibling donor HCT compared outcomes with conditioning regimes of CY and TBI ($n = 451$) or BU and CY ($n = 176$) [117]. The 3-year probability of LFS was significantly higher in the group receiving CY and TBI than in the group receiving BU and CY. The risk of relapse was similar in the two groups, but TRM was higher in children receiving BU and CY. Bunin et al. [118] reported similar inferior outcomes of a BU and CY conditioning regimen compared with TBI and CY in a small but prospective trial conducted by the Pediatric Blood and Marrow Transplant Consortium.

Together, these reports indicate that, in the past, modifications of conditioning regimens for children with ALL had limited success in improving outcomes. Inclusion of additional chemotherapeutic agents led, in some instances, to increased morbidity and mortality without significant improvement in long-term DFS. In addition, current data suggest reduced survival with radiation-free regimens.

A recent analysis of myeloablative TBI-containing conditioning regimens for pediatric ALL reported to the CIBMTR compared four commonly used regimens: (1) TBI 1000 or 1200 cGy + CY, (2) TBI 1000 or 1200 cGy + CY + etoposide, (3) TBI 1320–1400 cGy + CY, and (4) TBI 1320–1400 cGy + CY + etoposide [119]. This study included 765 patients with ALL aged <18 years at the time of HCT who received a graft from an HLA-matched sibling or an unrelated donor. Unrelated donor grafts included BM and UCB. All HCTs were performed between 1998 and 2007. All patients received myeloablative conditioning with a TBI-containing regimen (TBI >1000 cGy). The 5-year probability of relapse was similar in all four groups. However, TRM was higher (35% vs. 25%, $p < 0.02$) and overall survival lower (36% vs. 48%, $p < 0.03$) in group 4 (CY, higher dose TBI and etoposide) compared with group 3 (CY and higher dose TBI). Based on these findings, it was suggested that the addition of etoposide to a TBI ≥1320 cGy + CY conditioning regimen increases mortality risk and should be avoided in children and adolescents with ALL. In this study, the effect of conditioning regimen on transplantation-related and overall mortality was independent of patient age, donor source, and performance score.

These findings differ in some respects with the previous report from Marks et al. [115], which showed that TBI-containing regimens with etoposide alone were associated with superior leukemia control after transplantation. Patient age varied among studies; however, with the latest series limited to children and adolescents, the reported differences in risk may be related to differences in the biology of pediatric and adult ALL and/or differences in intensity of up-front chemotherapy regimens used to induce a second remission.

The inclusion of TBI in the preparative regimen is clearly associated with significant late effects, which are accentuated in pediatric populations, increasing reluctance to transplant very young children in particular, because of concerns regarding long-term growth and cognitive function [120–123].

Perkins et al. evaluated 13 children who were transplanted under the age of 3 years (range 0.58–2.93 years), with follow-up between 3 and 22 years, 11 of whom had received TBI [124]. All had IQs in the normal range. Phipps et al. performed a prospective study of neurocognitive outcomes of transplant [125]. A battery of neurocognitive measures were administered before admission and at 1, 3, and 5 years post-SCT. A total of 158 patients who survived were evaluated at 1 year after HCT. Subgroups of patients at relatively higher risk of poor outcomes were those undergoing URD transplantation, receiving TBI, and who experienced GVHD. Differences were seen in verbal IQ ($p < 0.01$), performance IQ ($p < 0.01$), reading ($p < 0.05$), and spelling ($p < 0.05$) and a marginal difference in math ($p = 0.06$) in recipients of TBI. The effects of TBI, although statistically significant, were small, reflecting a between-group difference of approximately three IQ points over a 5-year period, suggesting that these changes may not have been of great functional consequence [125]. These data suggest that TBI can be considered for young children with ALL, although careful follow-up and management of remediable late effects are essential.

The use of reduced-intensity transplant in ALL has lagged behind experience in AML, with enthusiasm limited by the mixed evidence for any benefit of allogenicity in disease control in ALL, and by concerns about disease progression in the period of establishment of donor hematopoiesis. Reduced-intensity transplantation is now being explored in adults with ALL, and is clearly feasible, although more detailed analyses of patients likely to benefit are ongoing [126–128]. Pediatric experience of reduced-intensity transplantation for ALL remains limited, as most children are able to tolerate myeloablative therapy. Verneris et al. described 38 reduced-intensity pediatric transplants for ALL reported to CIBMTR, with 30% EFS [129]. However, in registry studies it is difficult to ascertain the reason why reduced-intensity conditioning was used and what comorbidities might have been present, so further studies are needed to determine if this is a viable option for disease control.

Alternative donor HCT for children with ALL

Mismatched family member transplantations in children with ALL

Many or most of the problems of hematopoietic cell donor availability could be addressed using one-, two- or three-antigen-mismatched family member donors, who are available for almost every patient [130]. Early studies of highly mismatched related donor transplantations reported high rates of graft failure and GVHD, limiting enthusiasm for this approach [131,132]. Although newer transplantation approaches have shown improvement in outcomes, experience with transplantation using mismatched family member donors remains limited, and to a large extent focused in a small number of centers with particular interest and expertise in this strategy.

There are few data regarding the use of these transplants for uniform patient populations, although there are several series summarizing overall experience at single institutions. In reports of heterogeneous patient groups, a number of authors have shown that results using a one-antigen-mismatched family member as a donor can be comparable to those achieved using a matched sibling donor, while risk of treatment failure increases incrementally with increasing HLA disparity [133–135].

Investigators in Perugia, Italy, have pioneered the use of a high degree of T-cell depletion together with infusion of a high dose of haploidentical $CD34^+$ cells to overcome the problems of GVHD and graft failure associated with highly mismatched haploidentical grafts [136]. They demonstrated that donor-versus-recipient natural killer cell alloreactivity can eliminate leukemia relapse and graft rejection, and protect patients against GVHD in cases with AML. Unfortunately, activity against leukemia cells appeared to be specific for AML and not ALL blasts, and outcomes were not satisfactory in cases of ALL in this series.

A pediatric study using a similar approach described outcomes in 27 children with ALL (seven in CR1, ten in CR2, four in CR3, and six with refractory disease) [137]. Engraftment was rapid in 26 patients. Two patients who rejected the first graft and one patient with initial non-engraftment were successfully retransplanted. Survival for the group overall was 33%, and 44% for children in remission at the time of transplantation; none of the children transplanted without achieving remission survived. Relapse and infection were important causes of treatment failure.

Investigators in Bristol, United Kingdom, reported their experience of using haploidentical family members as donors for 34 children with leukemia, of whom 14 had ALL [151]. The conditioning regimen included CY and TBI (14.4 Gy in eight fractions). All patients received T-cell-depleted peripheral blood stem-cell grafts with a median CD34 cell dose of 13.8 (range 4.2–35.1) $\times 10^6$/kg, and 0.7×10^4 $CD3^+$ cells/kg. The actuarial survival at 2 years was 26% (95% CI 13–41%). Eight patients survived disease free with a median follow-up of 62 months.

A recent retrospective, multicenter, registry-based study analyzed the outcomes of children receiving a T-cell-depleted allograft from HLA-mismatched related donors, transplanted in European transplant centers, reported to the Pediatric Diseases and the Acute Leukemia Working Parties of the European Group for Blood and Marrow Transplantation (EBMT) registry [138]. A total of 127 children with ALL were transplanted from mismatched relatives from 1995 to 2004 in 36 EBMT centers. Twenty-two received a transplant in CR1, 48 in CR2, and 32 in CR3, and 25 were not in remission with 5-year DFS of 30% (±10%), 34% (±7%), 22% (±7%), and 0%, respectively. Acute GVHD was absent or grade I in 80 children, grade II in 13, grade III in six, and grade IV in three cases, and chronic GVHD was observed in 14 of the 84 patients at risk. The cumulative incidence of hematologic relapse at 5 years was 36% (±4%). The cumulative incidence of NRM at 5 years was 37% (±4%). In a multivariate analysis, only previous autograft was associated with increased NRM [hazard ratio (HR) 3.01, 95% CI 1.16–7.84, $p = 0.024$]. The estimated probability of OS at 5 years was 29% (±5%) for the whole cohort, 28% (±10%) for patients transplanted in CR1, 39% (±7%) for those in CR2, and 32% (±8%) for those in CR3.

Outcomes for children with ALL treated with haploidentical transplantation are perhaps disappointing when compared with well-matched unrelated donor marrow or cord blood grafts, but may represent the only treatment option for children with infrequent tissue types. New directions in the use of adoptive cellular immunity [139] and improved preemptive monitoring and treatment of viral infections appear to be promising in this respect.

Unrelated donor HCT

Donor selection

Historically, outcomes of URD transplantation have been inferior to those of sibling donor transplantation, and recommendations for transplant for ALL have differentiated between those with and without a matched sibling donor. The use of phenotypically matched URDs has increased in parallel with the remarkable growth of registries of HLA-typed potential BM donors, such as the National Marrow Donor Program (NMDP) in the United States, which facilitate the process of donor identification and marrow acquisition [140,141]. Additionally, refinements in HLA-typing technologies have allowed for more accurate matching at class I and class II loci [142,143]. Despite these improvements, donor identification can still be a logistic challenge, particularly for patients in whom remissions are likely to be short, such as those with ALL beyond first remission.

An improved search algorithm based on the conversion of HLA assignments of patients and donors into search determinants, and on allele and haplotype frequencies (Hap-Logic), is now routinely used by the NMDP registry, and greatly facilitates the identification of eight-allele-matched donors [144]. In a recent CIBMTR study of children with AML, ALL, chronic myelogenous leukemia (CML), and MDS undergoing allogeneic T-replete, myeloablative HCT between 1993 and 2006 included patients receiving BM from 1208 MSDs, 266 8/8 allelic-matched URDs and 151 0–1 HLA-mismatched related donors. Multivariate analysis showed that recipients of MSD transplants had less TRM, acute GVHD, and chronic GVHD, along with better DFS and OS, than the URD and mismatched related donor groups. Survival rate was lower with mismatched related donors compared with MSD (RR 1.55, 95% CI 1.21–1.98, $p < 0.001$). No statistical difference was found between URD and mismatched related donor transplantations in OS (RR 0.94, 95% CI 0.71–1.26, $p = 0.686$) [145].

Outcomes

Initial reports of the results of URD transplantations necessarily included small, heterogeneous groups of patients. As donor availability has improved, larger single-center experiences and registry studies are now being reported. Outcomes from different centers show some variability, which may reflect heterogeneous patient populations or differing treatment strategies and provision of supportive care. Taken together, DFS for children transplanted in first and second remission is generally in the range 40–50%, and outcomes are not markedly different from those reported for related donor HCT.

A CIBMTR report compared long-term survival after unrelated and sibling donor HCT for acute leukemia (ALL and AML) in children younger than 18 months [146]. Although leukemia recurrence was lowest after URD HCT in first clinical remission, the OS and LFS rates were similar after matched sibling and URD HCT, after adjustment for disease status. Relapse, OS, and LFS did not differ by graft type (BM versus UCB) or type of leukemia. This was confirmed in the recent update of experience in Bristol, United Kingdom; 2-year OS was identical for matched sibling and well-matched URD transplants (Figure 53.3) [147].

Taken together, these data indicate that in children, good outcomes can be achieved with URD HCT. Improvements in HLA typing have facilitated optimum donor selection, and currently a URD molecularly matched at HLA-A, -B, -C, and -DRB1 is considered ideal and likely equivalent to a genotypically matched sibling donor.

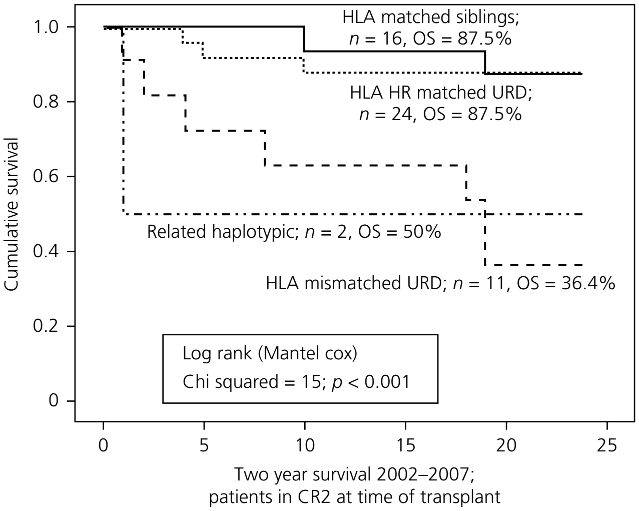


Figure 53.3 Two-year overall survival of patients in CR2 at time of transplant (2002–2007). HLA-matched sibling and URD transplants had identical OS at 87.5%.

As larger series of URD HCTs are being reported, it is now possible to evaluate prognostic factors influencing the outcome of URD HCT for children with ALL, although there is some variation between studies, perhaps due to variability in sample size and the population studied [148].

The impact of acute GVHD on relapse risk in ALL remains a controversial topic, as evidence for an important graft-versus-leukemia (GVL) effect has been at best mixed over the years, with generally poor responses to donor lymphocyte infusions [149]. Recent analysis of a large pediatric URD cohort showed decreased risk of relapse in children who developed acute GVHD, supporting the belief that there may be a benefit of GVHD in disease control [150].

The possible role of killer immunoglobulin-like receptor (KIR) mismatch in control of ALL has also been controversial. Early studies showed some benefit of KIR mismatch as imputed from HLA typing in AML, but not in ALL [136]. Additional studies using more direct measurement of KIR genotype and phenotype (a receptor–ligand model) support an important biologic effect in vitro and in vivo [151,152].

In contrast, other clinical studies have shown no impact of KIR mismatch, perhaps due to variability in the methodology used to determine KIR incompatibility, and in transplant technique, particularly the degree of T-cell depletion. Currently, there is insufficient evidence to recommend the selection of KIR-mismatched donors in children with ALL.

The increased mismatch associated with URDs might be hoped to bring with it more GVL effect and hence less relapse than sibling donors. Unfortunately, a CIBMTR analysis did not support this hypothesis for adults with ALL, AML, or CML [153].

The effectiveness of the GVL effect after cord blood transplant has been a concern in light of the generally reduced incidence of GVHD. However clinical studies have not demonstrated increased relapse associated with use of cord blood compared with sibling or URD transplants [154,155]. These data indicate that URD transplantation offers a cure for some children with ALL who are unlikely to be cured with chemotherapy. Challenges that remain, all of which are shared with sibling donor transplantation to a greater or lesser extent, include a high rate of GVHD (particularly with unmanipulated grafts), early and late infections (particularly in T-cell-depleted grafts), and the continuing problem of relapse. More intensive initial chemotherapy regimens may yield a population of children with highly drug-resistant relapsed disease, requiring novel approaches for cure.

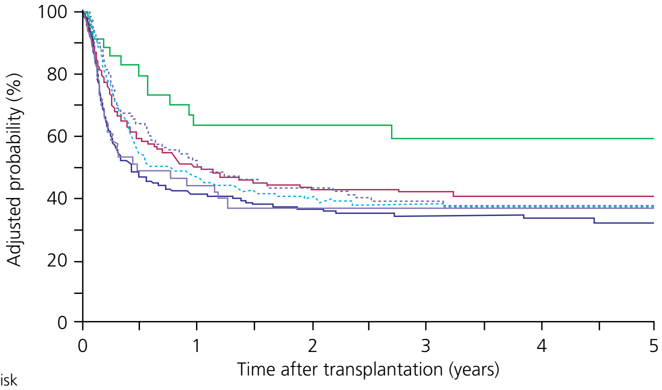
UCB transplantation for children with ALL

The establishment of banks of cryopreserved UCB for use in transplantation has been an important development in the treatment of children with ALL [156,157]. Potential advantages of UCB include immediate availability of stored units for transplantation once identified (particularly valuable for patients in whom remissions are likely to be short) and reduced GVHD, a major reason for treatment failure in recipients of URD marrow. In the last two decades, with the improvement in our understanding of factors implicated in expanding the availability of not just URD, but especially unrelated cord blood unit, has significantly shortened the search time to identify a compatible unrelated marrow donor or cord blood [158]. This is particularly advantageous in children with ALL, in whom achievement of remission can be difficult, and the ability to move to transplantation as soon as remission is achieved can be valuable. Initial reports of outcomes of UCB transplant procedures have been limited to relatively small series, although larger single-center experiences and registry series are now being reported.

As experience with UCB transplantations has increased, it has become possible to compare outcomes of UCB transplants with those of other graft sources to assist physicians in donor selection. A registry comparison of URD marrow (T-cell-depleted and T-cell-replete cases analyzed separately) and UCB transplantations in children with leukemia indicated equivalent survival among the three categories. Acute GVHD was reduced in UCB recipients compared with recipients of T-cell-replete marrow, and more relapses occurred in the T-cell-depleted marrow group than in the cord blood grafts [159,160]. In an important study that has influenced clinical practice, Eapen et al. [161] compared 503 children with acute leukemia transplanted with UCB with 282 BM recipients between 1995 and 2003. A total of 495 patients with ALL were included, 186 in the BM group and 309 in the UCB group. Clinical data were obtained from the CIBMTR and the National Cord Blood Program of the New York Blood Center. In comparison with allele-matched BM transplantations, 5-year LFS was similar after transplantation of UCB mismatched for either one or two antigens, and possibly higher (numbers were very small) after transplants of HLA-matched UCB (Figure 53.4). TRM rates were higher after transplantation of two-antigen HLA-mismatched UCB (RR 2.31, $p = 0.0003$) and possibly after one-antigen HLA-mismatched low cell dose ($<0.3 \times 10^5$/kg) UCB transplants (RR 1.88, $p = 0.0455$). Relapse rates were lower after two-antigen HLA-mismatched UCB transplants (54%, $p = 0.0045$). These data support the use of one- or two-antigen HLA-mismatched UCB with units of adequate size in children with acute leukemia who need transplantation.

A report from the IBMTR and National Cord Blood Program [161], including a large number of patients, showed similar GVHD rates and a lower relapse rate in patients receiving two-antigen HLA-mismatched UCB transplantation compared with matched BM transplantations, possibly indicating a greater, or at least equivalent, GVL effect. Adding another layer of complexity, a preliminary study comparing single unit cord blood transplant with double cord blood transplant suggested reduced relapse with the use of double cord blood transplants[162]. The mechanism of such an effect is unclear, although the incidence of acute GVHD was higher in recipients of double cord blood transplants, and these two observations may be related. This initial study included mostly adults in the double unit group and mostly children in the single unit group, as assignment was based on available cell dose, complicating interpretation of the data.

To explore this question further, a recent prospective pediatric study compared overall survival for recipients of a single UCB unit treated for hematologic malignancy on the original Cord Blood Transplantation (COBLT) Study ($n = 191$) conducted from 1999 to 2004 with the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0501 study ($n = 113$) conducted from 2006 to 2012. All patients received TBI 1350 cGy and cyclophosphamide 120 mg/kg. Patients enrolled in COBLT also received equine ATG 90 mg/kg and those on BMT CTN 0501 fludarabine 75 mg/m^2 with GVHD prophylaxis consisting of cyclosporine/methylprednisone and cyclosporine/mycophenolate mofetil, respectively. The 1-year



Numbers at risk		0	1	2	3	4	5
HLA-matched cord blood	—	35	20	17	13	11	8
HLA-matched bone marrow	·····	116	62	45	35	29	24
One-mismatched cord blood (high cell dose)	—	157	72	55	44	32	25
HLA-mismatched bone marrow	·····	166	77	60	53	44	30
One-mismatched cord blood (low cell dose)	—	44	19	13	12	10	6
Two-mismatched cord blood	—	267	100	67	49	34	21

Figure 53.4 Probability of leukemia-free survival after BM and UCB transplantation adjusted for disease status at transplantation. (For a color version of this figure, please see the color plate section.)

probabilities of overall survival were 57% (95% CI 50–64%) in COBLT and 71% (95% CI 62–79%) in BMT CTN 0501 ($p = 0.01$). Although it is not possible to prove which factor(s) played the greatest role, it is the "package" including conditioning and GVHD prophylactic regimens, supportive care, method of unit selection, and transplantation period combined that contributed to the improved survival observed in recipients of a single UCB unit [163].

Autologous transplantation for children with ALL

Autologous transplantation has generally proved disappointing in childhood ALL and is currently infrequently performed. Early, relatively small studies, generally from single centers, described fairly encouraging results [164,165]. However, these studies commonly included children with both early and late relapses, and those with isolated extramedullary relapses, some of whom would be expected to have a good outcome without transplantation of any kind, making interpretation of the data difficult.

Purging of the autograft of contaminating leukemia cells is an attractive concept, and has been attempted by a number of investigators. Overall, the outcomes of these transplantations were disappointing, and there were no clear data that outcomes were superior to those which could be achieved with chemotherapy alone or with unpurged grafts.

Increasing availability of URD marrow and cord blood has significantly reduced the number of children with no available allogeneic stem-cell source. In addition, continuing improvements in outcomes of allogeneic transplantations in children, particularly using URD stem-cell sources, with TRM of <15% now achievable, leaves little place for autologous transplantation in children with ALL.

Special pediatric considerations

Transplantation of infants with ALL

Treatment of infant ALL presents particular challenges because of variability in outcomes between the various genetic subtypes. Some children with infant ALL have a reasonable prognosis with chemotherapy (those with biologic characteristics similar to those of older children), whereas others (with very young age, high WBC count at diagnosis, and the *MLL* rearrangement) have possibly the most disappointing outcomes in childhood leukemia [14]. In addition, late adverse effects of treatment are a particular concern for this vulnerable group of small children. As a result, transplantation decisions are especially critical in infants.

Molecular rearrangements that involve the *MLL* gene occur in a high proportion of leukemias arising in infants less than 1 year of age (Table 53.1). Fusion between the *MLL* gene and the *AF4* gene as a consequence of a t(4;11)(q21;q23) translocation is the most frequent rearrangement. *MLL* gene-rearranged infant leukemias frequently have marked leukocytosis and a mixed-lineage phenotype (expressing both early B-cell and monocyte markers) [166].

Because of the poor response to chemotherapy, infants with *MLL*-rearranged ALL are often considered as candidates for early transplantation. Unfortunately, there are no prospective series of HCTs for infants, and outcomes are difficult to compare with those achieved with chemotherapy because of lack of uniform criteria for transplantation eligibility, varying donor sources, and the relatively small numbers of patients transplanted.

In the CIBMTR study discussed above, Eapen et al. [146] showed comparable long-term survival after sibling, URD, and UCB transplantation for acute leukemia (49% ALL and 51% AML) in children younger than 18 months. These data suggest that if transplantation is indicated, related and unrelated donors can be considered to be equivalent, but they do not address the issue of the relative roles of HCT and chemotherapy in CR1.

Establishing without doubt that HCT can improve outcome compared with chemotherapy in high-risk infants in CR1 requires a randomized study, and such a trial remains challenging owing to the rarity of this disease and to the logistic challenges of performing such studies, as discussed earlier. Recently, Dreyer et al. reported on data from infant ALL trials of the Children's Cancer Group (CCG 1953) and Pediatric Oncology Group (POG 9407) that used a similar chemotherapy backbone and advised allogeneic HSCT to be performed in *MLL*$^{+}$ cases. The 5-year EFS rates were 50.9% in the transplantation group and 48.7% in the chemotherapy-only group, suggesting no clinical benefit for HSCT in CR1 for infants with *MLL*$^{+}$ ALL [167].

The larger international Interfant-99 study enrolled 482 infants with ALL from 22 countries between 1999 and 2005. Eligible patients were risk stratified according to their peripheral blood response to a 7-day prednisone prophase, and then given a hybrid regimen based on the standard protocol for ALL, with some elements designed for treatment of AML (containing intensified cytarabine). In an ITT analysis, EFS and OS at 4 years were 46.0% and 55%, respectively. Factors prognostic of poor outcome included *MLL* gene rearrangements, very high WBC count ($>300 \times 10^{9}$/L), age younger than 6 months, and a poor response to the prednisone prophase. The 4-year DFS did not differ significantly in high-risk patients treated with chemotherapy alone (37.4%) versus chemotherapy plus HCT (50.2%) when adjusted for time to transplantation ($p = 0.19$). However, in a subset analysis, HCT in CR1 was associated with a significantly better DFS in infants with *MLL* translocations, aged <6 months at diagnosis and either a poor response to steroids at day 8 or a leukocyte count ≥300 g/L. In this subset of infants, HCT in CR1 was associated with a 64% reduction in the risk of failure resulting from relapse or death compared with chemotherapy alone, suggesting that this subgroup of high-risk infants may benefit from HCT [168,169].

While data from these multicenter reports are perhaps the best available, interpretation is limited by the small number of patients transplanted, multiple donor types, absence of standardized eligibility requirements, and absence of standardized treatments. There is a clear need for further prospective studies to define the role of HCT in infants with genetically defined leukemias that are at high risk for treatment failure. Such studies will need to be multi-institutional and possibly multinational (as was Interfant-99), as infant leukemia is rare. Studies should include evaluation of the late consequences of therapy in survivors, as morbidity can be significant in young children.

Transplantation of children with Down syndrome and ALL

Children with Down syndrome have an approximately 20-fold increased risk of leukemia, and historically reported to have increased treatment related toxicities, potentially related to increased cellular sensitivity to chemotherapeutic agents. However, some studies have shown chemotherapy outcomes as good as those seen in children with ALL without Down syndrome [170]. A review of the CCG experience, the largest analysis to date of this population, demonstrated decreased EFS and OS in children with Down syndrome and ALL enrolled in CCG trials when compared with children with ALL without Down syndrome [171]. Subset analysis revealed that poorer outcomes were restricted to the NCI standard-risk children (OS 70% vs. 85%, $p = 0.001$; EFS 56% vs. 74%, $p = 0.001$). The NCI high-risk subset of ALL and Down syndrome patients receiving more intensive therapy fared as well as their non-Down syndrome counterparts (OS 63% vs. 66%, $p = 0.7$; EFS 62% vs. 59%, $p = 0.9$), suggesting that, with modern supportive care, children with Down syndrome can tolerate intensive therapy.

Rubin et al. [172] reported 27 patients with Down syndrome (18 collected from a survey of BM transplantation centers and nine from the literature). Sixteen received a graft from an HLA-matched sibling, two from non-sibling family members, and four from URDs, and five received autologous grafts. This study included 14 children with ALL, nine in second remission and five in third remission. Five of the 14 ALL patients were surviving 9–60 months post-transplantation. The authors identified a high rate of regimen-related toxicity in these patients (39% cumulative risk of death in remission at 3 years). A high frequency of lung problems and airway problems, including upper airway obstruction due to severe mucositis, was common, perhaps associated with the use of methotrexate. Despite the significant toxicity, relapse-free survival for the whole group was 44% at 3 years, comparable to results seen in children with ALL without Down syndrome.

A retrospective case series of 11 children with Down syndrome who underwent HCT showed improvement in TRM, with a day 100 mortality of 18% [173]. Relapse was the most frequent cause of post-transplant mortality, with a 45% incidence of death due to recurrent disease. The decrease in TRM was possibly attributable to improved supportive care, advances in HLA typing, and relatively increased experience with HCT in children with Down syndrome.

Role of second transplant procedures for relapse

The most important cause of treatment failure following transplantation for childhood ALL is recurrent leukemia. Additional chemotherapy can induce further remission in a proportion of patients, although very few will survive long term. Experience with the use of second transplants following relapse after BM transplantation is limited. The CIBMTR reported on 114 recipients of HLA-identical sibling grafts who received a second allogeneic HCT for treatment of relapse between 1978 and 1989 [174]. Twenty-nine of these patients had ALL. LFS for all the ALL cases was 21% (95% CI 14–30%); however, outcomes were extremely poor in patients who had relapsed less than 6 months after their first transplant, in whom LFS was only 7% (95% CI 2–19%), largely as a consequence of high TRM. Patients with long remissions after their first transplant, those with good performance status, and those achieving remission after their initial post-transplant relapse had the best outcomes. Series of second transplantation procedures, each including a small number of ALL cases, also reported high early TRM and poor long-term survival [175–177]. A common theme of these reports is the importance of length of remission after transplant, with poor survival after a second HCT in those experiencing early relapse [178]. Although these procedures remain challenging, in the current era, both the careful assessment and eradication of MRD prior to second HCT can lead to better patient selection, and improved outcome in those with a long remission after the first HCT.

Conclusion

The treatment of childhood ALL with chemotherapy has been a story of remarkable success, with survival rates in excess of 80% in the majority of studies. This success is counterbalanced by the fact that current chemotherapy is still inadequate for the almost 20% of cases who will relapse. As ALL is the most frequent malignancy in children, relapsed ALL remains one of the most frequent causes of death from malignant disease in childhood. Determining the appropriate role for HCT in childhood ALL has been a dynamic process, as success with chemotherapy has increased and additional hematopoietic cell sources have become available.

Serial, large-scale, essentially population-based randomized studies have led to the improvements in outcomes of chemotherapy for ALL. Application of a similar rigorous approach to evaluation of the role of HCT has not proved possible, and significant uncertainty remains in some areas. Access to HCT has been enhanced by improvements in the availability and selection of unrelated hematopoietic cell donors and the development of URD UCB as a hematopoietic cell source. Indeed, outcomes with URD HCT have improved such that results are now equivalent to those for sibling donor HCT in well-matched cases.

Future improvements in outcome and applicability of HCT for childhood ALL may result from the development of methodologies to identify at the earliest possible time children who are destined to relapse, allowing HCT in first remission when outcomes are superior. The detection of MRD in children receiving chemotherapy, either as a harbinger of relapse or as a measure of inadequate early response to therapy, will allow early referral for HCT as intensification therapy [70,72,179–181]. It is important to know that the significance of MRD varies in different genetic subtypes of leukemia. For example, MRD is cleared more promptly in children with ALL and ETV6-RUNX1 compared with children with favorable trisomies, both groups having an excellent long term prognosis [182]. Some studies have also shown association between gene expression profiling of ALL blasts at diagnosis and distinct immunophenotypic and genetic subtypes of ALL, treatment outcomes, and risk of relapse [78,183–185]. Further validation of these and similar findings in the future could allow early referral of children with ALL destined to relapse for HCT in first remission.

In recent years, similarly there has been growing interest in the role of MRD as a predictive marker for relapse following allogeneic transplantation. Most studies have shown that MRD detection in pre- and/or post-HCT samples predicts increased risk of relapse following HCT [69,71,74,186,187].

Different approaches have been suggested to help improve transplantation outcomes using MRD detection to modify either pre- or post-transplant therapy, for example, additional cytoreductive therapy pre-transplant to reduce the malignant clone and render the patients MRD negative, designing transplant protocols favoring the development of GVHD to increase the GVL effect, the use of donor lymphocyte infusion, etc.

Some of these interventions are limited by the practicality of these approaches. For example, heavily pretreated patients have limited options for further chemotherapy prior to transplantation, and rapid withdrawal of immunosuppression and donor lymphocyte infusions is associated with the risk of life-threatening GVHD and has very limited efficacy.

Therefore, although it is possible to identify patients at risk of relapse with MRD detection, strategies to respond to that information are limited. Also, many questions remain unanswered; for example, should MRD-negative status be used to assign patients to non-transplant treatment options to prevent TRM and long-term toxicity? Efforts to assess the clinical significance of pretransplant MRD in children with ALL are hampered by the lack of prospective studies in large cohorts of homogeneously treated patients.

Improved strategies for donor selection using molecular methods of HLA typing, or examination of non-HLA loci that influence HCT, also have potential to improve outcomes [188,189]. Inclusion of chemotherapy agents such as etoposide or thioTEPA (*N*-triethylenethiophosphoramide) in the conditioning regimen may reduce relapse and improve survival. Lastly, targeting of conditioning therapy with radioimmunoconjugates offers the possibility of reducing radiation toxicity while maintaining the therapeutic effect [190].

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CHAPTER 54

Hematopoietic Cell Transplantation for Myelodysplastic Syndromes and Myeloproliferative Neoplasms

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Introduction

Myelodysplastic syndromes (MDSs) and myeloproliferative neoplasms (MPNs) represent two broad categories of chronic myeloid malignancies. Both are clonal diseases of hematopoietic precursors/stem cells. In contrast to acute leukemias, the course of MDS and MPN may extend over years or even decades. However, both disorders have the potential of evolving to acute leukemia, typically with myeloid characteristics. Alternatively, patients will have progressive blood cytopenias and hematopoietic failure. Chronic myelomonocytic leukemia (CMML) may have more dysplastic or more proliferative features and, in the World Health Organization (WHO) classification, has been placed in the category "Myelodysplastic/myeloproliferative neoplasms" [1]. Whether these diseases evolve towards acute leukemia or severe cytopenias, both paths eventually prove fatal. Chronic myeloid disorders can occur at any age, but the incidence increases progressively with age.

General considerations for hematopoietic cell transplantation in patients with MDS and MPN

Hematopoietic cell transplantation (HCT) has proven curative potential for patients with MDS and MPN, but the indications for and the optimal timing of HCT are controversial. The frequently indolent course often renders physician and patients reluctant to accept the potential risks associated with HCT. Although the day 100 mortality has declined to less than 10% or even 5%, non-relapse mortality (NRM) overall has been in the range 20–25%, causes including organ toxicity, graft-versus-host disease (GVHD), and infectious complications. Particularly in older patients, with reduced biologic reserve and tolerance to certain therapies, those issues are most relevant. Comorbid conditions in older patients contribute to the problem. Sorror et al. developed an HCT-specific comorbidity index (HCT-CI), which provides useful guidance for risk assessment before HCT [2]. Another risk classification, proposed by Parimon et al. [3], heavily based on pulmonary function, also considers the donor type in the risk assessment.

A Markov decision analysis by Cutler et al. [4] showed that in patients with MDS the International Prognostic Scoring System (IPSS) was useful in determining the timing of HCT in patients with human leukocyte antigen (HLA)-identical sibling donors and were transplanted after receiving high-intensity ("myeloablative") conditioning regimens: the higher the IPSS risk score, the more likely patients were to extend their life expectancy by HCT. A more recent analysis in older individuals transplanted after reduced-intensity conditioning (RIC) reached similar conclusions: patients with low-risk MDS (IPSS low or intermediate-1) are unlikely to benefit from HCT (quality or prolongation of life). Patients with higher risk disease stand to benefit from HCT, although a significant gain was apparent only after several years of follow-up [5]. Cytofluorimetric analyses of marrow cells indicated that IPSS intermediate-1 and low-risk patients can be further subdivided, at least with regard to relapse risk, on the basis of phenotypic aberrancy of marrow cells [6]. A subcategorization of patients with "low-risk disease" in the revised IPSS (IPSS-R), distinguishes patients with <5% myeloblasts in the marrow and those with >2% and <5% [7] (Table 54.1). For patients with MPN, a decision analysis for HCT is currently under way; two recent studies did find a highly significant correlation of HCT outcome with DIPSS (Dynamic IPSS; see below) [8,9].

Granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood progenitor cells (PBPCs) are currently the most widely used source of stem cells and are associated with decreased rates of graft rejection and relapse in patients with MDS or MPN (compared with marrow cells). However, the results of a randomized trial comparing marrow with PBPCs from unrelated donors in patients with various diagnoses showed a higher incidence of chronic GVHD with PBPCs [10]. There was no significant difference in the relapse rates between the two arms, but lower relapse rates with PBPCs have been observed in other studies [11]. Nevertheless, chronic GVHD may severely reduce the quality of life, and the use of PBPCs over marrow is being reassessed. Umbilical cord blood is an alternative stem-cell source, and successful HCT has been reported in patients with MDS [12]. Advantages of umbilical cord blood include the rapid availability and greater tolerance for HLA disparity, allowing for the use of mismatched units, thereby increasing the donor pool. However, cell numbers in cord blood units (along with the degree of HLA mismatching) are important determinants of engraftment. The strategy of infusing two units of cord blood has resulted in accelerated engraftment; more studies are needed to examine this modality in patients with MDS [13]. There is little experience with cord blood in patients with MPN.

The selection of donors on the basis of histocompatibility [14] is discussed in Chapters 44 and 45. Patients with MDS or MPN have

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Table 54.1 IPSS-R prognostic scores

Variable	**Score**						
	0	**0.5**	**1**	**1.5**	**2**	**3**	**4**
Cytogenetics[a]	Very good		Good		Intermediate	Poor	Very poor
Marrow Blasts (%)	≤2		>2–<5		5–10	>10	
Hemoglobin (g/dL)	≥10		8–<10	<8			
Platelets (× 10^9/L)	≥100	50–<100	<50				
Neutrophils (× 10^9/L)	≥0.8	<0.8					

IPSS-R scores and expected survival (years)

Patient age (years)	**Risk category (score)**				
	Very low (≤1.5)	**Low (>1.5–3)**	**Intermediate (>3–4.5)**	**High (>4.5–6)**	**Very high (>6)**
Any	8.8	5.3	3.0	1.6	0.8
≤60	NR	8.8	5.2	2.1	0.9
>60	7.5	4.7	2.6	1.5	0.7

[a] Categorized according to Schanz et al. [29].

shown virtually identical results with HLA-identical siblings and with unrelated donors selected by high-resolution HLA typing (DNA sequencing). Promising results are also being obtained with transplants from HLA-haploidentical related donors.

As with other indications, GVHD has remained the most frequent complication after allogeneic HCT for MDS and MPN. Approximately 40–50% of patients transplanted from HLA-identical siblings and 50–60% transplanted from unrelated donors will develop acute GVHD requiring therapy. Chronic GVHD occurs in 30–70% of patients. Calcineurin inhibitors combined with methotrexate, mycophenolate mofetil, or sirolimus are widely used for prophylaxis. Glucocorticoids remain front-line therapy for treatment of established GVHD (see Chapters 83 and 84).

High-dose conditioning regimens are offered to patients up to 65 or 60 years of age, with HLA-identical siblings or unrelated donors, respectively [15]. RIC regimens allow the acceptable upper age for HCT to be expanded; even patients in their 70s have been transplanted successfully [16]. This is an important development as patients with MDS or MPN tend to be in their 60s or even 70s. However, although treatment-related mortality (TRM) is lower with RIC, the relapse incidence tends to be higher than with high-dose regimens, an important consideration since patients with MDS (or MPN) have generally not been treated aggressively with cytotoxic therapy before HCT [9]. Patients with the lowest disease burden tend to enjoy the highest success rates, raising the issue of pre-transplant debulking chemotherapy [17,18]. Generally, patients who have elevated myeloblast counts (≥5% in the marrow) receive therapy with demethylating agents or with induction-type chemotherapy before HCT with RIC. The approach has been less consistent in patients who are prepared for HCT with high-intensity regimens. A beneficial effect of debulking in overcoming disease-associated risk factors, such as adverse cytogenetics, is doubtful.

A French study showed that patients with secondary/treatment-related MDS who achieved remissions after chemotherapy had higher relapse-free survival (RFS) after HCT than patients who did not respond [19]. Similar observations were reported by others [20,21]. However, patients who did not achieve remissions had lower RFS than patients who had not received chemotherapy, suggesting that pre-HCT therapy selected for "treatment-responsive" patients.

Data on the role of debulking chemotherapy in MPN are sparse. However, one report describing a cohort of 14 patients with transformation of MPN to leukemia suggested that patients did benefit from pre-HCT chemotherapy [22].

Myelodysplastic syndromes

Presentation and terminology

Approximately 20–25% of individuals with MDS undergoing HCT have antecedent diseases, malignant or non-malignant, for which they received radiation/chemotherapy (secondary or treatment-related MDS); these patients may be rather young. However, for all patients with MDS, the median age at diagnosis is 70–75 years and no etiology can be identified (de novo MDS). "Secondary" MDS is also used to describe MDS evolving from a preceding hematologic diagnosis, for example, aplastic anemia. The pathophysiology of MDS is incompletely characterized [23]. With low-risk or early-stage disease such as refractory anemia (RA) or refractory cytopenia with multilineage dysplasia (RCMD) (Table 54.2), apoptosis, contributing to peripheral blood cytopenias and possibly the containment of the clone(s), is upregulated. In advanced, MDS clonal precursors become increasingly apoptosis-resistant, and proliferative features are prominent. Long considered a cell-autonomous disease, there is evidence for contributions of the microenvironment and immune mechanisms [24].

Most patients with MDS present with anemia, and only few patients with isolated neutropenia or thrombocytopenia. About 40–50% of patients with de novo MDS have clonal chromosomal abnormalities (by classic banding or fluorescent in situ hybridization); as many as 75% or more have clonal chromosomal markers as detected by molecular techniques [25].

Classification systems

The French–American–British classification [26], considering dysplasia and the proportion of myeloblasts, has been replaced by the WHO classification (Table 54.2) [1]; patients with ≥20% myeloblasts are considered to have AML. CMML, as indicated, has been reclassified in a separate MDS/MPN category [27]. These staging systems are used with the IPSS to stratify patients into four prognostic groups [28]. The original IPSS, incorporating cytogenetics, marrow myeloblast count, and blood cytopenias, is being replaced

Table 54.2 WHO classification and criteria for MDS[a]

Classification	Peripheral blood	Bone marrow
Refractory anemia (RA)	Anemia No or rare blasts	Erythroid dysplasia only <5% blasts <15% ringed sideroblasts
Refractory anemia with ringed sideroblasts (RARS)	Anemia No blasts	Erythroid dysplasia only <5% blasts ≥15% ringed sideroblasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Bi- or pan-cytopenia	Dysplasia in ≥10% of cells in two or more myeloid cell lineages
	No or rare blasts No Auer rods Monocytes <1000/μL	<5% blasts No Auer rods <15% ringed sideroblasts
Refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS)	Bi- or pancytopenia	Dysplasia in ≥10% of cells in two or more cell lineages
	No blasts	<5% blasts ≥15% ringed sideroblasts
MDS-unclassified (MDS-U)	Cytopenias	Unilineage dysplasia in granulocytes or megakaryocytes
	No or rare blasts No Auer rods	<5% blasts No Auer rods
MDS with del(5q) (5q– syndrome)	Anemia	Normal to increased megakaryocytes with hypolobulated nuclei
	<5% blasts Platelets normal or increased	<5% blasts No Auer rods Isolated del(5q)
Refractory anemia with excess blasts1 (RAEB-1)	Cytopenias	Unilineage or multilineage dysplasia
	<5% blasts No Auer rods Monocytes <1000/μL	5–9% blasts No Auer rods
Refractory anemia with excess blasts2 (RAEB-2)	Cytopenias	Unilineage or multilineage dysplasia
	5–19% blasts Auer rods ± Monocytes <1000/μL	10–19% blasts Auer rods ±

[a] In 2008, the WHO added some modifications [1]. A subtype of RARS-T (refractory anemia with ring sideroblasts and thrombocytosis) was recognized, which is often associated with a JAK2 mutation [121]. Patients with persistent cytopenias, lacking sufficient dysplasia, but with typical MDS-associated cytogenetic abnormalities [–7; del(7p); –5; del(5q); t(17q); t(17p); –13; del(13q); del(11p); del(12p)] or rare balanced translocations including t(11;16); t(3;21); t(1;3); t(6;9); inv(3), can be considered under MDS. If only cytopenias are present, these patients are said to have ICUS (idiopathic cytopenia of undetermined significance). Also, RCMD and RCMD-RS are being combined into one group.

Table 54.3 WHO Classification Based Prognostic Scoring System (WPSS) for MDS

Parameters and severity scores

Prognostic parameter	Score			
	0	1	2	3
WHO category	RA, RARS, 5q–	RCMD, RCMD-RS	RAEB-1	RAEB-2
Karyotype[a]	Good	Intermediate	Poor	–
Transfusion requirement[b]	No	Regular	–	–

WPSS risk and outcome

Risk group[c]	Composite score	Survival (years)	AML evolution
Very low	0	11.3	7% at 10 years
Low	1	5.3	–
Intermediate	2	3.7	–
High	3–4	1.6	–
Very high	5–6	0.7	50% at 8 months

[a] Good = normal, –Y, del(5q), del(20q); poor = complex (≥3 abnormalities) or chromosome 7 anomalies; intermediate = other abnormalities.

[b] In a modification (Malcovati et al. [31]), transfusion dependence was replaced by the degree of anemia, with hemoglobin <9 g/dL for men and <8 g/dL for women representing the significant cut-off.

[c] Scores for risk groups as follows: very low = 0, low = 1, intermediate = 2, high = 3–4, very high = 5–6 [30].

by the IPSS-R [7] (Table 54.1). The composite score also considers the *severity* of cytopenias and the new five-group cytogenetic risk classification [29], and stratifies patients into five categories, very low (score ≤1.5), low (>1.5–3), intermediate (>3–4.5), high (>4.5 -6), and very high risk (>6), with median life expectancies of 8.8, 5.3, 3.0, 1.6, and 0.8 years, respectively [7]. Five risk groups also compose the WHO classification-based Prognostic Scoring System (WPSS) (Table 54.3) [30,31], which incorporates transfusion dependence as a risk factor. The five WPSS categories predict median survivals of 11.3, 5.3, 3.7, 1.6, and 0.7 years, respectively. The probability and rate of progression to leukemia increase with increasing scores [7,30]. Marrow fibrosis, flow-cytometric aberrancies, or molecular markers [25,32] are currently not reflected in any classification schemes. Patient age has been included as a risk factor in one scoring system [33].

Transplantation specific risk assessment

Comorbid conditions [34] and a risk score based primarily on pulmonary function [3] have proven to be important tools in determining the probability of transplant success [34–36]. Iron overload (e.g. serum ferritin >1000 or 2000 μg/L; high liver iron content) also are associated with increased NRM and inferior survival [37,38], problems including include increased infection risk and, possibly, sinusoidal obstruction syndrome [39]. No prospective trials are available [40].

Non-transplant therapy and the decision on transplantation

Several treatment modalities other than HCT are available. For patients with a del(5q), lenalidomide (Revlimid) is considered first-line therapy, inducing transfusion independence in two-thirds of patients, for a median duration of 2–2.5 years; many experience morphologic and cytogenetic remissions [41]. Two DNA methyltransferase inhibitors, 5-azacitidine (Vidaza) and 2-deoxy-5-azacitidine (Dacogen), induce hematologic improvement in about half of the patients, and may delay leukemic transformation, and 5-azacitidine prolongs survival by 9–10 months [42]. Therapy with antithymocyte globulin (ATG), alone or combined with other agents, induces hematologic responses in 10–30% of patients with low-risk MDS, lasting in some patients for years [43]. Erythropoietin combined with G-CSF is effective in raising hemoglobin levels in a proportion of patients with low endogenous erythropoietin levels, particularly those with a high proportion of ring sideroblasts [44]. Other therapies are being tested in clinical trials.

The availability of these drugs affects the decision about HCT: should a responding patient be transplanted when a response is achieved or should HCT only be carried out if and when such therapy has failed?

Allogeneic transplantation

Patients with lower risk MDS (by any classification) are generally managed conservatively and HCT is foregone or delayed [4,5] as these patients tend to have a more indolent course. However, isolated manifestations such as severe neutropenia, thrombocytopenia, or red blood cell transfusion dependence may well represent indications for HCT, even though risk scores by IPSS-R may be low (see Table 54.1) [7,37,45–47]. Patients transplanted at an early stage of their disease have superior outcomes. Relapse incidence increases progressively with increasing IPSS or WPSS scores, the major determinants being karyotype and myeloblast count [30,48]. Figure 54.1 illustrates the impact of pre-HCT cytogenetic risk on post-HCT outcome in patients with HLA-matched related or unrelated donors. Similar results have been reported for the impact of WPSS [30]. Some reports of allogeneic HCT in patients with MDS are summarized in Table 54.4.

The International Bone Marrow Transplant Registry (IBMTR) presented results in 452 patients with MDS transplanted from HLA-identical siblings, showing a 3-year RFS of 40% (72% for patients aged ≤18 years); 140 patients (31%) had fewer than 5% blasts at HCT. TRM was 37% and the relapse rate was 23%. High marrow blast counts and high IPSS scores were significantly correlated with relapse. Among 512 patients with MDS transplanted from unrelated donors, those conditioned with high-dose oral busulfan (BU) (1 mg/kg × 16 doses over 4 days) and IV cyclophosphamide (CY) (60 mg/kg/day ×2) fared better than those given high-dose total body irradiation (TBI) (e.g., 6 × 2 Gy) combined with CY [49]. With BU/CY (BU prescribed at 16 × 1 mg/kg), with BU dose adjustment to achieve target steady-state levels of 800–900 ng/mL (targeted BU/CY), investigators at the Fred Hutchinson Cancer Research Center (FHCRC) observed a 3-year RFS of 68% with related donors and 70% with unrelated donors among patients with RA or Refractory anemia with ringed sideroblasts (RARS); the NRM was 31%, and relapse occurred in 5% of patients [48].

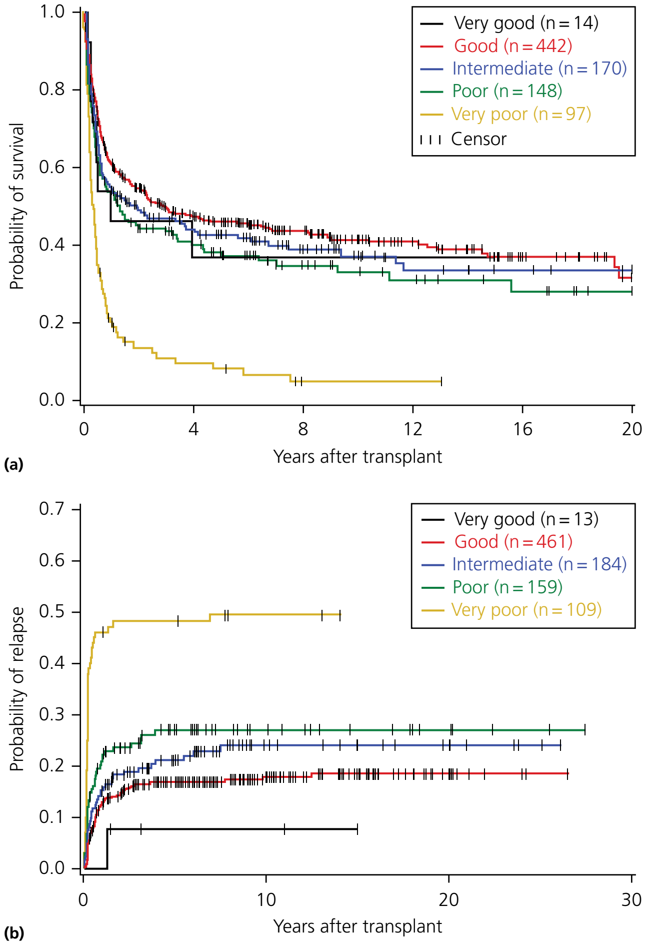

Figure 54.1 Impact of the five-group cytogenetic risk classification (incorporated into the IPSSR) [7,29] on transplant outcome. (a) Surivival. (b) Cumulative incidence of relapse. +, Censored patient. (For a color version of this figure, please see the color plate section.)

Patients with pre-HCT neutropenia tend to have inferior outcomes. Among 469 patients, 178 (61%) with less than 1.5 × 10^9 neutrophils/L had significantly higher NRM ($p = 0.01$), and a lower probability of survival, mostly related to infections [50]. Neutropenia was associated with higher risk karyotype. Iron overload has a negative impact on HCT outcome [37,45–47]. Transfusion dependence is linked to marrow fibrosis, which is associated with more rapid progression of MDS [51]. The potential benefit of chelation therapy remains to be determined [51].

Among 471 patients with MDS undergoing HCT, 113 had marrow fibrosis of various degrees and 358 did not. Patients with marrow fibrosis showed a significant delay in neutrophil and platelet recovery [hazard ratio (HR) 0.4, $p < 0.001$]. Survival, RFS, and NRM between the two groups were similar, but patients at intermediate-2 or high risk by IPSS with marrow fibrosis had greater NRM ($p = 0.04$) and reduced survival ($p = 0.03$) and RFS ($p = 0.04$). Similar results have been reported by others [52].

The success rates of HCT decline as the prognosis by WHO or IPSS criteria worsens, driving factors being marrow myeloblast count and high-risk cytogenetics (Figure 54.1). The EBMT reported a 5-year RFS with related donors of 34, 19, and 26% for refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEB-T), and therapy-related acute myeloid leukemia (t-AML), respectively; the relapse rate was 50% for the entire cohort. The 2-year RFS for patients transplanted from HLA-matched unrelated donors was 27%, 8%, and 27% for RAEB, RAEB-T, and t-AML, respectively [53]. In a study from FHCRC in patients with RAEB transplanted after conditioning with targeted BU/CY, the 3-year RFS was 45% with related and 40% with unrelated donors. The corresponding figures for RAEB-T/t-AML were 33 and 17%, respectively. The IPSS score correlated strongly with outcome [48].

In an effort to reduce toxicity, the tolerability and efficacy of intravenous fludarabine (4 × 30 to 5 × 50 mg/m^2) have been tested in combination with targeted oral BU [20,54]. The M.D. Anderson team used a regimen of intravenous fludarabine (40 mg/m^2/day) followed by intravenous BU (130 mg/m^2/day) on days −6 to −3 before HCT in patients with advanced myeloid malignancies (22 with MDS). Unrelated recipients also received equine ATG. The 1-year RFS and TRM were 52% and 3%, respectively [20]. Similar data were reported by Russell et al., using intravenous BU (3.2 mg/kg/day for 4 days) and rabbit ATG (Thymoglobulin, 4.5 mg/kg). The addition of 4 Gy TBI to such a regimen reduced the incidence of relapse [55]. Another 37 patients with MDS or t-AML were transplanted after conditioning with fludarabine (120–180 mg/m^2) and reduced-dose BU (8 mg/kg orally or 6.4 mg/kg intravenously) from related ($n = 19$) or unrelated ($n = 18$) donors (Table 54.4). TRM was 27% and 3-year estimated RFS 38%, with a relapse rate of 32% [56]. Whether ATG preparations should be used regularly with RIC regimens has been questioned in recent analyses [57]. The anti-CD52 antibody alemtuzumab was used as part of an RIC regimen for in vivo T-cell depletion. Among 75 patients conditioned with fludarabine/BU/alemtuzumab and given unrelated donor transplants, 3-year survival was 43%. Disease status at HCT and comorbidities were independent risk variables for overall survival [58]. In a prospective phase II study using fludarabine, melphalan, and alemtuzumab in 52 patients with AML and MDS, the relapse incidence

Table 54.4 Allogeneic HCT for MDS

Study	Year	No. of patients/ age (years) range (median)	Disease (No. of patients)	Donor (No. of patients)		Source of stem cells (No. of patients)		Conditioning	Disease category	Outcome (at years)		
				Rel	URD	BM	PB			NRM (%)	Relapse (%)	RFS (%)
High-dose regimens												
de Witte et al. [122]	2000	885/NA	MDS/t-AML	885	0	885	0	Various regimens	Untreated RA/RARS	37 (3)	13 (3)	55 (3)
			(1° = 818; 2° = 67)^{c}						Treated CR1	37	30	44
									Treated No CR	45	42	32
									Other	52	43	28
Guardiola et al. [11]	2002	234 4–59 (41) BM 12–60 (47) PB	MDS	234	0	132	102	Various regimens	RA	38 (2)	13 (2)	52 (2)
									RAEB	39	28	43
									RAEB-T		38	39
Sierra et al. [123]	2002	452/2–64 (38)	MDS	452	0	452	0	Various regimens (high-dose TBI 44%)	All patients	32 (3)	23 (3)	40 (3)
Bornhäuser et al. [54]	2003	42/12–65 (52)	MDS/CML	16	26	0	42	FLU/tBU	RA (high risk)	24 (1.5)	29 (1.5)	42 (1.5)
									RAEB/RAEB-T		52	22
									CML		75	0
de Lima et al. [20]	2004	96/19–66 (45)	MDS/AML	60	36	47	49	FLU + IV BU	All patients	4 (1)	43	52 (1)
									In CR		26	75
Deeg et al. [124]	2006	56/9–65 (50)	MDS/AML/MPD	30	26	0	56	tBU/CY + THY	RAEB	23 (2)	35 (2)	55 (2)
									AML		24	58
		27/11–64 (51)	RA	14	13	0	27	tBU/CY	RA	22 (1)	0	78 (1)
RIC												
Kröger et al. [56]	2003	37/23–72 (55)	MDS/t-AML	19	18	8	29	FLU (120–180 mg/m^2) + BU (8 mg/kg PO) (or 6.4 IV) + ATG (in 25)	RA RAEB RAEB-T Other	12 (Rel) (2)/ 45 (URD)	32 (3)	31/25 (3)
de Lima et al. [125]	2004	94/ 27–74 (61) [FAI] 22–75 (54) [FM]	MDS (26)/ AML (68)	65	29	53	41	FLU/Ara-C/Ida [FAI]		15 (3)	61 (3)	30 (3)
								FLU/Melphalan [FM]		39	30	35
van Besien et al. [59]	2005	52/17–71 (52)	MDS/AML	27	25	NA^{d}	NA^{d}	Flu (150 mg/m^2 IV) + melphalan (140 mg/m^2 IV) + Campath (100 mg)	Standard risk^{a}	22 (1)	16 (1)	61 (1)
									High risk^{b}	39	39	37
Lim et al. [58]	2006	75/24–68 (52)	MDS	0	75	28	47	FLU (150 mg/m^2 IV) + BU (4 mg/kg PO) + Campath (100 mg IV)	RCMD	24 (3)		55 (3)
									RAEB-1/2	44	43 (3)	18
									t-AML	21		47
									CMML	65		14
Laport et al. [60]	2008	148/50–75 (59)	MDS/MPN	75	73	4	144	TBI 2 Gy 5 TBI 2 Gy + FLU 90 mg/m^2	RA/RS 21 RAEB 16 CMML 7 PMF 12 tMDS 25 Other 18	32 (3)	41 (3)	27 (3)
High-dose or RIC												
Martino et al. [74]	2006	38 RIC/ 27–72 (56)	MDS (2° = 41%)	215	0	27	188	FLU + TBI (2–4 Gy) ± ATG/Campath or FLU+ alkylator	MDS	22 (3)	45 (3)	33 (3)
		621 Other/18–67 (45)	MDS (2° = 37%)	621	0	305	316	CY + TBI (≥8 Gy) ± ATG; Other or BU/CY	MDS	32	27	41
Scott et al. [61]	2006	38 RIC/ 40–72 (62)	MDS/t-AML (2° = 13%)	26	12	2	36	FLU (90 mg/m^2) + TBI (2 Gy)	RA/RARS	33 (3)	33 (3)	33 (3)
									RAEB	78	22	0
									RAEB-T/t-AML	31	34	35
		112 Other/ 40–65 (53)	MDS/t-AML (2° = 27%)	66	46	21	91	tBU/CY	RAEB	36 (3)	10	55
									RA/RARS	31	22	45
									RAEB-T/t-AML	31	40	29
Warlick et al. [73]	2009	84/18–69 (50)	MDS/CMML	47	37	16 Cord 26	42	HDC 52	RA 1	HDC 42 (1)	HDC 16 (1)	HDC 42 (1)
								RIC 32	RCMD/RS 23	RIC 34 (1)	RIC 35 (1)	RIC 31 (1)
									RAEB1 22 RAEB2 13 CMML, other 25			

(*Continued*)

Table 54.4 (*Continued*)

Study	Year	No. of patients/ age (years) range (median)	Disease (No. of patients)	Donor (No. of patients)		Source of stem cells (No. of patients)		Conditioning	Disease category	Outcome (at years)		
				Rel	URD	BM	PB			NRM (%)	Relapse (%)	RFS (%)
Lim et al. [21]	2010	1333/50–74 (56)	MDS/AML	811	522 (409=; 113≠)	256	1077	HDC 500	RA/RS (313)	HDC 44 (4)	HDC 33 (4)	HDC 30 (4)[e]
								RIC 833	RAEB (471)	RIC 32 (4)	RIC 41 (4)	RIC 32 (4)[e]
									RAEB-T (215)	HLA = sib: 34		
									AML (334)	URD=: 40 URD≠: 54		
Deeg et al. [127]	2012	1007/1–75 (45)	MDS/AML	547 (477=; 77≠)	460 (266=; 158≠)	502 Cord 21	484	HDC 906	RA/RS 392	40 (5)[f]	25 (5)[f]	35 (5)[f]

AML, acute myeloid leukemia; Ara-C, cytosine arabinoside; ATG, antithymocyte globulin; BM, bone marrow; BU, busulfan; CY, cyclophosphamide; FLU, fludarabine; Ida, idarubicin; IV, intravenous; MDS, myelodysplastic syndrome; NA, not available; NRM, non-relapse mortality; PB, peripheral blood; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RCMD, refractory cytopenia with multilineage dysplasia; Rel, related; RFS, relapse-free survival; RIC, reduced intensity conditioning; URD, unrelated donor; tBU/CY, targeted BU/CY; THY, Thymoglobulin.

[a] Defined as AML in first CR, 2° CR, MSD <5% blasts.

[b] All other patients.

[c] 1° = de novo; 2° = secondary

[d] PB was the stem cell source of choice, but numbers were not provided.

[e] Overall survival.

[f] The focus of this analysis was on the impact of cytogenetics. There was a strong direct correlation for relapse and a reverse correlation of RFS with the new five-group cytogenetic risk classification [29].

was 27%, TRM 33%, and RFS 38%. Disease risk and low performance status were the major adverse factors [59]. Even lower intensity conditioning regimens use TBI doses of 2–4.5 Gy plus fludarabine 3 × 30 mg/m^2, yielding a 2-year survival of 40% in patients with low-risk and 25% in patients with high-risk MDS [60,61]. The addition of iodine-131-labeled anti-CD45 monoclonal antibody for targeted delivery of radiation to hematopoietic cells has intensified the regimen, and may be associated with lower relapse rates [62]. As exportation of the ^{131}I method is challenging, newer protocols are using ^{90}Y, which may lend itself more readily to wider distribution.

In an analysis of results in more than 1000 patients with MDS aged 1–75 years, the impact of the newly established five-group cytogenetic risk classification [29] on outcome after HCT was determined. Patients were transplanted from related (n = 547) or unrelated (n = 460) donors. Cytogenetic risk was significantly associated with relapse and mortality (Figure 54.1). Monosomal karyotype tended to increase rates of relapse and mortality further even after considering the IPSS or five-group classifications. Mortality was also impacted by patient age, donor type, conditioning regimen, platelet count, and etiology of MDS. The best results were obtained with a regimen of fludarabine with treosulfan ± 2 Gy TBI [63,64]. Mortality declined significantly in recent years, despite the fact that patient age increased from a median of 31.7 years before 1990 to a median of 54.3 years in the interval 2006–2010. The proportion of unrelated donor HCT increased from 6 to 57%. There were 254 patients in whom the disease progressed or relapsed after HCT. With a median follow-up of 7.1 years, the 5-year estimates of relapse, NRM, survival, and RFS were 25, 40, 38, and 35%, respectively.

Hence the success of HCT in patients with MDS is determined primarily by the proportion of myeloblasts in the marrow and the karyotype. An additional important factor is patient co-morbidities (discussed in Chapter 28). Depending on these parameters, as many as 70–80% or as few as 15–20% of patients may be cured by HCT; some patients have been followed now for more than 25 years after HCT. Results with unrelated donors who are HLA matched by high-resolution typing are comparable to those with HLA genotypically identical sibling donors. Data with cord blood or HLA-haploidentical transplants are emerging.

Secondary/treatment-related MDS

Friedberg et al. analyzed outcomes in 552 patients after autologous HCT for non-Hodgkin lymphoma, 41 of whom developed MDS at a median follow-up of 47 months, for an incidence of 19.8% at 10 years [65]. None of the 13 patients who received allogeneic HCT survived long term. Yakoub-Agha et al. reported results in 70 patients with secondary MDS or t-AML [19], who received allogeneic HCT following conditioning with high-dose TBI or BU-containing regimens. RFS at 2 years was 28% for the entire cohort, 45% for patients in complete remission, and 18 and 26% for patients with active MDS and t-AML, respectively. Woodard et al. [66] presented results in 38 children given allogeneic HCT for secondary MDS. Overall survival and RFS were similar at 15.4% at 3 years; NRM was 60% and relapse incidence 24%. The proportion of marrow blasts at the time of HCT was the strongest risk factor for relapse.

In an FHCRC analysis of 257 patients aged 3–72 years (median 43 years) and with secondary MDS, the rate of HCT success was similar to that with de novo MDS when adjusted for risk factors, in particular cytogenetics. The five-year RFS was 43% and NRM 28% among patients conditioned with targeted BU/CY. The relapse incidence was lower (p = 0.003) and RFS superior (p = 0.02) with unrelated donors. Litzow et al. summarized CIBMTR data on 868 patients with treatment-related MDS or AML [67], and showed an NRM at 1 year (5 years) of 41% (48%) and a relapse incidence of 27% (31%). The 5-year RFS was 21%. Older age, high-risk cytogenetics, AML not in remission or advanced MDS, and the use of HLA non-identical donors were the major risk factors, with 5-year survivals of 50, 26, 21, 10 and 4% among patients with no, one, two, three, or four risk factors, respectively (Figure 54.2). The impact of cytogenetics was not reported.

The search for the ideal regimen

The RIC regimens discussed have the advantage of low regimen-related toxicity, allowing their use in older patients or patients with severe comorbid conditions [68,69]. The disadvantage (in MDS) is higher relapse rates [70–73]. An ongoing Blood and Marrow Transplant Clinical Trials Network (BMT CTN)-sponsored phase III trial is comparing toxicity and efficacy of high-intensity and RIC regimens in patients with MDS or high risk AML (BMT CTN protocol 0901). A large retrospective study by the EBMT group in 836 patients with MDS given various high-dose (n = 621) or RIC regimens (n = 215) showed no significant difference in survival; relapse rates were significantly lower in patients receiving high-dose conditioning, but this advantage was canceled out by a higher TRM [74]. An updated analysis of results in patients more than 50 years of age, basically confirmatory, showed that disease stage was the determining factor for outcome. Age had a minor effect, and results were comparable for RIC and high-intensity regimens [21] (Figure 54.3).

More conventional high-intensity regimens also have been progressively modified, aiming at low toxicity combined with high efficacy. Encouraging results have been observed with regimens incorporating treosulfan [75,76]. In a trial conducted at FHCRC, 60 patients (primarily with MDS or AML) were conditioned with fludarabine (30 mg/m^2 × 5) plus treosulfan (12 or 14 g/m^2 × 3) [63]. NRM by 1 year was less than 10%, and 2-year survival for patients with standard- or intermediate-risk cytogenetics was 80%. Patients with high-risk karyotype, in contrast, showed long-term RFS of 35–40%, the difference being due primarily to a higher relapse incidence. Preliminary data from a successor protocol in which 2 Gy of TBI was added to fludarabine/treosulfan suggested that this combination reduced the relapse risk even in patients with high-risk cytogenetics, although the frequency of toxicity (skin rashes and mild mucositis) was increased [64].

MDS in children

MDS is rare in children, accounting for 5–7% of hematologic malignancies. There are similarities to but also major differences from MDS in adults, and a separate classification has been proposed (Table 54.5) (the MDS/MPN group is discussed in Chapter 51). Patients with Down syndrome who developed MDS/leukemia are no longer considered in the MDS category.

Children with MDS present with thrombocytopenia or neutropenia more frequently than with anemia, and the term "refractory cytopenia of childhood" (RCC) is preferred. Also, the category of RAEB-T has been retained [77]. About 50–60% of the patients have clonal cytogenetic abnormalities, the most frequent one being monosomy 7 (in ~25% of cases). In patients with monosomy 7, the disease progresses faster than with other karyotypes. In secondary MDS, as in adults, about 80% of patients have cytogenetic abnormalities [78]. In contrast to adults, however, about 75% of children with MDS have hypocellular marrows, and the distinction from aplastic anemia may be difficult. In fact, about 10–15% of children with aplastic anemia develop MDS within 3 years, and "aplastic anemia" may simply be the initial presentation of MDS.

Chemotherapy is not useful in pediatric MDS, and HCT should be considered early in the course. Several studies have been reported from Europe, Japan, and the United States. Kardos et al. [79] reported on 67 children, 41 of whom underwent allogeneic HCT. Among children in whom the disease had not progressed from refractory cytopenia, 76% were surviving, compared to 36% with advanced MDS (>5% myeloblasts). Sasaki et al. [80] presented data on 189 patients [122 primary MDS, 24 with constitutional predisposition (see Chapter 75) and 43 with therapy-related MDS]. The 4-year survival among patients with refractory cytopenia was 79%, compared with less than 40% in all other patients. Children who underwent HCT had a significantly higher survival probability. Yusuf et al. reported results in 94 children receiving allogeneic HCT for MDS [81]. The 3-year probabilities of NRM, relapse, and RFS were 28, 29, and 41%, respectively. RFS was 74% in patients with refractory cytopenia, 68% with RAEB, and 33% with juvenile myelomonocytic leukemia. Patients with RAEB-T were 5.5 times more

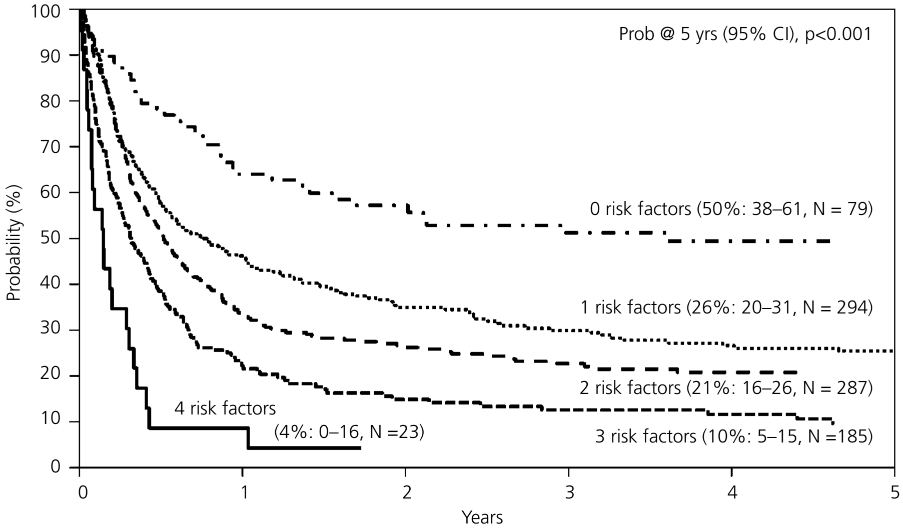

Figure 54.2 Probability of overall survival after allogeneic HCT for therapy-related AML and MDS, by risk factors: age older than 35 years; poor-risk cytogenetics; t-AML not in remission or advanced t-MDS; donor other than an HLA-identical sibling or well-matched unrelated donor. Source: Litzow et al. 2010 [67]. Reproduced with permission of the American Society of Hematology.

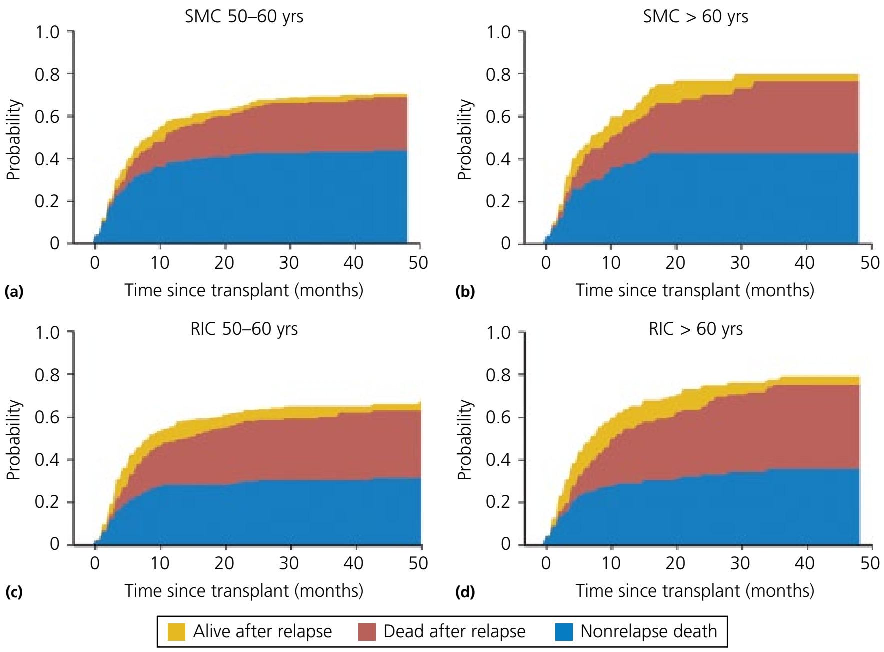

Figure 54.3 Stacked cumulative incidence curves from a competing risk model with relapse and death as competing risks, with the study population substratified according to (a) age 50–60 years, standard "myeloablative" conditioning (SMC); (b) age >60 years, SMC; (c) age 50–60 years, reduced-intensity conditioning (RIC); (d) age >60 years, RIC. Source: Lim et al. 2010 [21]. Reproduced with permission of the American Society of Clinical Oncology.

Table 54.5 Classification of MDS and MDS/MPN of childhood

I. MDS/MPN
  - Juvenile myelomonocytic leukemia
  - Chronic myelomonocytic leukemia (secondary only)
  - BCR-ABL-negative chronic myeloid leukemia

II. Down syndrome disease
  - Transient abnormal myelopoiesis
  - Myeloid leukemia of Down syndrome

III. MDS
  - Refractory cytopenia (peripheral blood blasts <2% and marrow blasts <5%)
  - Refractory anemia with excess blasts (RAEB) (peripheral blood blasts 2–19% or marrow blasts 5–19%)
  - RAEB in transformation (RAEB-T) (peripheral blood or marrow blasts 20–29%)

likely to relapse than other patients. More recently, Parikh et al. [82] presented results in 23 patients transplanted with umbilical cord blood cells. Children were aged 1.6–12.4 years (median 5.3 years) and had RA (*n* = 12), RAEB (*n* = 8), or RAEB-T (*n* = 3). Donors were matched for HLA at 4/6 antigens in 16 cases and 5/6 antigens in seven cases. Most patients were conditioned with TBI-based regimens; 0.2–28.5 (median 1.7) × 10^5 CD34$^+$ cells/kg were infused. All but three patients engrafted, albeit with very delayed platelet recovery, and RFS at 1 and 3 years was 70 and 61%, respectively. Causes of death, in addition to graft failure, included relapse (*n* = 4), infections (*n* = 2), and a lymphoproliferative disorder (*n* = 1). Factors associated with superior survival were younger age (p = 0.05) and lower body weight (p = 0.03).

Myeloproliferative neoplasms

The scope of MPN, presentation, and staging

The second category of chronic myeloid disorders is known as myeloproliferative neoplasms (MPNs). MPNs include chronic myeloid leukemia (CML) (discussed in Chapter 48), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). According to the WHO classification, other, rare disorders such as chronic neutrophilic leukemia, chronic eosinophilic leukemia (and hypereosinophilic syndrome), and chronic myeloproliferative diseases, unclassifiable, are included in the MPN category (mast cell disorders are classified separately) [27].

Patients with PV or ET in many instances have a rather indolent course over decades, until they develop severe marrow fibrosis and splenomegaly, along with peripheral blood cytopenias, or progress to acute leukemia. Patients with PMF present with marrow fibrosis, may have leukocytosis or elevated platelet counts, and generally proceed to develop single- or multilineage peripheral blood cytopenias. In 2005, an activating mutation (V617F) in the JAK-2 kinase was identified in >95% of patients with PV, and about half of the patients with ET or PMF [83]. This mutation contributes to the disease, but recent findings indicate that other mutations, for example in TET2, precede the JAK2 mutation, and the development of leukemia not showing the JAK2 clone in patients previously JAK2 (V617F)-positive patients, supports that concept. Also, additional mutations have been identified, for example, in exon 12 of the gene or in the *Mpl1* gene, that contribute to the disease [84,85].

Patients with MPN also develop complications in nonhematopoietic organs, including hepatic fibrosis, portal hypertension, heart failure, and infectious complications due to cytopenias [86]. Several prognostic scoring systems have been developed for PMF, and are also applied to patients with PV or ET once they develop marrow fibrosis. The Lille (or Dupriez) score has been replaced by the DIPSS, which considers patient age, hemoglobin levels, leukocyte count, circulating myeloblasts, and patient symptoms, and on that basis distinguishes four risk groups (Table 54.6) with median life expectancies not yet reached (at the time of reporting) for the good-risk group, and 14.2, 4, and 1.5 years, respectively, for the remaining three risk groups. In a refinement (DIPSS-plus) [87], platelet counts less than 100,000, cytogenetic abnormalities such as +8, −7/7q−, i(17q), −5/5q−, 12p−, inv(3), 11q23, and complex karyotype, and transfusion dependence, have been added as risk factors (see Table 54.6).

Non-transplant therapy

Hydroxyurea and interferon (IFN) are time-honored modalities of treatment for MPN. A pegylated form of IFN is better tolerated than older preparations of IFN, leading to gratifying responses in spleen size reduction and blood cell counts [88]. IFN may also lead to regression of marrow fibrosis. However, with the identification of the JAK2 (V617F) mutation, the focus has been on "JAK2 inhibitors," and one preparation, ruxolitinib (Jakafi), has been approved by the US Food and Drug Administration (FDA) for patients with intermediate- and high-risk MPN. Major benefits have been improvement or resolution of patient symptoms (such as night sweats or bone pain) and regression of spleen size [89,90]. Although not without controversy, at least one randomized trial suggested prolongation of survival [89]. Additional JAK2 inhibitors with different clinical profiles are currently being investigated. The availability of these agents is leading to discussions similar to those on hypomethylating agents in patients with MDS, and how the use of those agents may impact the decision about HCT. It will be important to monitor patients closely; there have been patients whose symptoms improved while the disease actually progressed, as indicated by rising blast counts. Other agents include thalidomide and pomalidomide that may improve anemia or anagrelide for the control of high platelet counts.

Transplantation for PMF, PV, and ET

Most patients with PV or ET are considered for HCT when they develop peripheral blood cytopenias or their disease progresses to myelofibrosis or AML develops. In PMF, indications for HCT are typically peripheral blood cytopenias or evidence of leukemic transformation. Splenectomy does probably not significantly alter the course of the disease [8,52]. Marrow fibrosis by itself does not interfere with engraftment of donor cells [91,92].

Table 54.6 Risk factors in PMF in the DIPSS classification[a] [126]

Risk factors		
Age >65 years	DIPSS	DIPSS-plus
Constitutional symptoms		
Hemoglobin <10 g/dL		
WBC count >25 × 10^9/L		
Blood myeloblasts >1%		
Platelet count <100 × 10^9/L		
Abnormal cytogenetics		
Transfusion dependency		

DIPSS risk categories and survival

Risk group	No. of factors	Proportion of patients (%)	Median survival (months)
Low	0	22	135
Intermediate-1	1	29	95
Intermediate-2	2	28	48
High	≥3	21	27

DIPSS-plus categories and survival

Risk group	Risk score	Median survival (months)
Low	0	180
Intermediate-1	1	80
Intermediate-2	2–2	35
High	4–6	16

DIPSS, Dynamic International Prognostic Scoring System.

[a] Unfavorable karyotype: complex karyotype or sole or two abnormalities that include +8, –7/7q-, i(17q), –5/5q1, 12p–, inv(3), or 11q23 rearrangement. DIPSS-plus is derived from the DIPSS by weighing: one point is added for intermediate-1 risk (by DIPSS) and for platelet counts <100 × 10^9/L, unfavorable cytogenetics, and red cell transfusion need, respectively; DIPSS intermediate-2 receives two points and DIPSS high receives three points.

Ballen et al. reported results in 289 patients with PMF, 18–73 years old, transplanted from HLA-identical siblings (n = 162), unrelated individuals (n = 101), or HLA non-identical family members (n = 26) [93]. Splenectomy had been performed in 65 patients. The day 100 mortality was 18, 35, and 19% and 5-year RFS was 33, 27, and 22% for the three donor groups, respectively.

Outcome data on 170 patients from FHCRC with PV, ET, or PMF, 12–78 years of age, transplanted from allogeneic (83 related, 84 unrelated) or syngeneic (n = 3) donors were recently presented [8]. Most patients (n = 106) received targeted BU/CY (combined with ATG in 15) for conditioning. Other regimens included intravenous fludarabine, 3×30 mg/m^2, and 2 Gy of TBI in 13 patients (see Chapter 21); in 18 patients CY was administered first, followed by BU, thereby avoiding altered CY metabolism (and toxicity) due to BU-induced enzyme activation. Most patients (n = 125) received PBPCs, and 45 received marrow. Eight of 167 allogeneic recipients experienced graft rejection/failure. The 5-year estimate of relapse/disease progression was 10%, and NRM was 34%. With a median follow-up of 5.9 years, the median survival was not reached for patients with low or intermediate-1 risk by DIPSS (survival estimated at 80 and 65%, respectively) and was 7 and 2.5 years for patients with intermediate-2 and high-risk disease, respectively [8]. The best tolerated regimen was the CY followed by BU combination, with a day 100 mortality of 0%. The overall and NRM (but not relapse by itself) increased significantly with DIPSS stage, presumably a reflection of the fibrosis affecting non-hematopoietic tissues (Figures 54.4 and 54.5). Low hemoglobin values had the most profound impact on outcome ($p < 0.001$ for overall mortality, "relapse or death" and NRM), consistent with the added factor "transfusion dependence" in the DIPSS-plus. Among parameters not included in the DIPSS, there was a suggestion that splenectomy was associated with reduced mortality ($p = 0.05$), a finding that must be interpreted with caution since the transplant center had no control over who underwent splenectomy and, in fact, another report suggested an inferior success rate in splenectomized patients [94]. The major causes of death were related to GVHD, infections, relapse, and graft failure. When adjusted for disease risk, the results show a clear improvement of transplant results in more recent years.

Ditschkowski et al. presented results in 76 patients with PMF (n = 47) or myelofibrosis after PV or ET (n = 29), transplanted from related (n = 30) or unrelated (n =4 6) donors [9]. The 1-year NRM was 28% and RFS was 50%, with a relapse rate of 19% at 5 years. Cytotoxic therapy pre-HCT (and the development of chronic GVHD) were adverse risk factor for survival. Patients in the DIPSS risk group "low" had significantly superior RFS ($p = 0.02$) compared with higher risk groups. The inclusion of the DIPSS-plus allowed four risk groups to be discriminated with 5-year survival probabilities of 100, 51, 54, and 30%, respectively. Relapse was significantly increased in patients without chronic GVHD.

Rondelli et al. presented data on 21 patients aged 27–68 years (median 54 years) with intermediate- or high-risk myelofibrosis (based on the Lille score) transplanted over an extended time interval, using several RIC regimens. All but one patient engrafted, and 18 patients were alive (17 in remission) with a median follow-up of 31 months [95].

Kröger et al. presented data from a prospective study in 103 patients aged 32–68 years (median 55 years) with PMF or fibrosis

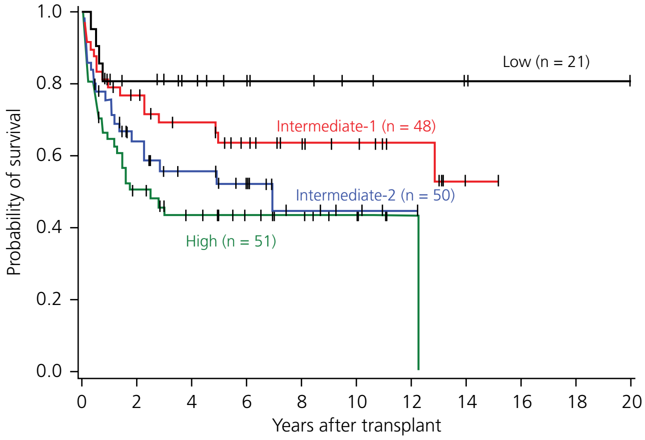

Figure 54.4 Overall survival of patients with PMF or myelofibrosis developing from PV or ET, by DIPSS category. Source: Scott et al. 2012 [8]. Reproduced with permission of the American Society of Hematology.

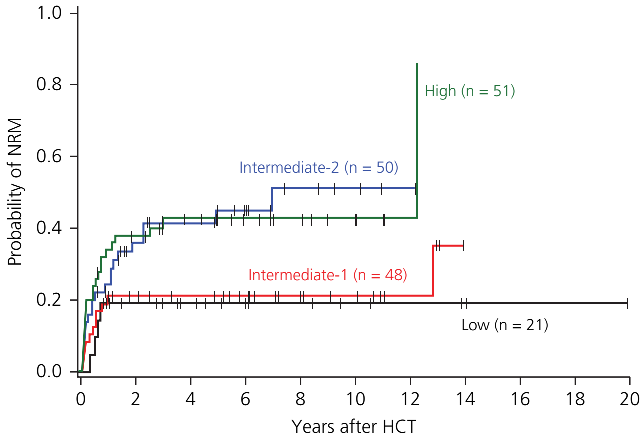

Figure 54.5 Non-relapse mortality of patients with PMF or myelofibrosis developing from PV or ET, by DIPSS category. Source: Scott et al. 2012 [8]. Reproduced with permission of the American Society of Hematology.

after PV or ET, conditioned with BU 10 mg/kg, plus fludarabine 180 mg/m^2 and ATG, and transplanted from related or unrelated donors [52]. Two patients failed to achieve leukocyte and platelet engraftment. NRM at 1 year was 16%, significantly lower in recipients of HCT from completely HLA-matched donors (12% vs. 38%, p = 0.003). The relapse incidence was 22% at 3 years and correlated with the Lille score. The estimated 5-year RFS was 51%. Age older than 55 years (HR 2.70) and HLA-mismatched donors (HR 3.07) were significant risk factors for survival. Investigators at City of Hope Medical Center presented results on nine patients aged 46–68 years (median 54 years). Eight patients received fludarabine 25 mg/m^2/day for 5 days followed by melphalan 140 mg/m^2 for conditioning, while one patient received fludarabine 30 mg/m^2 for 3 days followed by 2 Gy TBI. All patients achieved engraftment, and the probability of 1-year survival was 56% [96].

An RIC regimen of fludarabine 90 mg/m^2 plus 2 Gy TBI yielded a survival rate of 56%, which did not differ from that achieved with higher dose regimens. However, this small cohort consisted of patients up to 70 years of age, most of whom had comorbid conditions [2]. Conceivably, conditioning regimens of intermediate intensity will offer the best outcome [97].

An analysis of results in 139 patients with advanced myelofibrosis, with known JAK2 status who were transplanted following RIC, showed a significantly inferior overall survival among those with wild-type JAK2 (HR 2.14, p = 0.01); allele burden did not appear to affect outcome among patients with mutated JAK2 [98]. However, in the study of 170 patients discussed above, who were conditioned with various regimens, there was a trend for inferior survival among patients with mutated JAK2 [8].

While generally HCT has been carried out for patients with PV or ET only when they developed marrow fibrosis or there was evidence of leukemic transformation, a recent analysis described 119 patients with PV or ET who were transplanted for various reasons [99]. Most patients received high-intensity conditioning; donors were HLA-identical siblings in 32 and unrelated individuals in 87 patients. The 1-year NRM was 27% for ET and 21% for PV patients, and the 5-year relapse incidence was 12 and 32%, respectively. Overall survival (RFS) at 5 years was 56% (48%) and 67% (47%) for ET and PV patients, respectively. As in all other studies, platelet and neutrophil recovery was significantly faster in patients with normal spleen size or after splenectomy.

Taken together, the results show that HCT provides effective therapy for patients with PMF, PV, or ET. Once leukemic transformation occurs, the probability of a successful HCT declines significantly to about half of what is achievable with earlier HCT.

Other MPNs

Experience with HCT for patients with chronic neutrophilic leukemia, chronic eosinophilic leukemia (hypereosinophilic syndrome), and unclassified MPN is very limited. The optimal therapy for chronic neutrophilic leukemia is not known. However, because of the potential for blastic transformation, HCT has been considered appropriate therapy, particularly for young patients [100]. Similarly, successful HCT has been reported in patients with chronic eosinophilic leukemia [101,102]. The recognition that about 10% of patients with this disorder have an interstitial deletion on chromosome 4q12 that leads to the expression of an FIP1L1–PDGFRα fusion gene with tyrosine kinase activity has made treatment with tyrosine kinase inhibitors an attractive alternative [103]. About 70–80% of these achieve complete remissions with imatinib [104,105]. Several patients with unclassifiable MPN have also been transplanted successfully [8,91].

Myelodysplastic/myeloproliferative neoplasms

The WHO has listed CMML, atypical CML, juvenile monocytic leukemia, and unclassifiable MDS/MPN as a separate category. As implied by the terminology, these disorders show both dysplastic and proliferative features [106].

Chronic myelomonocytic leukemia

Persistent peripheral blood monocytosis (>1000/μL) is the hallmark of CMML. Depending on the myeloblast count, CMML is divided into CMML-1 (blood <5%, marrow <10%) and CMML-2 (blood 5–19%, marrow 10–19%). The WHO has also defined a separate category of CMML with eosinophilia when there is an accompanying peripheral blood eosinophilia. Patients in this category, representing a small proportion of patients with CMML, often have a t(5;12)(q33;p13) translocation, which results in an abnormal platelet-derived growth factor receptor fusion gene, and those patients are likely to respond to tyrosine kinase inhibitors

Table 54.7 M.D. Anderson criteria for CMML risk category

No. of risk factors[a]	Risk group	Median survival (months)
0–1	Low	24
2	Intermediate-1	15
3	Intermediate-2	8
4	High	5

[a] Risk factors: hemoglobin <12 g/L; absolute lymphocyte count >2.5 × 10^9/L; immature myeloid cells circulating in peripheral blood; ≥10% myeloblasts in bone marrow [107].

[106]. Other clinical features include splenomegaly, lymphadenopathy, and skin rashes. The M.D. Anderson group has developed a scoring system for CMML based on four clinical parameters: hemoglobin <12 g/L, the presence of circulating immature myeloid cells, an absolute lymphocyte count of over 2.5 × 10^9/L, and marrow myeloblasts over 10% predict a poor prognosis (Table 54.7). Based on the presence of one or several of those findings, patients are divided into low, intermediate-1, intermediate-2, and high-risk categories, with median survivals of 24, 15, 8, and 5 months, respectively, in patients who are not transplanted [107].

Despite the term "chronic," CMML can show fairly rapid progression to acute leukemia. About half of the patients with transformation will achieve complete remissions with intensive induction chemotherapy. On average, remissions are sustained for 4–5 months. Thus, except for patients with a t(5;12) translocation, the majority of whom will respond to tyrosine kinase inhibitors, the only promising therapy is HCT.

An EBMT series reported transplant results in 50 patients, about half of whom were conditioned with TBI-based regimens (n = 26). Most patients (n = 38) were transplanted from HLA-matched siblings. Forty patients received marrow, nine received PBPCs, and one received both. RFS at 2 years was 18%, with an NRM of 55% and a relapse incidence of 42%. Patients who developed acute GVHD had a lower rate of relapse [108]. A report from FHCRC included 86 patients , 1–69 years of age, 38 transplanted from related and 47 from unrelated donors [35]. The source of stem cells was marrow in 32 and PBPCs in 53 patients. Sustained neutrophil engraftment was achieved in 91% of the patients; however, 18 patients died between days 11 and 115 without having achieved platelet reconstitution. The major causes of death were relapse and infections ± GVHD; the relapse incidence at 10 years was 27% and RFS was 38%, Mortality was increased with low pre-HCT hemoglobin (p = 0.007), high-risk cytogenetics (p = 0.02), higher HCT-CI (p = 0.0008), and older age (p = 0.02). Survival at 10 years was 53% in patients with HCT-CI 0–2 and 27% with HCT-CI ≥3 (Figure 54.6). Outcome did not correlate with the WHO classification or with any other currently used classification system.

Atypical CML

Atypical CML is a rare disorder with poor prognosis. Koldehoff et al. reported results with HCT in nine patients (four transplanted from HLA-identical siblings, four from HLA-matched unrelated donors, and one from an identical twin brother) [109]. One patient died at 9 months from cerebral toxoplasmosis. The remaining patients are alive and in remission (the twin transplant recipient after a second HCT from the same donor following relapse) at a median of 55 months after HCT. These data suggest that HCT offers curative therapy for patients with atypical CML.

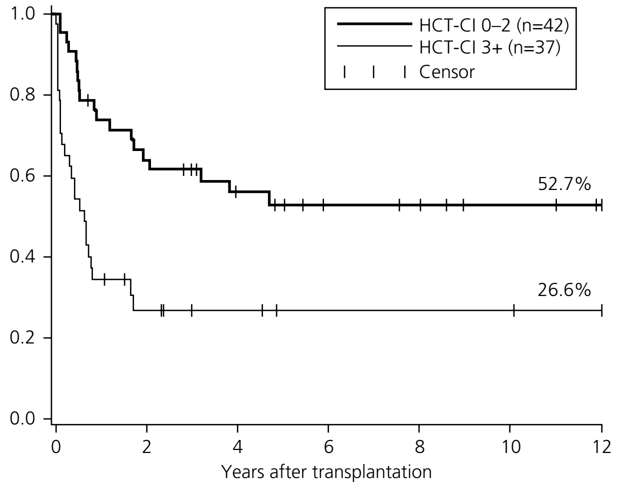

Figure 54.6 Survival dependent upon pre-transplant comorbidities. Shown are the probabilities of survival for 42 patients with HCT-CI scores 0–2 (52.7%) and for 37 patients with scores of ≥3 (26.6%). Source: Eissa et al. 2011 [35]. Reproduced with permission of Elsevier.

Juvenile myelomonocytic leukemia

Studies in patients with juvenile myelomonocytic leukemia are described in Chapter 49.

Systemic mastocytosis

The WHO has classified mast cell disorders in a separate category [27]. Patients with indolent mastocytosis have an excellent prognosis. However, patients with aggressive systemic mastocytosis or mast cell leukemia may have a life expectancy of only 1 year or less [110]. Nakamura et al. reported on three patients who were transplanted from HLA-identical siblings, following conditioning with an RIC regimen [111]. All patients achieved engraftment; however, all experienced disease progression, with the longest response interval being 39 months. Sperr and co-workers reported a successful HCT following high-intensity conditioning in an 18-year-old boy with myelomastocytic leukemia [112,113]. Other successful HCTs for mastocytosis have been reported by Hennessy et al. [114] and Przepiorka et al. [91].

Autologous HCT

Several reports have shown potential benefits and even cure with autologous HCT in patients with MDS and MPN. However, successes have been few, and with the development of various conditioning regimens and the availability of many allogeneic donor options, autologous HCT is currently not a recommended strategy for these patients.

Relapse after HCT

Post-HCT relapse has remained a challenging problem, particularly in patients with MDS. Discontinuation of GVHD prophylaxis (or therapy) and the infusion of viable donor lymphocytes can induce remissions in a small proportion of patients. A prospective trial in pediatric patients tested the efficacy of preemptive donor lymphocyte infusion in 65 patients with refractory cytopenia or advanced MDS who showed mixed chimerism with increasing proportions of host cells after allogeneic HCT [115]. Sequential chimerism testing showed that 48 patients became complete chimeras, and five had increasing proportions of donor cells, while 12 continued to show increasing numbers of host cells. RFS was 50%, compared with 0% in historical controls not treated with donor lymphocyte infusions. However, in a study of 194 adult patients with MDS or acute or chronic leukemia, originally conditioned with high-dose or RIC regimens, only 20–30% achieved complete remission following discontinuation of immunosuppression or donor lymphocyte infusion. Further, 2-year survival was 0–4% for patients who had relapsed within 100 days of HCT, and 5–12% if the relapse occurred after day 100 [116]. Platzbecker et al. monitored CD34 chimerism post-HCT and initiated treatment with 5-azacitidine when donor CD34 chimerism declined to <80% [117]. Among 20 patients so treated, four (20%) achieved complete and sustained remissions and a further six (30%) achieved major hematologic responses, which, however, were not sustained. Second transplants, particularly with RIC regimens, are an option. However, the success rates have been low, related to NRM or refractoriness of the disease [118]. Ongoing studies are investigating whether adjuvant or preemptive therapy, in particular with DNA methyltransferase inhibitors [119], may reduce the relapse incidence in high-risk patients. Other strategies include the infusion of donor natural killer (NK) cells, T cells sensitized against patient leukemia cells, or vaccination approaches.

GVHD

GVHD is common after allogeneic HCT for MDS or MPN. The incidence of acute GVHD has been reported in the range 25–70%, typically somewhat higher for unrelated than for related donor HCT, and chronic GVHD occurs in a similar proportion of patients. The incidence is higher with the use of PBPCs than with marrow, not only for related but also for unrelated donor HCT [10]. Although survival rates have been similar, the impact of chronic GVHD on quality of life cannot be neglected.

Conclusions and perspective

The past 15 years have seen major developments with regard to optimizing conditioning regimens with the goal of reducing regimen-related toxicity and mortality without increasing the risk of relapse. One basic principle has been the reduction of cytotoxic intensity in favor of regimens focused on immunosuppression, sufficient to allow donor cells to become established and rely on donor cell-mediated allo-immune effects to eradicate the patient's disease. With this strategy, it is essential to consider both pre- and post-HCT treatment modalities in the overall approach to HCT (e.g., patients with MDS who have received little or no cytotoxic therapy before HCT may be at higher risk of graft failure and disease recurrence than patients with AML who received aggressive induction chemotherapy) (Figure 54.7) [61,74].

HCT is the only currently available treatment with curative potential for patients with MDS and MPN. The prognostic scoring systems discussed here are helpful in deciding which patients should be transplanted and provide a guide to the optimum timing. Results from controlled prospective trials comparing different intensity regimens are currently not available, although one such study is under way in patients with AML or MDS and l<5% marrow myeloblasts at the time of HCT (BMT CTN protocol 0903). Retrospective studies have generally failed to show significant differences between results obtained with different intensity conditioning regimens, possibly related to the fact that the selection of a conditioning regimen was subject to selection bias. Typically, older patients who appear biologically younger and have no major comorbid conditions will undergo HCT. Also, often the response to pre-HCT therapy will be a factor leading to the decision to proceed to HCT whereas patients whose disease shows no response will not.

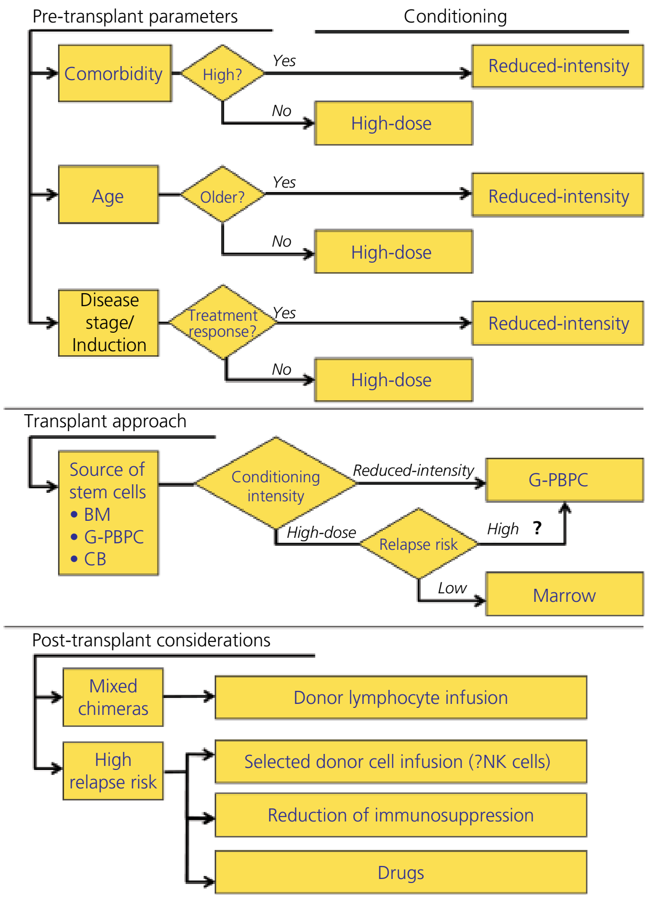

Figure 54.7 HCT for patients with MDS and MPD is an evolving field. This algorithm provides some guidance regarding consideration of patient age, comorbid conditions, disease stage (and the possible advisability of induction therapy) when deciding upon a conditioning regimen. For patients with high-risk disease and with reduced-intensity/non-myeloablative transplant regimens, G-CSF-mobilized peripheral blood progenitor cells (G-PBPCs) are currently the preferred source of stem cells. Post-transplant manipulations depend upon the type of transplant, the degree of donor cell chimerism that is achieved, and the remission status. BM, bone marrow; CB, cord blood; NK, natural killer.

We need novel strategies to overcome the hurdle of high-risk cytogenetics; targeted approaches with radioimmunotherapy may be such a modality. As multiple genomic mutations in clonal hematopoietic precursors are being identified in MDS and MPN, these mutations are likely to be incorporated into new classification schemes. Further, the impact of these mutations on transplant outcome will need to be determined and therapeutic compounds targeting the pathways affected by these mutations will have to be developed. As new agents for non-HCT therapy become available, the question of timing of HCT will have to be readdressed [120].

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CHAPTER 55

Hematopoietic Cell Transplantation for Multiple Myeloma

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General aspects of myeloma

Epidemiology and etiology of myeloma

Multiple myeloma (MM) is a clonal disorder of plasma cells affecting approximately 50,000 people in the United States, with an annual incidence of about 15,000 new cases. Median age of onset is 72 years, and it is rarely seen in patients under the age of 40 years. Men are slightly more commonly affected than women, and African-Americans have nearly twice the incidence of Caucasians. Thus MM represents a relatively common hematologic disorder and has emerged as the most common indication for stem-cell transplantation (SCT) today in most of the world [1,2].

Although the causes of myeloma are not known, there is evidence that the pathogenesis of myeloma is a multistep process. The disease often evolves from a premalignant condition called monoclonal gammopathy of undetermined significance (MGUS) [3]. Clonal plasma cell proliferation in MGUS is triggered by translocations involving the immunoglobulin heavy chain (IgH) locus on chromosome 14q32 [4]. Secondary genetic abnormalities involving other oncogenes and tumor suppressor genes (e.g., *ras*, *p53*, *RB-1*) and changes in the bone marrow microenvironment are believed to play a role in the evolution of MGUS into MM [4,5]. No common molecular pathway for transformation has yet been described [6].

Molecular and cellular biology

The malignant plasma cell migrates from the germinal centers of the lymph nodes to the bone marrow, which provides a microenvironment conducive to terminal plasma cell differentiation [6,7]. Malignant transformation in MM appears to start with genetic abnormalities late in B-cell differentiation [8–10]. The presence of genetic abnormalities have been demonstrated by fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH), and gene expression profiling (GEP) in most patients with MM [10].

The evolution from normal to malignant plasma cells has been extensively reviewed elsewhere [11]. In brief, in an early stage of MGUS or smoldering MM, critical chromosomal abnormalities may occur, leading to one of the five known IgH translocations.The five recurrent IgH translocations seen in MGUS and MM involve these chromosomal partners: 4p16, 6p21, 11q13, 16q23, and 20q11. The identified genes include cyclin D1 and other growth factors (11q), the fibroblast growth factor receptor 3 (4p), the basic zipper C-MAF transcription factor (16q), and interferon (IFN) regulatory factor 4 (6p). The IgH translocation increases the expression of an oncogene that is positioned near a strong immunoglobulin enhancer. The malignant clone derived from the transformed cell is capable of further differentiation and division. During the evolution of MM to advanced stages, additional genetic events, including loss of chromosome 13, activation of other oncogenes such as *Ras*, loss of tumor suppressor gene *p53* and *Rb*, and dysregulation of the cell cycle may take place. Once in the bone marrow, myeloma and stromal cells generate a supportive microenvironment with a network of cytokines and adhesion molecules that promote MM proliferation and perpetuation by promoting growth and preventing apoptosis. Increased osteoclast activation, likely mediated by increased levels of macrophage inflammatory protein 1 (MIP-1) and receptor activator of nuclear factor kappa B ligand (RANKL), and decreased levels of osteoprotegerin (OPG), the decoy receptor for RANKL, results in lytic bone lesions and osteoporosis [11].

Clinical description

Bone pain affects approximately 70% of all myeloma patients [12], the pain generally being localized to the back and ribs. The bone lesions of myeloma are caused by the proliferation of tumor cells and the activation of osteoclasts that destroy the bone. The bone destruction results in severe bone pain, pathologic fractures, hypercalcemia, and nerve compression syndromes. A unique feature of myeloma bone lesions is that the lesions rarely heal even when the patient is in complete remission (CR) [12–14].

Recurrent bacterial infections constitute another common clinical problem in myeloma. The most common infections are pneumonias and pyelonephritis, and the most frequent pathogens are *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* in the lungs and *Escherichia coli* and other Gram-negative organisms in the urinary tract. The susceptibility to infection has several contributing causes, including suppression of normal antibody synthesis, poor antibody response to polysaccharide antigens, and abnormalities in granulocyte and complement functions [15].

The serum creatinine level is elevated in almost 50% of patients and renal failure is seen in about 25% of patients. Many factors contribute to renal dysfunction, including hypercalcemia, hyperuricemia, amyloid deposition, and recurrent infections. However, tubular damage associated with light-chain excretion is the predominant cause [12,16]. Anemia is present in 80% of patients at diagnosis and mostly is independent of renal function [12]. It is

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usually normocytic and normochromic and related both to the replacement of normal marrow by expanding tumor cells and to the inhibition of hematopoiesis by factors secreted by the tumor.

The diagnosis of symptomatic MM requires the presence of clonal plasma cells on bone marrow examination or biopsy-proven plasmacytoma, monoclonal (M) protein in the serum and/or urine, and evidence of end-organ damage (hypercalcemia, renal insufficiency, anemia, or bone lesions) believed secondary to the underlying plasma cell disorder [17].

Conventional radiography shows skeletal abnormalities in approximately 80% of patients. With magnetic resonance imaging (MRI), it is possible to determine the extent of marrow infiltration in the axial skeleton despite having normal plain radiological examinations [18]. Positron emission tomography (PET) is now also being used in the evaluation of myeloma as it has improved sensitivity over skeletal X-rays. The M protein can be detected by serum protein electrophoresis in >80% of patients and by immunofixation in >90% [12,19]. Up to 20% of patients with MM are considered to have light-chain MM. The M protein in these patients is always detected in urine but can be absent in serum, even by immunofixation.

Table 55.1 New International Staging System for myeloma

Stage	Criteria	Median survival (months)
I	Serum β_2-microglobulin <3.5mg/L Serum albumin ≥3.5g/dL	62
II	Not stage I or III[a]	44
III	Serum β_2-microglobulin ≥5.5mg/L	29

[a] There are two categories for stage II: serum β_2-microglobulin <3.5mg/L but serum albumin <3.5g/dL, and serum β_2-microglobulin 3.5 to <5.5mg/L irrespective of serum albumin level.

Source: Rajkumar et al. 2010 [46]. Reproduced with permission of Elsevier.

Among patients with MM, 3% have no detectable M protein on serum or urine immunofixation and are considered to have non-secretory MM. In these patients, the serum free light chain (FLC) assay, which measures the level of free (unbound) kappa and lambda light chains in the serum, is helpful for diagnosing and monitoring response to therapy. The FLC kappa/lambda ratio is used to distinguish polyclonal elevations seen with renal dysfunction from monoclonal elevations that occur in clonal plasma cell disorders [20,21].

Staging, prognosis, and response

Median survival has traditionally been quoted as approximately 3 years. However, improvements in therapies and supportive care have dramatically improved the prognosis.[22]. Survival depends on both tumor burden and intrinsic biologic features. Tumor burden has traditionally been measured using the Durie–Salmon staging system. This staging system has limitations, especially in the categorization of bone lesions [23]. The more recent International Staging System (ISS), which was a collaborative effort by investigators from 17 institutions worldwide based on data from more than 11,000 patients, overcomes the limitations of the Durie–Salmon staging and divides patients into three distinct stages and prognostic groups on the basis of serum β_2-microglobulin and albumin levels (Table 55.1) [24]. Selected clonal chromosomal abnormalities have also emerged as adverse prognostic markers. These include hypodiploidy, deletions involving chromosomes 1, 13s and 17, and translocations involving IgH locus on chromosome 14 [25].

New uniform response criteria are required to assess clinical outcomes in myeloma adequately. Table 55.2 shows the European

Table 55.2 Definitions for response categories

Response subcategory	Response criteria[a]
CR	Negative immunofixation on the serum and urine and Disappearance of any soft tissue plasmacytomas and ≤5% plasma cells in bone marrow [b]
sCR	CR as defined above plus Normal FLC ratio and Absence of clonal cells in bone marrow [b] by immunohistochemistry or immunofluorescence [c]
VGPR	Serum and urine M-component detectable by immunofixation but not on electrophoresis or 90% or greater reduction in serum M-component plus urine M-component <100mg per 24 h
PR	≥50% reduction of serum M-protein and reduction in 24-h urinary M-protein by ≥90% or to <200mg per 24h If the serum and urine M-protein are unmeasurable [d], a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria If serum and urine M-protein are unmeasurable, and serum free light assay is also unmeasurable, 50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma cell percentage was 30% In addition to the above criteria, if present at baseline, a 50% reduction in the size of soft tissue plasmacytomas is also required
SD (not recommended for use as an indicator of response; stability of disease is best described by providing the time to progression estimates)	Not meeting criteria for CR, VGPR, PR, or progressive disease

CR, complete response; PR, partial response; sCR, stringent complete response; SD, stable disease; VGPR, very good partial response; FLC, free light chain.

[a] All response categories require two consecutive assessments made at any time before the institution of any new therapy; complete and PR and SD categories also require no known evidence of progressive or new bone lesions if radiographic studies were performed. Radiographic studies are not required to satisfy these response requirements.

[b] Confirmation with repeat bone marrow biopsy not needed.

[c] Presence/absence of clonal cells is based upon the k/λ ratio. An abnormal k/λ ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is k/λ of >4:1 or <1:2. Alternatively, the absence of clonal plasma cells can be defined based on the investigation of phenotypically aberrant plasma cells (PCs). The sensitivity level is 10^{-3} (less than one phenotypically aberrant PC within a total of 1000 PCs). Examples of aberrant phenotypes include (1) $CD38^{+dim}$ and $CD56^{+strong}$ and $CD19^{-}$ and $CD45^{-}$; (2) $CD38^{+dim}$ and $CD138^{+}$ and $CD56^{++}$ and $CD28^{+}$; (3) $CD138^{+}$, $CD19^{-}$ $CD56^{++}$, $CD117^{+}$.

[d] *Definitions of measurable disease*

Response criteria for all categories and subcategories of response except CR are applicable only to patients who have "measurable" disease defined by at least one of the following three measurements:

Serum M-protein ×1g/dL (×10gm/L) [10 g/L];

Urine M-protein ×200mg/24h;

Serum FLC assay: involved FLC level ×10mg/dL (×100mg/L) provided serum FLC ratio is abnormal.

Source: Durie et al. 2006 [26]. Reproduced with permission of Nature Publishing Group.

Group for Blood and Marrow Transplantation/International Bone Marrow Transplant Registry (EBMT/IBMTR) criteria that have been expanded, clarified, and updated to provide an improved comprehensive evaluation system [26].

Myeloma therapy

Importance of achieving a complete response

Achievement of a complete remission as defined by the International Myeloma Working Group (IMWG) criteria has emerged as the single most important surrogate prognostic factor for survival [27]. A meta-analysis of 21 studies demonstrated that maximal response was associated with overall survival (OS) and event-free survival (EFS) benefit [28]. Likewise, Martinez-Lopez et al. demonstrated that outcomes were significantly better for patients achieving a CR compared with those who only achieved a very good partial remission (VGPR) or a near CR [29]. CR does not imply eradication of the malignant clone. Sensitive techniques such as flow cytometry and polymerase chain reaction (PCR) routinely show significant residual disease in patients achieving CR by IMWG criteria [30,31]. Reduction of tumor burden as documented by multiparameter flow cytometry has been shown to be an important prognostic factor for progression-free survival (PFS) (71 months vs. 37 months) among patients treated uniformly on the GEM2000 protocol [32,33]. CR duration is emerging as an important prognostic factor for survival. Hoering et al. reported that for patients with CR durations of more than 3 years the 10-year survival estimate was 54% versus 17% for patients losing their CR within 3 years and 35% for patients never achieving a CR [34].

Therapeutic approaches for SCT-eligible patients

Only patients with symptomatic myeloma require initiation of therapy. Once a decision has been made to initiate therapy, a decision should be made whether a patient is a potential candidate for an autologous SCT. Age by itself can no longer be considered a criterion for transplant eligibility since most centers can perform autologous SCT safely in patients in their 70s and 80s if physically fit and medically stable [35–39]. Therapy for myeloma is commonly divided into three phases: induction, consolidation, and maintenance. For transplant-eligible patients, it is important to avoid treatment with alkylating agents such as melphalan that are toxic to progenitor cells, and may interfere with adequate hematopoietic cell (HC) mobilization and collection [40–42].

Induction therapy

Goals of induction therapy in the transplant-eligible patient are based on evidence demonstrating an association between depth of response and PFS and, more importantly, OS. Prior to the advent of immunomodulatory drugs (IMIDs) and proteosome inhibitors, the superior survival seen with high-dose chemotherapy and autologous ASCT was related to the higher CR rates seen with high-dose therapy [43]. Depth of response prior to transplant, that is, after induction therapy, can improve PFS and possibly OS post-transplant [44]. However, it is not only the achievement of a CR but also the duration of CR that is important in long-term survival. Patients with high-risk disease, classically defined by cytogenetics, can achieve rapid responses with modern chemotherapy regimens, but these remissions are often short lived [34]. Hence the heterogeneity of myeloma underscores the challenges of treatment decisions for induction, transplant, and post-transplant therapy.

The combinations of vincristine, adriamycin, and dexamethasone (VAD) or thalidomide and dexamethasone were considered for many years the gold standards for induction therapy for transplant-eligible patients [45]. Lenalidomide has a more favorable toxicity profile than thalidomide, which made it a more attractive induction choice. The ECOG trial of lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone (40mg weekly) confirmed the safety and low toxicity of both the choice of lenalidomide as induction and the improved safety profile of lowering the dexamethasone dose [46]. One-year survival was over 90% in both older and younger patients in the low-dose dexamethasone arm. This led to a major paradigm shift in the United States with regard to both the dexamethasone dose and s the use of lenalidomide upfront preferentially over thalidomide.

For certain subgroups of patients such as those with renal insufficiency or specific cytogenetic abnormalities such as t(4;14), bortezomib-based induction may be a more favorable option. Several large randomized trials have studied bortezomib-based induction compared with traditional regimens. The IFM 2005 trial compared bortezomib plus dexamethasone with VAD followed by autologous SCT. The primary trial end-point was complete/near complete response (nCR) rate. The bortezomib arm had a CR/nCR rate of 14.8% compared with 6.7% in the VAD arm. There was also a non-significant trend towards a longer PFS in the bortezomib arm [47]. Analysis of patients with t(4;14) by retrospective comparison of bortezomib plus dexamethasone-treated patients including those from the IFM 2005 trial compared with a similar group induced with VAD showed a superior EFS of 28 months for the bortezomib-treated patients compared with 16 months for VAD-treated patients [48].

Three-drug combinations are also an induction choice for patients. The most common regimens are VTD (Velcade/bortezomib–thalidomide–dexamethasone), RVD (Revlimid/lenalidomide–Velcade/bortezomib–dexamethasone), and CyBord (cyclophosphamide–bortezomib–dexamethasone). Of these, only VTD has phase III results in comparison with a two-drug combination. A study of VTD versus TD (thalidomide–dexamethasone) as induction and consolidation post-autologous ASCT showed a better response rate and PFS with the three-drug arm but no overall survival advantage [49]. The recently completed SWOG S0777 trial of lenalidomide plus low-dose dexamethasone versus RVD will also provide further evidence either for or against a three-drug regimen. The Evolution trial, a randomized phase II trial of RVD versus VCD (Velcade/bortezomib–cyclophosphamide–dexamethasone) versus VDCR (Velcade/bortezomib–dexamethasone–cyclophosphamide–Revlimid/lenalidomide) showed comparable VGPR/CR rates and 1-year PFS for the VCD and RVD arms and the VDCR arm [50].

In addition to efficacy, toxicity must also remain a consideration when choosing a regimen, This applies to patient comorbidities present at the start of induction and also the potential for long-term toxicity. For example, bortezomib can produce severe peripheral neuropathy. This risk is greater in patients with underlying neuropathy such as diabetics, and also in older patients. Hence the choice of a bortezomib-based induction must weigh this risk in addition to consideration of myeloma-related factors such as cytogenetics The use of alternative bortezomib schedules such as weekly dosing and alternative routes such as subcutaneous administration have also been shown to reduce the incidence of neuropathy. Hence this provides further options to patients at risk for neuropathy [51–53]. One advantage of bortezomib is that it does not require dose reduction in the setting of renal insufficiency, which makes it an attractive choice for renally impaired patients. In addition, there is no concern about stem-cell yields with long-term bortezomib use.

IMIDs, on the other hand, offer the advantage of an oral regimen. In the case of lenalidomide, the incidence of neurotoxicity is low. The ECOG E4A03 trial underscores the high tolerability and excellent long-term survival of lenalidomide upfront when combined with low-dose dexamethasone. However, because of the thrombotic risk of IMIDs, all patients require antithrombotic prophylaxis – this is generally with aspirin in low-risk patients, but in patients at high risk for thrombosis either low molecular weight heparin or full-dose warfarin is required [54]. The other concern with lenalidomide in particular is its effect on stem-cell yields. Retrospective studies have raised the concern of impaired stem-cell collection from patients who have been on long term lenalidomide. This may be overcome to some extent by using either chemotherapy mobilization or plerixafor, a receptor chemokine receptor 4 (CXCR4) inhibitor. This led to the IMWG consensus recommendations that stem cells be collected with cyclophosphamide plus granulocyte colony-stimulating factor (G-CSF) for patients with over 4 months or more of lenalidomide exposure, and for patients over 65 years of age cyclophosphamide or plerixafor plus G-CSF-based priming should be considered [42].

In general, patients are treated with three or four cycles of an induction regimen before proceeding to transplant. The optimal number of cycles prior to transplantation is unknown. Although all of the aforementioned regimens have high response rates, the depth of response deepens with continued therapy [55]. For example in the phaseI/II trial of RVD, the quality of response continued to improve between cycles 4 and 8 [56]. Although it would theoretically be ideal to achieve a minimal residual disease state prior to stem-cell transplantation, the implications of delaying SCT until a plateau have never been studied. With the advent of more potent induction regimens, this may be less of a controversy. Jakubowiak et al. recently reported that the combination of a new proteasome inhibitor, carfilzomib, together with lenalidomide and dexamethasone resulted in an nCR rate of 62% with a stringent CR rate of 42%, with major responses achieved within 6 months of initiation of therapy [57]. In summary, induction therapy with a combination of an IMID and a proteosome inhibitor has become the standard of care for myeloma patients, replacing thalidomide and dexametahsone and also VAD.

Autologous stem-cell transplantation for myeloma

High-dose melphalan without autologous hematopoietic cell transplantation (HCT) was first reported in 1983 by McElwain et al. at the Royal Marsden Hospital [58]. Patients received 100–140mg/m^2 of melphalan, and three previously untreated patients achieved a CR. High-dose therapy with autologous HCT to reduce the regimen-related toxicity was first reported by Barlogie et al. in 1986 [59]. They used up to 140mg/m^2 of melphalan intravenously. Over the next 25 years, high-dose therapy and autologous HCT evolved into a safe and common therapeutic approach for patients with MM. Compared with chemotherapy alone, intensified chemotherapy followed by autologous HCT has been shown to prolong both EFS and OS in previously untreated patients with myeloma. Various randomized trials and non-randomized analyses have shown both survival and PFS benefits in favor of autologous HCT of approximately 12 months (reviewed in [60,61]). All of these studies are now considered historical since none of them included modern induction therapy with a proteosome inhibitor and an IMID [35,62]. Hence the role of high-dose therapy with autologous SCT as consolidation for all patients with myeloma needs to be re-explored. Three randomized trials are looking at this question and their results are eagerly awaited [55].

Preparative regimens

Many different preparative regimens have been assessed over the last 25 years. Only one prospective, randomized trial by a French Cooperative group has directly compared two different preparative regimens. In 282 newly diagnosed symptomatic patients <65 years of age, they reported that high-dose melphalan at 200mg/m^2 is superior to a combination of melphalan 140mg/m^2 + 8Gy of total body irradiation (TBI) mainly by reducing toxicity including mucositis and transplant-related mortality (TRM) [63]. Melphalan 200mg/m^2 was associated with a faster hematologic recovery, reduced transfusion requirements, and a shorter hospitalization. Although the median duration of EFS was similar in both arms (21 months), survival at 45 months was significantly better in the melphalan-alone arm (66% vs. 46%, $p = 0.05$). As a result, melphalan 200mg/m^2 is the standard conditioning regimen used for autologous HCT in myeloma, with dose reductions based on age and renal function. In two non-randomized studies and a registry analysis, use of more intensive preparative regimens, such as busulfan with melphalan [64], thioTEPA (*N*-triethylenethiophosphoramide), busulfan, and cyclophosphamide [65] or high-dose idarubicin, cyclophosphamide, and melphalan [66] did not result in better outcomes than melphalan at a dose of 200mg/m^2. Recently, Blanes et al. compared SCT outcomes for 51 newly diagnosed patients with MM who received intravenous busulfan in combination with melphalan with the outcomes for 102 patients receiving melphalan 200mg/m^2 in a 1:2 matched control analysis. After a median follow-up of 63 and 50 months in control and IV busulfan, the median PFS was 24 and 33 months ,respectively [67]. Other recent work has focused on combining proteasome inhibitors with melphalan. Lonial's group demonstrated that the combination was feasible – in a group of 39 patients the response rate was 87% with 51% achieving a VGPR or greater [68]. In addition, pharmacodynamic studies demonstrated that sequencing of the drug may be important with greater plasma cell apoptosis seen in patients who received bortezomib after the high dose melphalan.

Relapse after autologous HCT remains the single most important cause of treatment failure in patients. Strategies aimed at improving the conditioning regimen have usually focused on intensifying the conditioning regimen either by increasing the dose of melphalan or by adding other alkylating agents, as summarized in Table 55.3. Of these, only tandem high-dose therapy has been reported to improve outcomes in randomized trials [69–72]. This benefit is limited because many patients do not want to undergo tandem transplantation, and it is possible that only patients failing to achieve a greater than 90% reduction of their myeloma burden after the first high-dose therapy benefit from a second intensification.

Various groups have explored dose intensification of melphalan with or without cytoprotectors such as amifostine. Phillips et al. demonstrated the feasibility of administering up to 280mg/m^2 of melphalan with amifostine. Significant mucosal toxicities were observed, and at the higher doses cardiotoxicity was dose limiting [73]. Moreau et al. explored the use of melphalan at a dose of 220mg/m^2 as part of a tandem transplantation strategy for patients with high-risk myeloma as defined by cytogenetics and β_2-microglobulin. CRs were seen in 30% of patients, with a further 18% achieving at least a 90% reduction in tumor burden [74]. To date, neither melphalan dose intensification nor the addition of

Table 55.3 Randomized trials addressing autologous stem cell transplant issues in myeloma

Ref.	*n*	Age limit (years)	Complete remission (%)	Median event-free survival (months)	Median overall survival (months)
[91]	200	≤65	5 vs. 22	18 vs. 27	37 vs. NR
[92]	401	<65	8 vs. 44	19 vs. 31	42 vs. 54
[93]	191	55–65	5 vs. 10	19 vs. 25	47.6 vs. 47.8
[94]	164	≤65	11 vs. 30	33 vs. 42	66 vs. 61
[95]	516	≤70	15 vs. 17	7-year event-free survival: 14 vs. 17%	7-year overall survival: 38% vs. 38%
[98,99]	399	60	42	25	48
Single			50	30	58
Tandem					
[100]	220	60	35	22	59
Single			48	35	73
Tandem					
[104]	597	<60	55–57(+ VGPR)	37	87% @ 4 years
No Thal			67 (+VGPR)	28	75% @ 4 years
Thal					
[105]	668	75	43	60	NR
No Thal			62	NR	NR
Thal					
[107]	557	<65	NS	23	75% @ 3 years
No Thal			NS	30	80% @ 3 years
Thal					
[108,109]	556	<60	54 (+VGPR)	22	60
IFN			66 (+VGPR)	34	73
Thal					
[110]	243	<70	44 (+VGPR)	18	79% @ 1 year
No Thal			65 (+VGPR)	32	77% @ 1 year
Thal					
[116]	827	<65	15	28	55% @ 5 years
VAD			31	35	61% @ 5 years
PAD					
[49]	480	<65	49	56% @ 3 years	84% @ 3 years
TD			34	68% @ 3 years	86% @ 3 years
VTD					
[123]	322	<65	46.6	32 months	88% @ 3 years
TD			60	60% @ 3 years (median NR)	90% @ 3 years
VTD					
[112]	614	<65	27	23	84% @ 3 years
Placebo			29	41	80% @ 3 years
Len					
[113]	460	<70	NS	27	80% @ 3 years
Placebo			NS	46	88% @ 3 years
Len					

NR, not reached; NS, not stated; Len, lenalidomide: Thal, thalidomide; for other abbreviations, see text.

other alkylating agents has resulted in large increases in post-transplantation CR rates, and novel conditioning regimens exploring different mechanisms of enhancing the antitumor effect of high-dose melphalan need to be further explored.

It is now well established that the interactions between malignant plasma cells and the bone marrow microenvironment that includes stromal cells, extracellular adhesion molecules, and secreted cytokines are important contributors to myeloma resistance and disease recurrence [11]. Novel therapies targeting these interactions have shown promising responses and outcomes. Bortezomib [68,75,76] and targeted skeletal radiotherapy with either holmium DOTMP (1,4,7,10-tetraazcyclododecane-1,4,7,10-tetramethylenephosphonate) or samarium EDTMP [ethylenediaminetetra(methylenephosphonic acid)] [77,78] have been studied as part of the conditioning regimen, with encouraging phase II results that need to be confirmed in larger phase III trials. Of these, bortezomib–melphalan combinations are being intensely explored. Roussel et al. reported on 54 patients who received bortezomib 1mg/m^2 on days –6, –3, +1, and +4 in addition to melphalan 200mg/m^2. VGPR and CR rates were 70% and 32%, respectively, with no increases in toxicity. Compared with a matched control group treated with high-dose melphalan alone, the CR rates were higher (35% vs. 11%) [76]. Lonial et al. performed a randomized phase I/II trial among patients who failed to achieve a VGPR after one or more induction regimens. Of the 39 patients randomized, 20 received bortezomib after melphalan and 19 received bortezomib before melphalan. The overall response rate for all patients was 87%, with 51% achieving a VGPR or better. Pharmacodynamic studies showed greater plasma cell apoptosis among patients who received bortezomib following melphalan [68]. Taken together, these studies demonstrate that adding bortezomib to high-dose melphalan is feasible but that randomized trials will be needed to demonstrate whether the combination is better than melphalan alone.

The tolerance to high-dose melphalan is extremely heterogeneous, with some patients exhibiting no symptoms whereas others are affected by serious life-threatening toxicities. Even without severe toxicities, patient-reported outcomes vary significantly, as found by the M.D. Anderson group [79]. To a certain extent, this variability is probably due to genetic polymorphisms among enzymes such as glutathione-*S*-transferase [80]. However, the practice of dosing melphalan according to body surface area, with arbitrary modifications for overweight patients, also suggests that melphalan dosing may be implicated, as reported by Grazziutti et al. [81]. Nath et al. reported that current dosing results in a 4.3-fold variation in exposure, with AUC$_{0-\infty}$ values ranging from 5.8 to 24.7mg/L/h (median: 12.7mg/L/h). This variation probably impacts toxicity and myeloma responses [82].

Lastly, Dimopoulos et al. demonstrated that the extent of damage and repair seen in the *p53* tumor suppressor gene of peripheral blood lymphocytes after exposure to melphalan is an important predictor of outcome, and could serve as a tool for developing a personalized or individually tailored conditioning regimen [83].

Patient selection

Older age

Most centers excluded patients over the age of 70 years, because of the potentially greater risk of regimen-related toxicities. However, advances in supportive care have made autologous HCT feasible in older patients. In a study of patients undergoing tandem autologous HCT, 49 patients aged 65 years or older were compared with 49 pair-mates matched for prognostically relevant disease drawn from 501 younger patients, CR duration, EFS, and OS were comparable in the two groups [36]. The prognosis was primarily determined by presence of unfavorable cytogenetics (11q breakpoints, monosomy 13 or deletion 13q, or any translocation), and serum β_2-microglobulin level of over 2.5mg/L. The authors concluded that age should not constitute a criterion for exclusion from high-dose regimens. Similar results were obtained in three other retrospective analyses, including a large registry analysis from the CIBMTR [37–39].

Renal failure

Although renal failure increases the morbidity and mortality of myeloma, at least two reports have shown that high-dose therapy and autologous HCT are feasible in patients with concurrent myeloma and renal failure. Renal failure apparently has no adverse effect on either the quality of hematopoietic cell (HC) collection or engraftment following autologous HCT, and the toxicity is acceptable, with survival comparable to that of other patients [84,85]. In a study of patients with MM and a serum creatinine level over 2.0 mg/dL undergoing autologous HCT, the median OS for the entire group of 81 patients was longer than 53 months, and was more than 51 months for the 38 patients who were dialysis dependent. In this study, only two dialysis patients recovered partial renal function post-transplantation [84]. In a second study, four of 17 evaluable patients became independent of dialysis at a median time of 5 months post-transplantation [85]. Patients on dialysis undergoing autologous SCT should be considered for a lower dose of melphalan (usually 140mg/m^2) owing to increased risk of serious adverse events with 200mg/m^2 in this population.

Stem-cell source

Peripheral blood hematopoietic cells (PBHCs) are preferred over bone marrow cells for transplantation because of rapid and predictable engraftment, and possibly less contamination of the graft with tumor cells [61]. The absolute number of CD34$^+$ cells/kg recipient weight is the most reliable and reproducible method for determining the dose of the HC product. PBHCs should be collected before the patient is exposed to alkylating agents. The current practice at many transplant centers is to harvest enough HCs for two transplantations; a single transplantation is performed with half of the collected cells, and the other half is cryopreserved. The minimum recommended CD34$^+$ dose to support one autograft is 2×10^6 CD34$^+$ cells/kg recipient weight; patients receiving 5×10^6 CD34/kg or more have more rapid platelet recovery [86]. Although retrospective analysis has shown that the risk of relapse is lower for recipients of syngeneic transplants than for autograft recipients, randomized trials of CD34 selection of autografts as a strategy for purging failed to show improved outcomes [87–90].

Current role of autologous HCT in myeloma

Since 1995, more than a dozen randomized trials have been performed addressing transplant-specific issues in myeloma. The most representative of these trials are summarized in Table 55.3. The first generation of randomized trials started with the IFM 90 trial. In this trial, high-dose chemotherapy supported by autologous bone marrow transplantation (BMT) was compared with conventional chemotherapy in 200 previously untreated patients less than 65 years of age with myeloma [91]. The results showed a higher CR rate (22% vs. 5%), a higher rate of 5-year EFS (28% vs. 10%), and greater OS (52% vs. 12%) in the autologous BMT group. There was a 13 months' longer median duration of OS in patients assigned to the BMT arm (57 months vs. 44 months) and improved median EFS (from 18 to 27 months). Soon afterwards, the the Medical Research Council Myeloma VII trial randomized 401 previously untreated patients less than 65 years of age with myeloma, with similar outcomes to the IFM 90 trial. CR rates were higher in the autologous HCT group (44% vs. 8%; 19 months vs. 31 months) [92]. Three other randomized studies, however, have not shown a survival benefit of autologous HCT. Comparison between these trials is difficult owing to variability in patient eligibility, including age, induction chemotherapy, the conditioning regimen for the HCT, and definitions of response [93–95].

Barlogie and co-workers were the first to report on the potential role of tandem SCT in the late 1990s. Among 231 patients with newly diagnosed MM who received tandem autologous HCT, second transplantations were performed in 71%. After the first autologous HCT, 41% achieved CR and 83% achieved CR or partial remission (PR). These numbers were increased to 51% CR and 95% CR or PR in patients completing a second transplantation. Median duration of remission in complete responders was 50 months. In an updated analysis, 15-year OS and disease-free survival (DFS) were 17 and 7%, respectively, and having received a tandem SCT was an important variable for survival in multivariate analysis [96,97]. Based on these results, the next generation of randomized trials explored the role of tandem versus single high-dose therapy as consolidation for upfront myeloma therapy. The IFM 95 trial randomized 399 previously untreated patients to a single versus a tandem SCT strategy. Long-term outcomes demonstrated a significantly improved 7-year EFS (20% vs. 10%) and OS (42% vs. 21%) in recipients of double versus single autologous HCT [98,99]. Cavo et al. reported on 321 patients randomly assigned to receive either a single course of high-dose melphalan at 200mg/m^2 or melphalan at 200mg/m^2 followed, after 3–6 months, by melphalan at 120mg/m^2 and busulfan at 12mg/kg [100]. Patients in the tandem autologous HCT arm had a significantly increased probability of attaining at least an nCR (33% vs. 47%, respectively; $p = 0.008$), prolonged relapse-free survival (median 24 versus 42 months, respectively; $p < 0.001$), and prolonged EFS (median 23 vs. 35 months, respectively; $p = 0.001$). OS, however, was not prolonged in this study, possibly because 50% of patients randomly assigned to receive a single autologous HCT received an autologous SCT as salvage and the appearance of novel agents such as bortezomib and thalidomide [100]. Thus, by 2005, high-dose melphalan with autologous SCT had emerged as the most effective strategy to obtain CRs in myeloma and was considered an established standard of care compared with the conventional chemotherapy regimens of the time [61]. However, with modern induction regimens resulting in CR rates of 30% or greater, the role of upfront high-dose therapy for all patients with myeloma is currently being readdressed through various large randomized trials [55,101,102].

The emerging role of maintenance therapy post-SCT

Improvements in induction therapy have led to higher response rates, and also improved PFS post autologous stem-cell transplantation. However, the majority of patients with myeloma will relapse post-SCT. Strategies to improve relapse include consolidation therapy and maintenance therapy. The definitionsof and line between consolidation and maintenance can be rather blurred. Generally, consolidation is thought of as a finite course of therapy administered relatively early post-transplant with the aim of deepening the response achieved with the transplant ideally to a minimal residual disease state. Maintenance is usually a less intense therapy but administered for longer periods, often in fact until disease progression. Early maintenance trials used interferon, with mixed results. It was poorly tolerated and on meta-analysis led to an approximately 6-month improvement in long-term survival [103]. The IFM 99-02 study randomized patients after tandem autologous transplant to thalidomide plus pamidronate, pamidronate alone, or observation [104]. An improvement in EFS and OS was seen in the thalidomide arm; however, this benefit was restricted to those with less than a VGPR post-transplant, without deletion 13 or high β_2-microglobulin. Barlogie and co-workers demonstrated that the impact of thalidomide maintenance may not be seen early on but only after long-term follow up [105,106]. The MRCIX trial demonstrated a significant PFS but not OS benefit to thalidomide maintenance [107]. The HOVON 50 study randomized patients from induction to a thalidomide arm and a thalidomide, adriamycin, and dexamethasone (TAD) versus VAD arm. Both arms then underwent single or tandem autologous transplant. The TAD arm went on to receive thalidomide maintenance and the VAD arm interferon maintenance. The TAD arm had a longer EFS (33 months vs. 22 months) and a non-significant trend to improved OS (62 months vs. 59 months). However, since thalidomide was included as part of induction, it is difficult to sort out what benefit this played in PFS post-transplant versus the maintenance thalidomide [108].

A meta analysis by the IMWG corroborated the finding of a significant reduction in risk of progression in patients on thalidomide maintenance [110]. However, the analysis revealed variability with regard to an vOS benefit (test for heterogeneity 0.03), although with a positive effect. The trialists explanation for this variability included the facts that elderly patients were part of two trials and the variability of the availability of novel agents at relapse depended on the country. It is clear from all trials that long-term thalidomide use is difficult owing to its toxicity profile, especially the cumulative peripheral neuropathy. Nonetheless, in certain countries where other novel agents are not available in the maintenance setting, the use of thalidomide maintenance in patients without deletion 13 can be considered. The optimal duration of use remains unknown. There was wide variability in the duration of thalidomide use in the many trials, in part due to patient tolerance of the drug. In the IFM 99-02 trial, the median duration of therapy was 15 months versus 7 months in the MRCIX trial.

Lenalidomide, with its more favorable side-effect profile, is a more attractive choice for maintenance. Two randomized trials have been completed, evaluating the benefit of lenalidomide post-autologous transplant versus observation. In the IFM 2005-01 trial, all patients received two cycles of lenalidomide consolidation at a dose of 25mg/day for 21 days, and subsequently they were placed on either placebo or lenalidomide until disease progression. Interim analysis demonstrated a significant benefit to the lenalidomide arm and the study was therefore unblinded, and no crossover from placebo was allowed [111]. There was a significant upgrading of response, with CR rates increasing from 14 to 20% in the lenalidomide arm ($p < 0.001$). PFS was 41 months in the lenalidomide arm and 23 months in the placebo arm ($p < 0.001$). This benefit was seen independent of response at randomization or β_2-microglobulin at study entry. In patients with poor risk cytogenetics, PFS and OS were shorter than in standard risk patients, even in the lenalidomide arm. So far there has been no demonstrable overall survival benefit. The 4-year estimated OS was 79% in the lenalidomide arm versus 73% in the placebo arm.

The CALGB 100104 trial also evaluated lenalidomide maintenance versus observation. There was no consolidation phase in this trial and patients were placed on lenalidomide from 10mg up to 15mg daily versus placebo post-transplant until disease progression. This trial also at interim analysis demonstrated a significant PFS benefit to the lenalidomide arm, and it was unblinded early. PFS was 46 months in the lenalidomide arm versus 27 months in the placebo group. In contrast to the French trial, patients in the placebo arm were allowed to cross over to lenalidomide. Even with this crossover, at a median follow-up of 34 months there was an overall survival advantage in the lenalidomide arm [112]. In the CALGB trial, lenalidomide maintenance was effective in patients with either high or low β_2-microglobulin levels and regardless of prior exposure to thalidomide or lenalidomide. The two studies demonstrated that lenalidomide was overall well tolerated, with only about 20% of patients in the lenalidomide arms coming off therapy because of adverse events. Both studies are too immature to assess if there is a shorter survival post-relapse in the lenalidomide-treated patients, and since the CALGB trial allowed a crossover for placebo patients, it may not be possible to assess this (Figure 55.1). Both trials demonstrated that lenalidomide maintenance was associated with higher rates of second cancers, both solid tumors and hematologic malignancies. The CALGB trial reported an 8% incidence versus 2% in the placebo arm, and the IFM trial 3.1 per 100 patient years versus 1.2 per 100 patient years in the placebo group. The reason for this increased risk is unclear. Multivariate analysis in the French trial identified age >55 years, male gender, DCEP (dexamethasone, cyclophosphamide, etoposide, and cisplatin) induction, and ISSIII as risk factors for development of a second primary cancer. Because of this finding, no firm recommendation was made by the IMWG group in their consensus statement on maintenance therapy [110–112]. In general, the risk of maintenance with regard to side effects, quality of life, and especially second malignancies must be weighed against the risk of myeloma disease progression.

Bortezomib maintenance post=transplantation has proven to be more challenging to explore owing to the parenteral route of administration and the high incidence of neuropathy with long-term use. However, its potential benefits as part of induction therapy, as seen in the IFM trials, in specific high risk cytogenetic subgroups (t4;14), make it an agent worthy of further study [113] Two trials, the HOVON 65 MM/GMMG-HD4 trial and the PETHEMA/GEM VTD vs TD trial, have used it post-transplant. However, the data with regard to the degree of benefit are difficult to evaluate, as bortezomib was also included in the induction. For example, the HOVON trial randomized patients to PAD (bortezomib, adriamycin, and dexamethasone) versus VAD [114] The VAD arm post-transplant received thalidomide maintenance and the PAD arm received bortezomib maintenance for 2 years. Patients with 17p deletion, a known poor-risk group, benefited the most from bortezomib treatment with a median PFS of 26.2 months versus 12 months in the VAD/thalidomide arm. Interestingly, only a marginal benefit was seen in t(4;14) patients (25.3 months vs. 21.7 months),

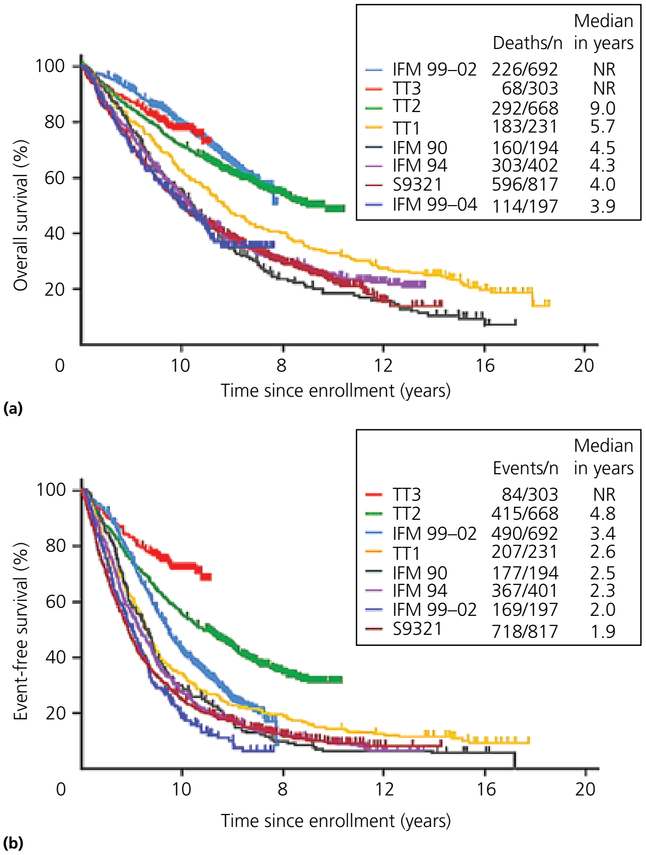


Figure 55.1 Long-term outcomes of large prospective autologous SCT trials. Source: Barlogie et al. 2010 [106]. Reproduced with permission of the American Society of Clinical Oncology. (For a color version of this figure, please see the color plate section.)

although the authors surmised that this was because chromosomal aberrations were analyzed in conjunction with ISS score, which may have diluted out the effects of t(4;14). However, since bortezomib was also given as induction but only in one arm, it remains unclear what influence this had on long-term outcome versus the maintenance portion. Also, the toxicity profile of bortezomib was an issue; 27% of patients required dose reductions and 47% went off bortezomib maintenance, with 9% discontinued due to bortezomib-related toxicity, with peripheral neuropathy predominating [115].

The PETHEMA/GEM trial randomized patients to induction with TD versus VTD versus VBMCP/VBAD/bortezomib, where VBMCP = vincristine, BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine)], melphalan, cyclophosphamide, and prednisone and VBAD = vincristine, BCNU, doxorubicin, and dexamethasone. The complete remission rate in the poor-risk cytogenetic groups was significantly higher in the VTD arm than the TD or VBMCP/VBAD arms. The maintenance portion consisted of thalidomide plus bortezomib (TV) versus thalidomide (T) versus α_2-IFN with a planned duration of maintenance of 3 years or until disease progression in all arms. At a median follow-up of 24 months, the PFS was significantly longer in the TV arm (78% vs. 63% T vs. 49% VBMCP), but OS was not different between the three arms. Interestingly, the discontinuation rate was significantly higher in the T arm than the TV arm (30% vs. 15.6%). So far there are no data on PFS in the high-risk subgroups. In addition, given the different induction regimens, it again begs the question of the role of bortezomib in induction versus maintenance [116,117]. The use of alternative routes of bortezomib administration such as subcutaneous dosing and weekly dosing have been shown to reduce rates of peripheral neuropathy. Notwithstanding, the long-term impact of these new schedules or routes of administration has not been well studied in the maintenance setting [118–120].

Role of consolidation post-SCT

Prior to the advent of novel agents, the approach to consolidation was tandem transplantation, with the second transplant consolidating response from the first, especially in patients with less than a VGPR to the first [96–100]. With the advent of novel agents, another approach has been to supplant the second transplant with treatment with either single agents or combination therapy in the early post-transplant period. Ladetto et al. demonstrated the feasibility and potential benefit of this approach [121]. They enrolled 39 patients who had achieved at least a VGPR post-transplant and who had an available molecular marker based on IgH rearrangement. They ultimately treated 31 patients with four courses of VTD in combination. The response to therapy was assessed with qualitative and quantitative PCR using tumor-specific primers. Traditional response criteria were also used. By these criteria, the CR rate increased from 15 to 49%. The molecular remission rate also increased from 3% post-transplant to 18% post-VTD. With a median follow-up of 42 months at the time of reporting, no patient in molecular remission had relapsed.

Single-agent bortezomib was used as consolidation in the Nordic Myeloma Group phase III trial [122]. In this large trial of 392 patients, there was a randomization post-transplant to no treatment or bortezomib for two 3-week cycles of a traditional day 1, 4, 8, 11 schedule and then four 4-week cycles of a day 1, 8, 15 schedule. The planned total number of bortezomib injections was 20. The median number delivered was 19, suggesting that it was feasible and well tolerated. The incidence of grade III/IV peripheral neuropathy was only 3%. There was an upgrading of response with a CR/nCR rate of 54% in the bortezomib arm versus 35% in the observation arm ($p < 0.005$). However, no data have yet been reported regarding PFS, OS, or MRD.

The largest trial reported to date analyzing the role of consolidation therapy with IMiDs and proteasome inhibitor has been the GIMENA trial, reported by Cavo and co-workers [49,123]. In the original report, 480 patients were randomly assigned to receive VTD ($n = 241$ patients) or TD ($n = 239$). After induction therapy, CR or nCR was achieved in 31% of patients receiving VTD and 11% receiving TD ($p < 0{\cdot}0001$). The 3-year PFS was significantly longer in patients receiving VTD than in those on TD (68% vs. 56%), with similar 3-year OS rates (86% vs. 84%) [49]. In a follow-up report of patients who received treatment per protocol, the outcomes for the 160 patients who received VTD were significantly better than for those who received TD consolidation with respect to CR rate (60.6% vs. 46.6%). With a median follow-up of 30.4 months, the 3-year PFS was also superior for patients receiving VTD, 60% versus 48% for autologous SCT for newly diagnosed myeloma patients [intent-to-treat (ITT) analysis; VTD, $n = 236$; TD, $n = 238$]. This per-protocol analysis (VTD, $n = 160$; TD, $n = 161$) specifically assessed the efficacy and safety of consolidation with VTD or TD. After consolidation, CR rates were significantly higher in the VTD arm (60.6% vs. 46.6%). With a median follow-up of 30.4 months from start of consolidation, 3-year PFS was significantly longer for the VTD group (60% vs. 48% for TD) [123].

The US BMT CTN 0702 trial will evaluate this concept in a prospective randomized fashion. In this three-armed trial, patients will be randomized to single transplant, tandem transplant, or single transplant plus RVD consolidation for four cycles. All arms will receive lenalidomide maintenance. The primary study end-point will by 3-year PFS, but the trial will also assess depth of remission including MRD analysis in each study arm [55].

Autologous SCT as salvage therapy

In contrast to the upfront setting in which the role of high-dose therapy as consolidation of a first remission is well established, the utility of a second course of high-dose therapy with autologous SCT has not been established. Although multiple retrospective series have been published and are summarized in Table 55.4, no prospective trials have been performed to date, but retrospective results suggest that this may be a reasonable consolidation strategy for patients who have a long initial remission from their first high-dose regimen [124–131].

Future directions for autologous HCT in myeloma

MM is currently the most common indication for high-dose therapy with autologous HCT in North America and Europe [132]. Despite the high response rates for IMIDs and proteosome inhibitor combinations, many patients and physicians opt for high-dose therapy as consolidation of an initial remission and until the results of the large randomized trials comparing early with delayed SCT in the context of modern induction therapy are known this strategy should continue to be considered the standard of care. High-dose melphalan is used in over 90% of patients, with the most common regimen used being single-agent melphalan at a median dose of 180–200mg/m^2 performed as a single transplantation (CIBMTR, unpublished observations). Notwithstanding, patients with high-risk disease still have a remission duration of less than 3 years after autografting with most patients dying from their disease. Hence novel post-remission strategies need to be continuously explored, and likewise identification of patients who may have long-term disease control without upfront SCT could reduce the burden of treatment in a significant proportion of patients.

Table 55.4 Retrospective analysis of salvage autologous SCT outcomes: series with more than 40 patients

Ref.	*n*	NRM	Median PFS (months)	Prognostic factor
[125]	44	2	12	1st RD
[126]	81	3	16	1st RD ≥VGPR
[127]	41	7	8.5	Age >65 years Lines of therapy
[128]	55	5	14	1st RD
[129]	83	NS	15.6	
[130]	60	NS	11	NS
[131]	98	4	10.3	1st RD
[132]	187	2	11	1st RD

NS, not stated; RD, relapsed disease.

Allogeneic HCT in myeloma

The existence of a graft-versus-myeloma effect was first documented by Tricot et al., and later confirmed in large single- and multi-institutional series of donor lymphocyte infusion [133–136]. Thus, with the potential benefits of a graft-versus-tumor effect and the use of a clean HC product, it would be expected that high-dose therapy with allogeneic HCT would be extremely useful in the treatment of MM. Unfortunately, that is not necessarily the case. The EBMT case-controlled series reported that the relapse rate after allogeneic HCT was not significantly lower than the relapse rate after syngeneic HCT, suggesting that the graft-versus-tumor effect is not as potent in myeloma as it is in chronic or acute myelogenous leukemia [87].

A large report from the EBMT registry showed disappointing results for patients undergoing allogeneic HCT. Median survival was only 18 months, and the TRM was prohibitive at 41%. TRM, however, has been substantially reduced over the last decade even in the context of high-dose conditioning regimens [137]. To try to overcome toxicity from high-dose regimens, and to extend the applicability of allografting to older patients with significant comorbidities, allogeneic HCT after reduced-intensity conditioning (RIC) regimens has been attempted in patients with MM. Several phase II non-randomized trials and also large registry trials have been published and reported (reviewed in [138]). Various prospective donor versus no-donor biologic assignment studies have been performed and published comparing allogeneic SC consolidation with either autologous SCT or other alternative therapies. These trials are summarized in Table 55.5.

Garban et al. published the first of these prospective trials in patients with high-risk disease as defined by high levels of β_2-microglobulin or deletion of chromosome 13 as detected by FISH [139]. Patients received an initial autologous HCT with melphalan 200mg/m^2. Sixty-five patients had an HLA-identical sibling donor, of whom 46 patients received an RIC regimen consisting of fludarabine, busulfan, and antithymocyte globulin (ATG). Patients without an HLA sibling donor received a second autologous HCT prepared with melphalan 220mg/m^2. On an ITT basis, the OS and EFS did

Table 55.5 Auto–allo versus tandem autologous SCT: results of biologic assignment trials among standard-risk patients

Ref.	*n*	Regimen	Response rate (complete response) (%)	Chronic GVHD (%)	Transplant-related mortality (%)	Disease-free survival	Overall survival
[139]	Allo 46	Flu/BU	82	43	11	25 months[a]	35 months[a]
	Auto 166	Mel 220			2	30 months[a]	41 months[a]
[140,141]	Allo 60	Flu/TBI	86	32	10	43 months[a]	NR
	Auto 59	Mel 200	89		4	33 months	56 months[a]
[142]	Allo 108	Flu/TBI	51	54	12	39% @ 5 years	65% @ 5 years
	Auto 249	Mel 200	41		3	19% @ 5 years	58% @ 5 years
[143]	Allo 122	Various	43	NS	17	28% @ 6 years	55% @ 6 years
	Auto 138	Various	37		1	22% @ 6 years	55% @ 6 years
[144]	Allo 189	Flu/TBI	NS	54	11	40% @ 3 years	67% @ 3years
	Auto 366	Mel 200			4	33% @ 3 years	59% @ 3 years

Flu/BU, fludarabine/busulfan; Mel, melphalan (mg/m^2); NS, not stated; TBI, total body irradiation.

[a] Median.

not differ significantly in both groups (median 35 and 25 months in the allogeneic HCT patients versus 41 and 30 months in the autologous HCT patients, respectively). There was a trend for a better OS in patients treated with tandem autologous HCT, with a median of 47.2 versus 35 months ($p = 0.07$) for patients who actually received a reduced-intensity allogeneic HCT.

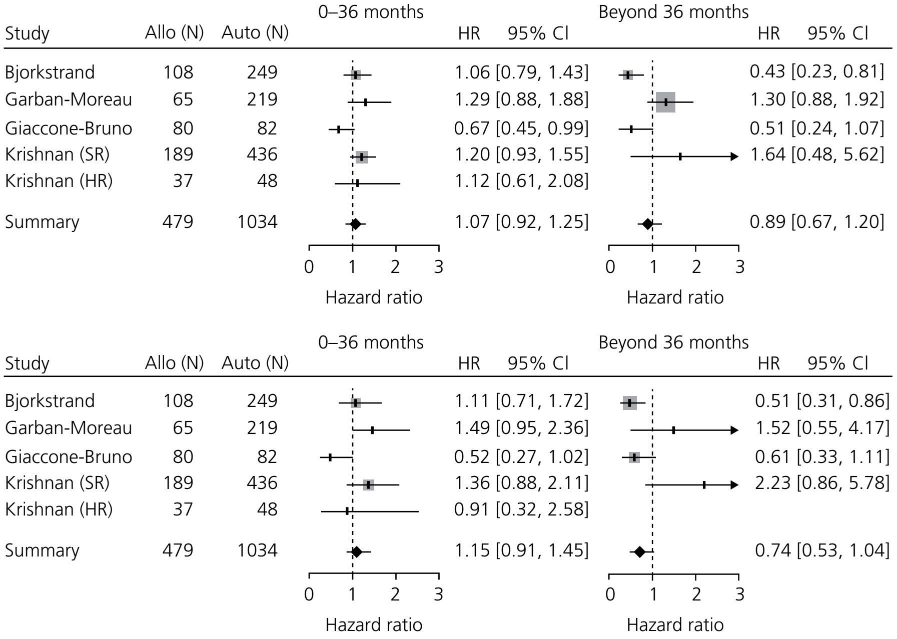
The Italian Cooperative Group performed a similar study, which was reported by Bruno et al. [140]. After a median follow-up of 3 years, non-relapse mortality (NRM) was 11% for the autologous + allogeneic group versus 4% for the tandem autologous group ($p = 0.09$). CR was significantly higher in the autologous + allogeneic group than the tandem autologous group (6% vs. 16%, $p = 0.0001$). OS and EFS rates were superior in the autologous + allogeneic group to those in the tandem autologous group (84% vs. 62%, $p = 0.003$, and 75% vs. 41%, $p = 0.00008$, respectively). The results have held over time with subsequent follow-up reports [141]. Positive outcomes for recipients of allogeneic SCT were also reported by Björkstrand et al. [142]. A total of 357 patients participated in this EBMT prospective trial. Similarly to other designs, patients with an HLA-identical sibling donor were allocated to the auto-allo arm ($n = 108$) and patients without a matched sibling donor were allocated to the auto arm ($n = 249$). PFS at 60 months was 35% for the auto-allo arm versus 18% for the auto-auto arm; survival rates were similar in both arms with significantly higher relapse rates for the tandem auto arm (78% vs. 49%) but higher NRM rates for the auto-allo arm (12% vs. 3%).

These results contrast with those of HOVON 50 and more recently those of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) Trial 0102. Lokhorst et al. reported on the donor versus no-donor analysis performed on patients treated in the HOVON 50 study. With a median follow-up of 77 months, no significant difference with regard to PFS was seen among the 138 patients without an HLA-identical sibling donor and 122 patients with a donor (6-year PFS 28% for donor, 22% for no donor). As with other studies, NRM was significantly higher in the donor arm (16% vs. 3%) [143]. Krishnan et al. reported on the transplant outcomes of 625 patients with standard-risk disease who received either an autologous hematopoietic stem-cell transplant (HSCT) ($n = 366$) or an allogeneic SCT ($n = 189$). Kaplan–Meier estimates of 3-year PFS were 43% in the auto-allo group and 46% in the tandem auto group; overall survival also did not differ at 3 years (77% vs. 80%) [144].

Hence the preponderance of data suggests that RIC allogeneic SCT after autologous HSCT is not more effective than tandem autologous HSCT for patients with standard-risk multiple myeloma. Further enhancement of the graft-versus-myeloma effect and reduction in TRM are needed to improve the allogeneic HSCT approach. Selecting patients who are more likely to benefit from a graft-versus-myeloma effect may improve the risk–benefit ratio for allografitng. Rosiñol et al. reported on SCT outcomes among 110 patients who had failed to achieve at least an nCR after a first autologous SCT who received either an RIC allograft ($n = 25$) or a second autograft SCT ($n = 85$) depending on the availability of an HLA-identical sibling donor. The CR rate was higher amongst the patients who underwent an allograft (40% vs. 11%, $p = 0.001$) and a trend towards a longer PFS (31 months vs. not reached), with no survival benefit due to the higher NRM rate (16% vs. 5%) [145]. A recent meta-analysis suggests lower rates of events in patients surviving at least 36 months post-allografting but until NRM rates are significantly decreased it will be difficult to justify upfront allograft in standard-risk patients (Figure 55.2) [146].

Alternative donor HCT

Ballen et al. reported on HCT outcomes for patients with MM undergoing unrelated donor HCT after high-dose conditioning. NRM was over 50% and PFS at 3 years was 15% [147]. Kröger et al. compared ATG with alemtuzumab in 73 patients with MM who underwent RIC with melphalan–fludarabine, followed by allogeneic HCT from HLA-matched or HLA-mismatched unrelated donors [148]. Alemtuzumab resulted in faster engraftment of leukocytes ($p = 0.03$) and platelets ($p = 0.02$), and in a lower incidence of acute graft-versus-host disease (GVHD) grade II–IV (24% vs. 47%, $p = 0.06$). More cytomegalovirus-seropositive patients in the alemtuzumab group experienced cytomegalovirus reactivation (100% vs. 47%, $p = 0.001$). The cumulative incidence of TRM at 2 years was 26% for ATG versus 28% for alemtuzumab ($p = 0.7$).



Study	Allo (N)	Auto (N)	0–36 months HR	0–36 months 95% CI	Beyond 36 months HR	Beyond 36 months 95% CI
Bjorkstrand	108	249	1.06	[0.79, 1.43]	0.43	[0.23, 0.81]
Garban-Moreau	65	219	1.29	[0.88, 1.88]	1.30	[0.88, 1.92]
Giaccone-Bruno	80	82	0.67	[0.45, 0.99]	0.51	[0.24, 1.07]
Krishnan (SR)	189	436	1.20	[0.93, 1.55]	1.64	[0.48, 5.62]
Krishnan (HR)	37	48	1.12	[0.61, 2.08]		
Summary	479	1034	1.07	[0.92, 1.25]	0.89	[0.67, 1.20]

Study	Allo (N)	Auto (N)	0–36 months HR	0–36 months 95% CI	Beyond 36 months HR	Beyond 36 months 95% CI
Bjorkstrand	108	249	1.11	[0.71, 1.72]	0.51	[0.31, 0.86]
Garban-Moreau	65	219	1.49	[0.95, 2.36]	1.52	[0.55, 4.17]
Giaccone-Bruno	80	82	0.52	[0.27, 1.02]	0.61	[0.33, 1.11]
Krishnan (SR)	189	436	1.36	[0.88, 2.11]	2.23	[0.86, 5.78]
Krishnan (HR)	37	48	0.91	[0.32, 2.58]		
Summary	479	1034	1.15	[0.91, 1.45]	0.74	[0.53, 1.04]

Figure 55.2 Meta-analysis of auto-RIC allogeneic versus tandem autologous trials. Source: Armeson et al. 2013 [146]. Reproduced with permission of Nature Publishing Group.

There was no significant difference in the estimated 2-year OS and PFS between ATG and alemtuzumab. Hence although feasible, alternative donor allografting should also be considered only in the context of a clinical trial primarily.

Future directions for allogeneic HCT in myeloma

Allogeneic SCT has been routinely associated with lower relapse rates than autografting [146]. Notwithstanding, relapse rates after allografting are still routinely 40% or more. Lenalidomide maintenance has also been used post-allogeneic transplant for myeloma. It theoretically appeared advantageous because of both its potential effects of enhancing the graft-versus-myeloma immune effect and also its direct anti-myeloma activity. The HOVON 76 phase II trial evaluated the safety and efficacy of lenalidomide 10mg daily following tandem autologous and non-myeloablative allogeneic SCT [149]. Thirty-five eligible patients were enrolled and 30 started on lenalidomide. However, after two cycles, 47% ($n = 14$) had to stop treatment, primarily due to flare of GVHD. Overall, 37% developed acute and 53% chronic GVHD. Despite the short duration and poor tolerability of therapy, 20% of patients upgraded response during lenalidomide therapy and an additional six patients improved their response after the lenalidomide was stopped, which made the total response rate 37%. The poor tolerability may be due in part to the timing of initiation of lenalidomide and also the dose. A US intergroup trial also used lenalidomide post-allogeneic transplant; results of this trial are awaited.

Conclusion

The treatment for myeloma continues to evolve. Thirty years ago, the standard treatment was melphalan and prednisone, with a response rate of only 40% and an average life expectancy of 3 years. During the last three decades, significant advances in therapy have been made. Today, transplantation-eligible patients receiving a standard induction therapy with thalidomide and dexamethasone followed by consolidation therapy with high-dose melphalan and one autologous HCT have more than an 80% chance of achieving a partial remission or better, and an average life expectancy of more than 5 years. Moreover, approximately one-third of patients treated will achieve CR, of whom 30% will remain without disease progression 10 years after HCT.

The advent of new agents such as bortezomib, carfilzomib, thalidomide, and lenalidomide, and the improved outcomes after induction therapy, should move the field to re-explore the role of high-dose melphalan as consolidation therapy for all patients with MM. Likewise, exploring strategies that could improve on the outcomes of high-dose melphalan are necessary. Post-transplantation therapy with consolidative chemotherapy and maintenance is also becoming an important adjuvant to improve outcomes. Although both allogeneic and autologous HCT can result in long-term disease control and potential cure, most patients still relapse. Therefore, caution needs to be exercised when recommending allogeneic HCT to patients with myeloma, since the risk–benefit ratio of this procedure still makes it difficult to recommend to all patients as upfront therapy. Likewise, recommending maintenance therapy to all patients regardless of their response to therapy or other risk factors will expose a substantial proportion of them to drug toxicities, who may have only a marginal benefit from this strategy. Hence further delineation of the prognostic value of gene expression profiling and other parameters will assist in developing true risk-tailored approaches for this disease, fulfilling the goal of delivering the longest life with the best quality and minimum burden of therapy for the greatest number patients with myeloma.

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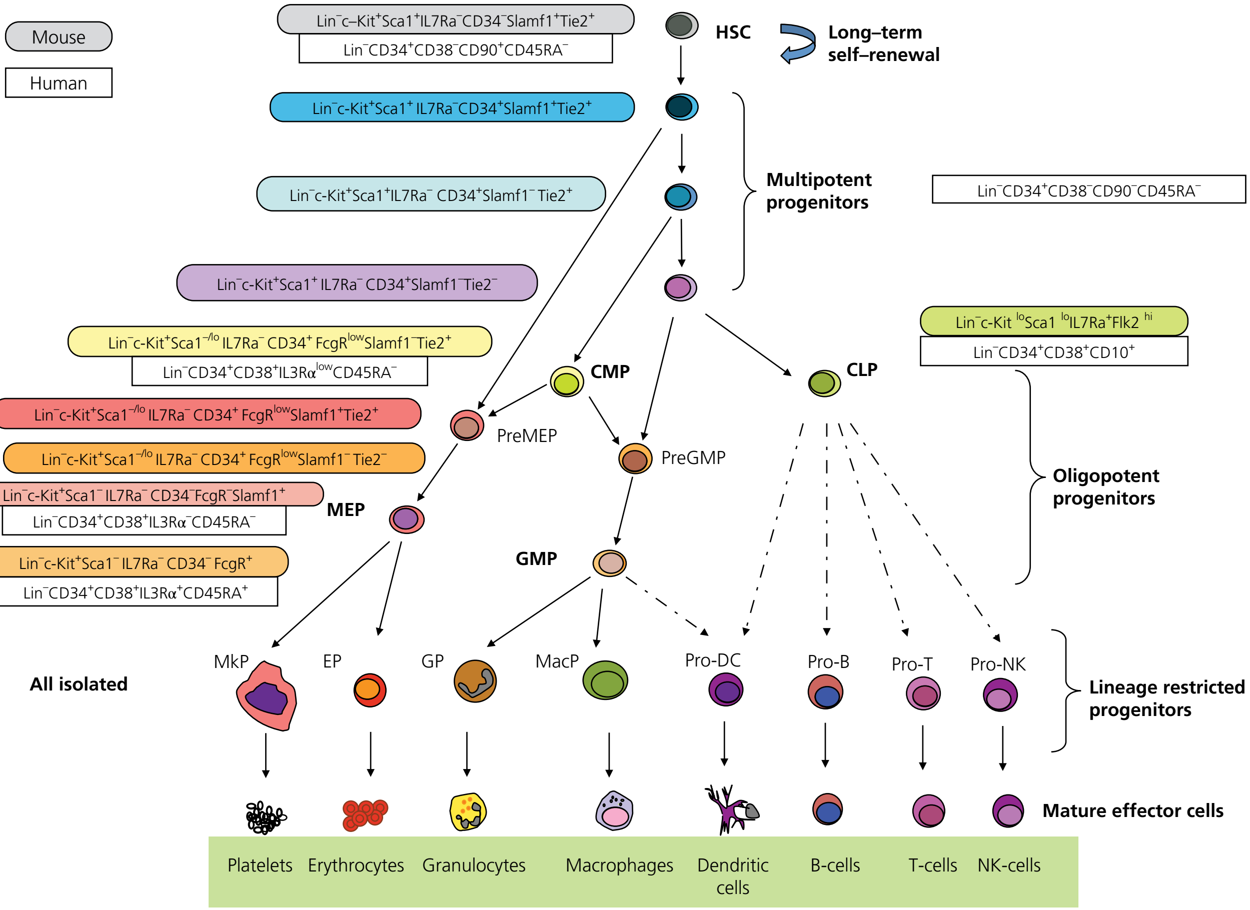
Mouse
Human
Lin$^-$c–Kit$^+$Sca1$^+$IL7Ra$^-$CD34$^-$Slamf1$^+$Tie2$^+$
Lin$^-$CD34$^+$CD38$^-$CD90$^+$CD45RA$^-$
HSC
Long–term self–renewal
Lin$^-$c-Kit$^+$Sca1$^+$ IL7Ra$^-$CD34$^+$Slamf1$^+$Tie2$^+$
Lin$^-$c-Kit$^+$Sca1$^+$IL7Ra$^-$ CD34$^+$Slamf1$^-$ Tie2$^+$
Multipotent progenitors
Lin$^-$CD34$^+$CD38$^-$CD90$^-$CD45RA$^-$
Lin$^-$c-Kit$^+$Sca1$^+$ IL7Ra$^-$ CD34$^+$Slamf1$^-$Tie2$^-$
Lin$^-$c-Kit$^+$Sca1$^{-/lo}$ IL7Ra$^-$ CD34$^+$ FcgR^{low}Slamf1$^-$Tie2$^+$
Lin$^-$CD34$^+$CD38$^+$IL3Rα^{low}CD45RA$^-$
CMP
Lin$^-$c-Kit ^{lo}Sca1 ^{lo}IL7Ra$^+$Flk2 ^{hi}
Lin$^-$CD34$^+$CD38$^+$CD10$^+$
CLP
Lin$^-$c-Kit$^+$Sca1$^{-/lo}$ IL7Ra$^-$ CD34$^+$ FcgR^{low}Slamf1$^+$Tie2$^+$
PreMEP
Lin$^-$c-Kit$^+$Sca1$^{-/lo}$ IL7Ra$^-$ CD34$^+$ FcgR^{low}Slamf1$^-$ Tie2$^-$
PreGMP
Lin$^-$c-Kit$^+$Sca1$^-$ IL7Ra$^-$ CD34$^-$FcgR$^-$Slamf1$^+$
Lin$^-$CD34$^+$CD38$^+$IL3Rα$^-$CD45RA$^-$
MEP
Oligopotent progenitors
Lin$^-$c-Kit$^+$Sca1$^-$ IL7Ra$^-$ CD34$^-$ FcgR$^+$
Lin$^-$CD34$^+$CD38$^+$IL3Rα$^+$CD45RA$^+$
GMP
All isolated
MkP
EP
GP
MacP
Pro-DC
Pro-B
Pro-T
Pro-NK
Lineage restricted progenitors
Mature effector cells
Platelets
Erythrocytes
Granulocytes
Macrophages
Dendritic cells
B-cells
T-cells
NK-cells

Figure 4.1 Hematopoietic lineage tree, showing cells at successive stages of differentiation with distinctive marker profiles (immunophenotypes) in mouse and human. The hematopoietic stem cell (HSC) has long-term reconstituting, self-renewing capacity; the multipotent progenitors have limited or no self-renewal leading to transient but multilineage reconstitution. CMP, common myeloid progenitor; CLP, common lymphoid progenitor; MEP, megakaryocyte/erythroid progenitor; GMP, granulocyte/macrophage progenitor; MkP, megakaryocyte progenitor; EP, erythroid progenitor; GP, granulocyte progenitor; MacP, macrophage progenitor; Pro-DC, dendritic cell progenitor; Pro-B, B lymphocyte progenitor; Pro-T, T lymphocyte progenitor; Pro-NK, natural killer cell progenitor.

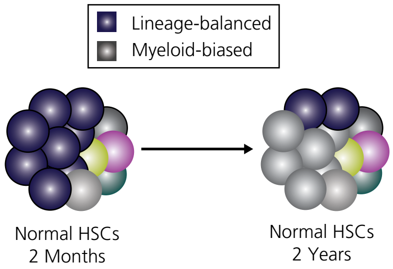
Lineage-balanced
Myeloid-biased
Normal HSCs
2 Months
Normal HSCs
2 Years

Figure 4.5 At birth, most of the hematopoietic stem cell (HSC) clones in mice are lineage-balanced, that is, equally capable of generating various lineages. However, with age, clones biased towards generating myeloid lineages (myeloid-biased) predominate, due to either clonal selection or epigenetic changes in the originally lineage-balanced clones.

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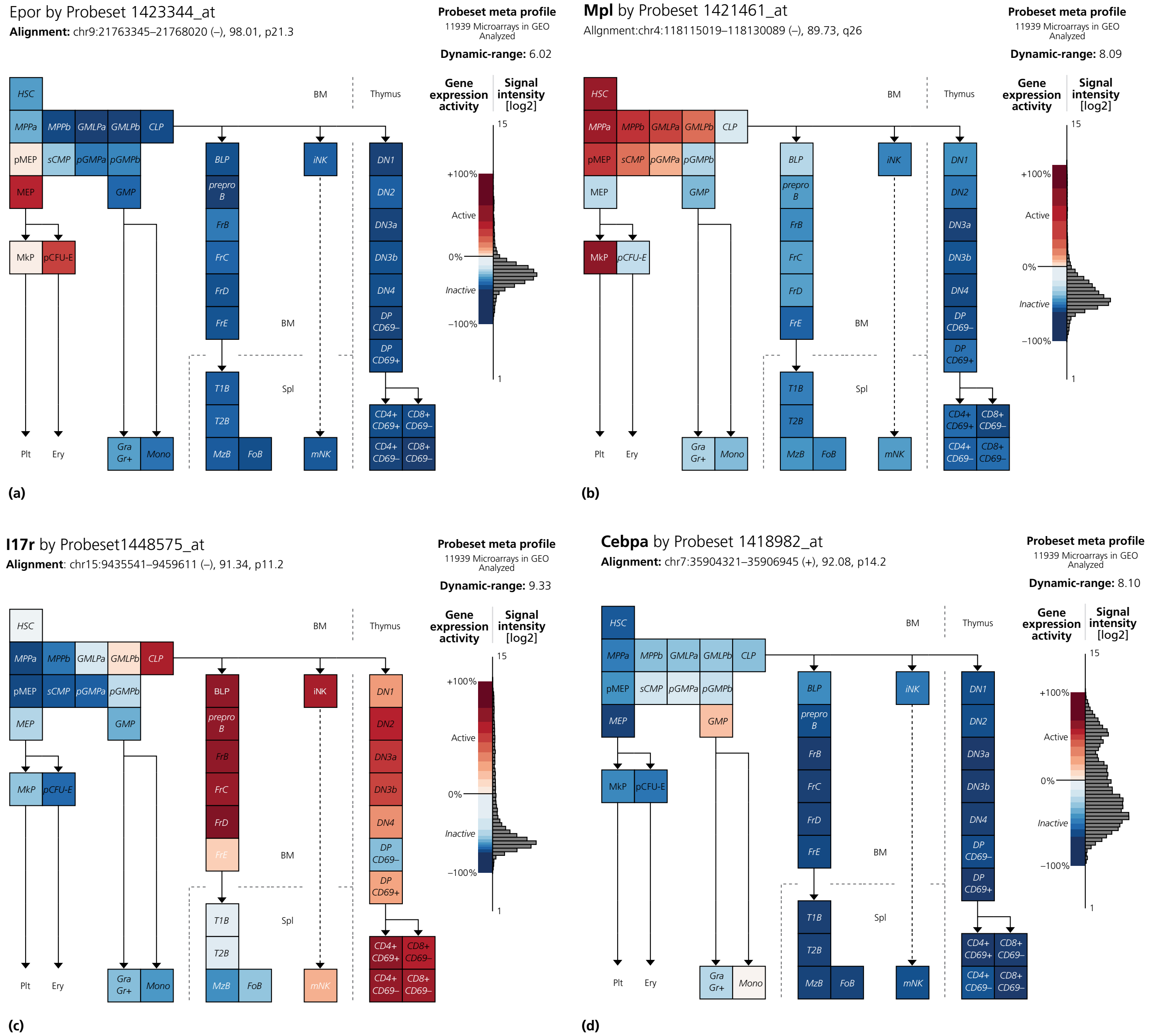

Epor by Probeset 1423344_at
Alignment: chr9:21763345–21768020 (–), 98.01, p21.3

Probeset meta profile
11939 Microarrays in GEO Analyzed
Dynamic-range: 6.02

(a)

Mpl by Probeset 1421461_at
Alignment:chr4:118115019–118130089 (–), 89.73, q26

Probeset meta profile
11939 Microarrays in GEO Analyzed
Dynamic-range: 8.09

(b)

I17r by Probeset1448575_at
Alignment: chr15:9435541–9459611 (–), 91.34, p11.2

Probeset meta profile
11939 Microarrays in GEO Analyzed
Dynamic-range: 9.33

(c)

Cebpa by Probeset 1418982_at
Alignment: chr7:35904321–35906945 (+), 92.08, p14.2

Probeset meta profile
11939 Microarrays in GEO Analyzed
Dynamic-range: 8.10

(d)

HSC
MPPa
MPPb
GMLPa
GMLPb
CLP
pMEP
sCMP
pGMPa
pGMPb
MEP
GMP
MkP
pCFU-E
BLP
prepro B
FrB
FrC
FrD
FrE
T1B
T2B
MzB
FoB
iNK
mNK
DN1
DN2
DN3a
DN3b
DN4
DP CD69–
DP CD69+
CD4+ CD69+
CD8+ CD69–
CD4+ CD69–
CD8+ CD69–
Plt
Ery
Gra Gr+
Mono
BM
Thymus
Spl
Gene expression activity
Signal intensity [log2]
15
+100%
Active
0%
Inactive
–100%
1

Figure 4.6 Images from Gene Expression Commons (https://gexc.stanford.edu/) showing absolute comparison of the array data for particular genes (*EpoR, c-Mpl, IL-7Rα*, and *c-Ebpα*). Raw microarray data are individually normalized against a large-scale common reference, then mapped onto each probe-set metaprofile. This strategy enables profiling of absolute expression levels of all genes on the microarray.

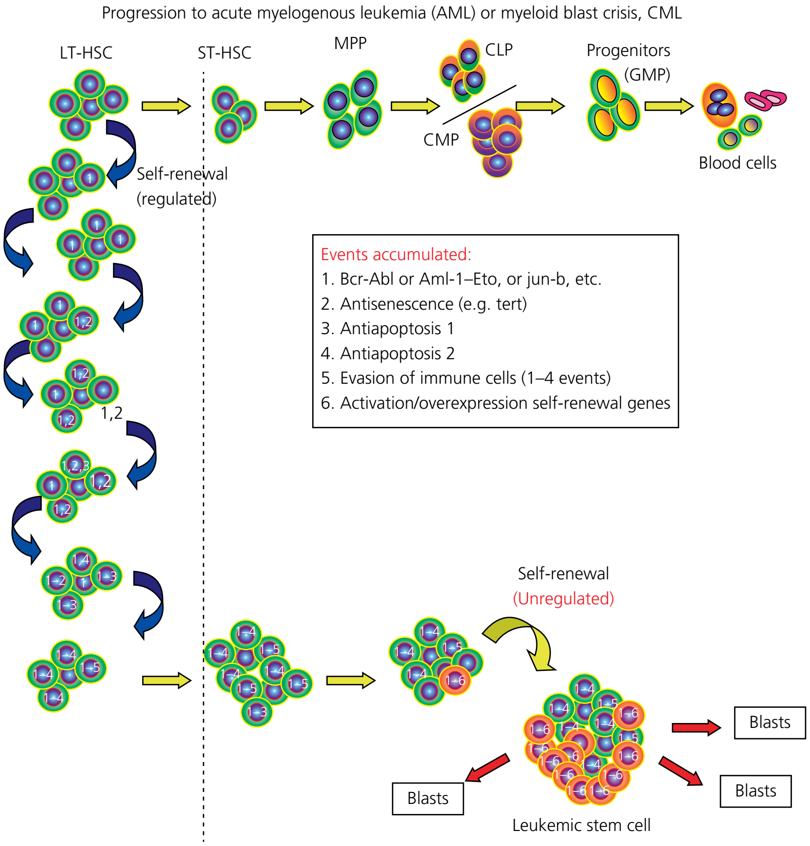

Progression to acute myelogenous leukemia (AML) or myeloid blast crisis, CML
LT-HSC
ST-HSC
MPP
CLP
CMP
Progenitors
(GMP)
Blood cells
Self-renewal
(regulated)
Events accumulated:
1. Bcr-Abl or Aml-1–Eto, or jun-b, etc.
2. Antisenescence (e.g. tert)
3. Antiapoptosis 1
4. Antiapoptosis 2
5. Evasion of immune cells (1–4 events)
6. Activation/overexpression self-renewal genes
1,2
Self-renewal
(Unregulated)
Blasts
Blasts
Blasts
Leukemic stem cell

Figure 4.11 Comparison of stem cell self-renewal during normal hematopoiesis and leukemic transformation. During normal hematopoiesis, the signaling pathways that regulate self-renewal are tightly controlled, allowing proper differentiation to each of the mature blood cell lineages (top). Accumulation of mutations in the self-renewing hematopoietic stem cells (HSCs) over time until dysregulated self-renewal ability is attained and leukemia propagation is initiated. Dysregulation of self-renewal mechanisms in transformed cancer stem cells leads to uncontrolled self-renewal and the production of leukemic blast cells. The leukemic population capable of sustaining disease is often distinct from the blast population, and may reside in the stem cell or progenitor cell compartment. Importantly, if the transformation event occurs in a progenitor cell, it must endow the progenitor cell with the self-renewal, because the progenitors would otherwise differentiate.

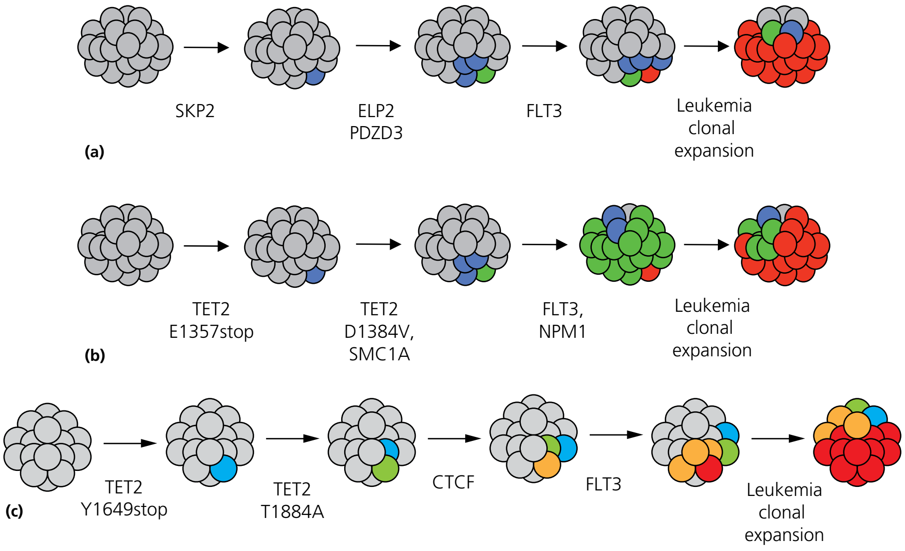

SKP2
ELP2
PDZD3
FLT3
Leukemia
clonal
expansion
(a)
TET2
E1357stop
TET2
D1384V,
SMC1A
FLT3,
NPM1
Leukemia
clonal
expansion
(b)
TET2
Y1649stop
TET2
T1884A
CTCF
FLT3
Leukemia
clonal
expansion
(c)

Figure 4.12 Clonal progression of preleukemic mutations in hematopoietic stem cell (HSC) clones, followed by emergence of the leukemia stem cell (LSC) in the multipotent progenitor (MPP) stage in three cases of FLT3td acute myeloid leukemia (AML) [84]. Exomic sequencing of the AML cells and T cells from the same patients allow listing of the mutations specific to the AML. Individual HSCs were isolated from the 3 patients and grown to colonies with hematopoietic growth factors, and the colonies analyzed with DNA primers specific for each of the mutations in the LSCs. Shown are the normal HSC clones (grey) and mutant HSC clones (with each successive color – blue, green, yellow – indicating the addition of a new mutation). Leukemic HSC clones (red) finally have all mutations. A TET2 loss of function mutation via a stop codon was the earliest preleukemic HSC event in 2 of the 3 patients (b) and (c). In the same individuals, the next event is the loss of TET2 by another stop codon in the other TET2 allele. Following that are clones with 3, or 4 or n mutations in the 3 patients. In all 3 the FLT3 internal tandem repeat occurred as the final mutation before the LSCs emerge. It is interesting that in 2 patients the loss of all TET2 function, which regulates CMEpG to hydroxymethyl cytosine, an epigenetic modifier of the CpG methylome, occurs before any of the other mutations. Source: Jan et al. 2012 [84]. Reproduced with permission of the American Association for the Advancement of Science.

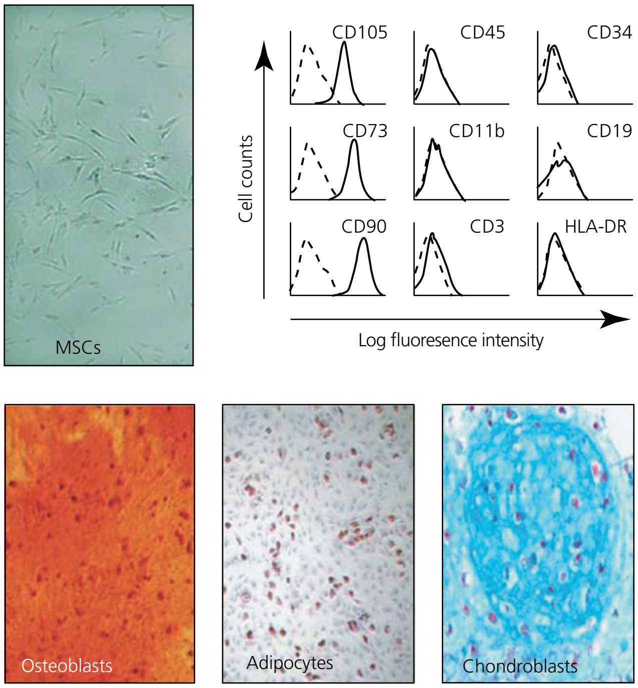

MSCs
CD105
CD45
CD34
CD73
CD11b
CD19
CD90
CD3
HLA-DR
Cell counts
Log fluoresence intensity
Osteoblasts
Adipocytes
Chondroblasts

Figure 7.1 A panel of data demonstrating the defining characteristics of MSCs: adherence, immunophenotype, and in vitro differentiation. (Top, left) Photomicrograph of undifferentiated MSCs showing the characteristic spindle shape and adherent properties of the cells. Original magnification, ×x40. (Top, right) Flow cytometry histograms demonstrating the typical expression pattern of surface antigens (—) and isotype control (- - -), as indicated. (Bottom) Immunocytochemical staining demonstrating the differentiation of MSCs into osteoblasts (Alizarin Red stain), adipocytes (Oil Red O stain), and chondroblasts (Alcian Blue stain). Source: Martinez C et al. Human bone marrow mesenchymal stromal cells express the neural ganglioside GD2: a novel surface marker for the identification of MSCs. *Blood* 2007; **109**(10): 4245–4248. Reproduced with permission of American Society of Hematology. (From Martinez et al. *Blood* **109**: 4245–4248, 2007, with permission)

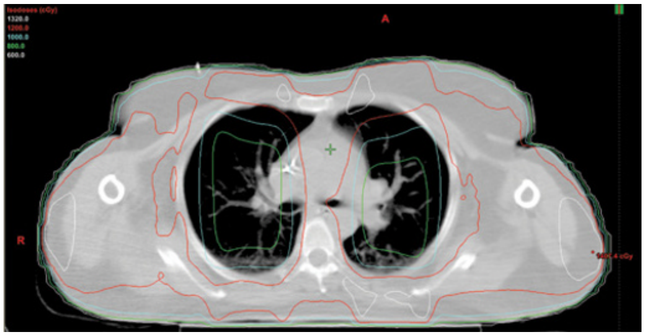

Isodoses (cGy)
1320.0
1200.0
1000.0
800.0
600.0
A
R

Figure 22.2 Dose distribution through T5–6 showing 6, 8, 10, 12, and 13.2 Gy isodose lines. The prescription dose was 12 Gy, with 50% attenuation lung blocks.

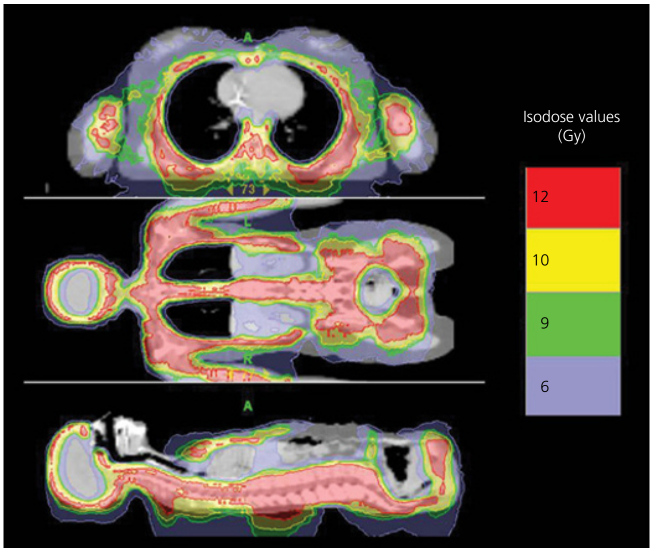

A
73
A
Isodose values (Gy)
12
10
9
6

Figure 22.4 Color wash demonstrating typical dose distribution in a patient treated with targeted TBI using tomotherapy. The target structure is skeletal bone. Source: Wong et al. 2006 [2]. Reproduced with permission of the American Society for Blood and Bone Marrow Transplantation.

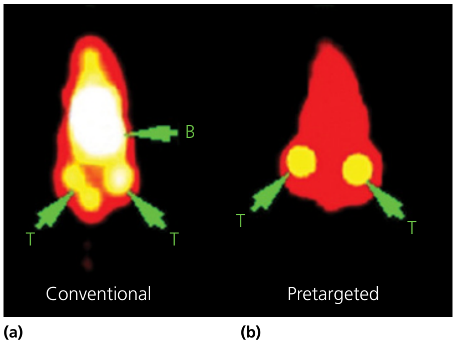


Figure 23.4 Efficacy of pretargeting as demonstrated by planar gamma camera images of Burkitt lymphoma xenograft tumors in athymic mice. Animals were injected with either directly labeled [^{111}In]anti-CD20 Ab (a) or pretargeted with anti-CD20–streptavidin conjugate followed 24 hours later by clearing agent and then by [^{111}In]DOTA–biotin (b). Arrows indicate radioactivity in tumors (T), and blood pool (B) 24 hours after injection of ^{111}In-labeled reagents. Bladder activity is also seen in the conventional mouse. Source: Subbiah K. et al. 2003. Comparison of immunoscintigraphy, efficacy, and toxicity of conventional and pretargeted radioimmunotherapy in CD20-expressing human lymphoma xenografts. *J. Nucl. Med.* 2003; **44**: 437–445. Reproduced with permission of the Society of Nuclear Medicine.

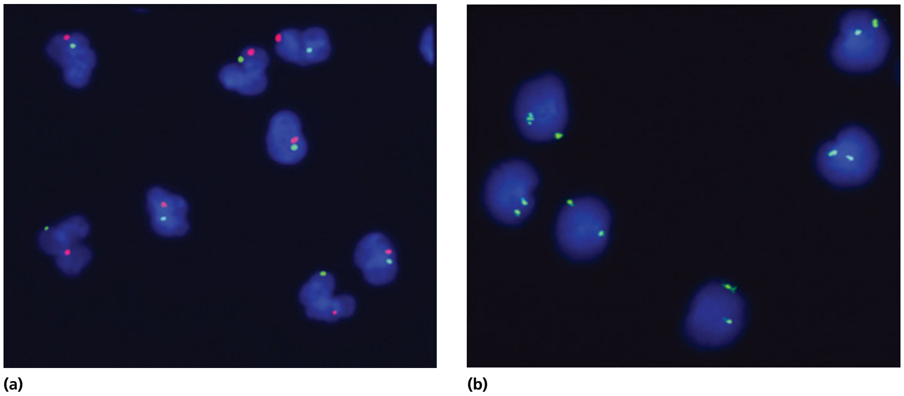
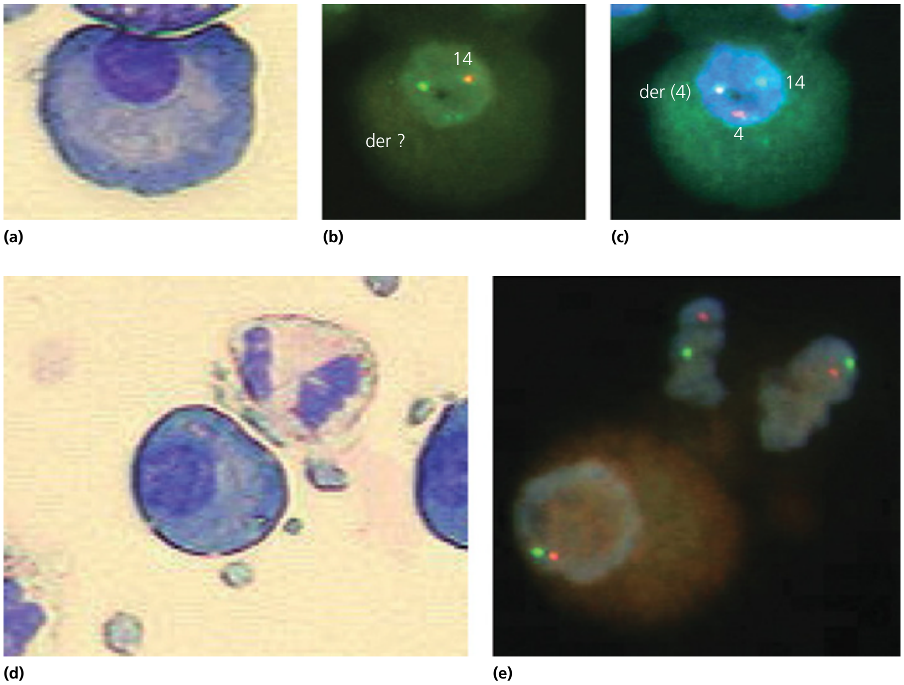


Figure 24.1 Assessment of chimerism in sorted granulocytes (a) and leukemic blasts (b) by dual-color fluorescence in situ hybridization (FISH). Hybridization was carried out with a biotin-labeled Y chromosome-specific DNA probe and a digoxigenin-labeled X chromosome-specific DNA probe. Hybridization was detected with Texas Red-conjugated avidin and fluorescein-conjugated antibody against digoxigenin. Cells were counterstained with 4′,6-diamidino-2-phenylindole (API). Male cells contain a single red fluorescent spot and a single green fluorescent spot, whereas female cells contain two green fluorescent spots and no red fluorescent spots. In this case, granulocytes were derived from a male donor and leukemic blasts were derived from the female recipient.



Targeted scanning with CD19 monoclonal antibody

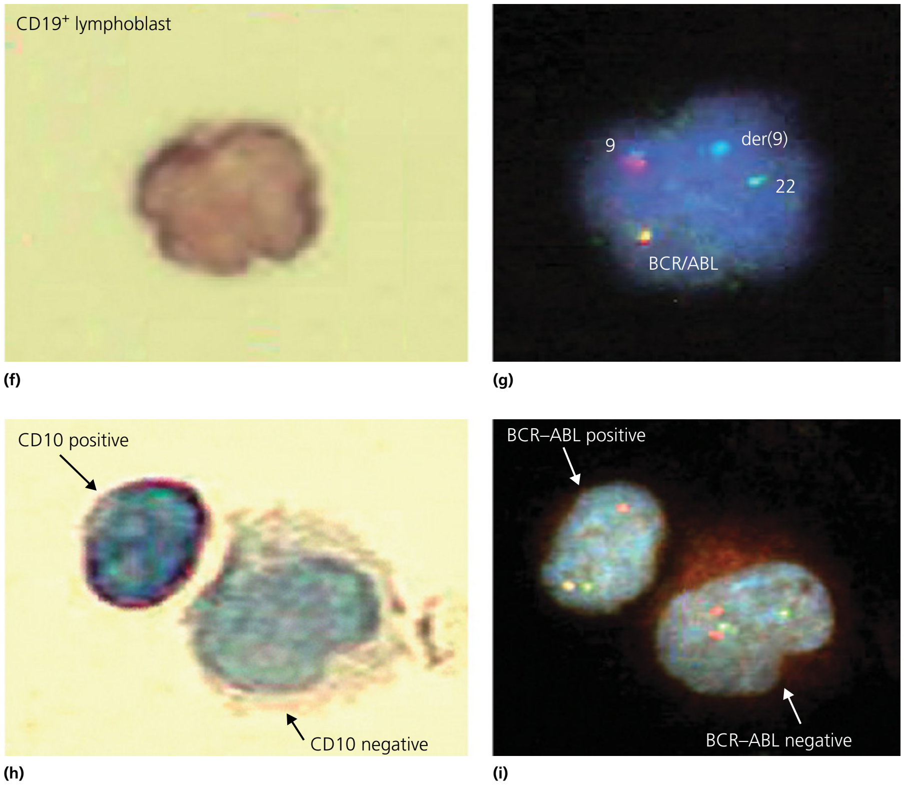


Figure 25.2 Plasma cell-specific fluorescence in situ hybridization (FISH) analysis. (a–e) Cytospin slides are made from the bone marrow aliquot and stained with May–Grünwald stain. After the cells have been mapped to the slide, the slide is destained and hybridized with the selected DNA probe. For details, see [5]. (a) A plasma cell that has been mapped. This same cell is seen in (b), showing a variant immunoglobulin H (*IGH*) pattern using a dual-color *5′IGH/3′IGH* break-apart probe. Once an *IGH* gene rearrangement is found in a patient with multiple myeloma, the same slide is rehybridized to determine its partner chromosome in the IGH translocation. (c) A fusion signal is detected with the *FGFR3* (fibroblast growth factor receptor-3)/*IGH* probe indicating the derivative chromosome 4 of the t(4;14) or der(4)t(4;14). The second fusion that would have indicated the der(14)t(4;14) is not present in this patient, the result of loss of the derivative 14 chromosome. In another slide from this patient [(d) and (e)], monosomy 13 is shown in the neoplastic plasma cells, but the segmented neutrophil adjacent to the plasma cells shows both chromosome 13 signals (one each in each of the segments of the nucleus). The chromosome 13 probe set used was composed of D13S319 probe (red), which maps to 13q14.3, and the *LAMP1* (lysosomal-associated membrane protein-1)/13q34 reference probe labeled in green. Sequential immunohistochemistry (IHC)/FISH is shown in (f)–(i). (f) A CD19$^+$ lymphoblast that is *BCR/ABL1* fusion gene positive (g), indicating residual disease and not a hematogone. Similarly, (h) and (i) show the cell that is CD10$^+$ is positive for the *BCR/ABL1* fusion gene, and the CD$^-$ cell is negative for the *BCR/ABL1* fusion gene. The *BCR/ABL1* probe used for the sequential IHC/FISH studies was the single fusion probe spiked with 9q34/argininosuccinate synthetase gene probe labeled in Spectrum aqua. All probes were obtained and reproduced with permission from Abbott Molecular Inc. (Des Plaines, IL, USA).

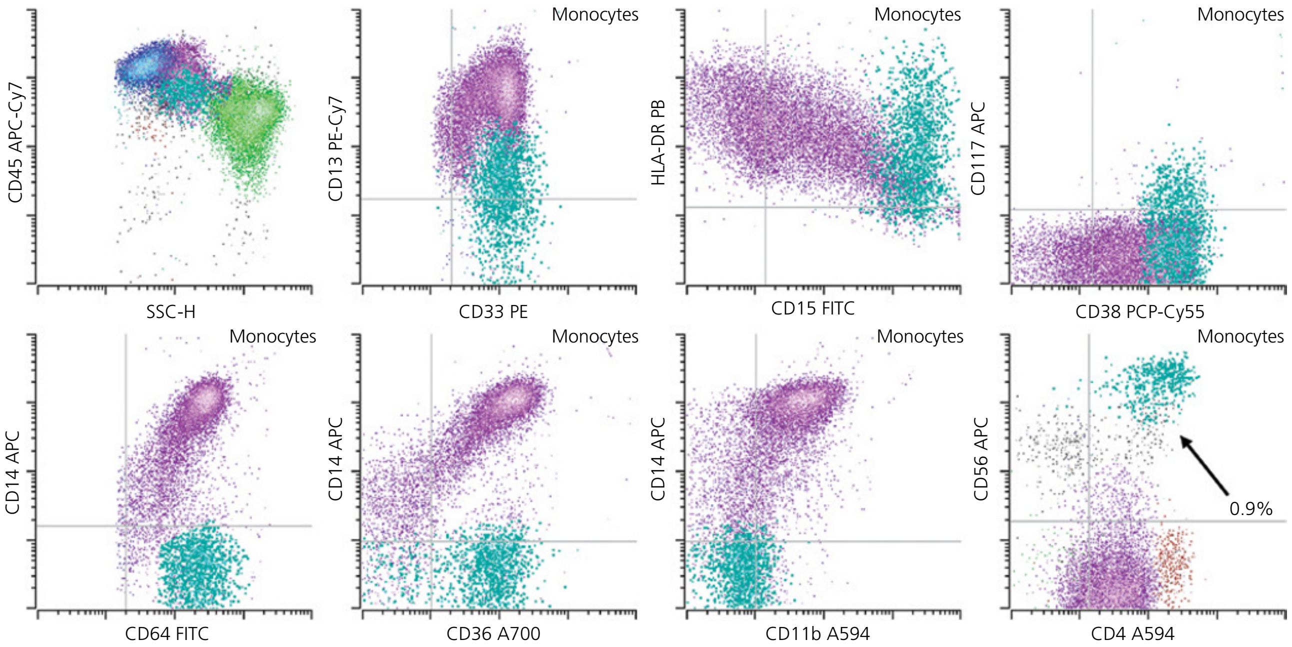


Figure 25.3 Flow cytometry assays for detection of minimal residual disease. Leukemia samples can be labeled with multiple fluorescent antibodies, and cells can be distinguished by the size of the cell and the types of antibody that bind the cell surface. Normal hematopoiesis has very stereotypical cell antigen expression linked to differentiation; leukemia cells are distinguished by inappropriate expression patterns of these antibodies. In the example, a residual acute monocytic leukemia is analyzed with multiple antigens. In the bottom left panel, the sample shows an inappropriate expression of CD56 and CD4 consistent with residual disease. In this example, these cells made up 0.9% of the total nucleated cells. Source: courtesy of Dr. Brent Wood, University of Washington.

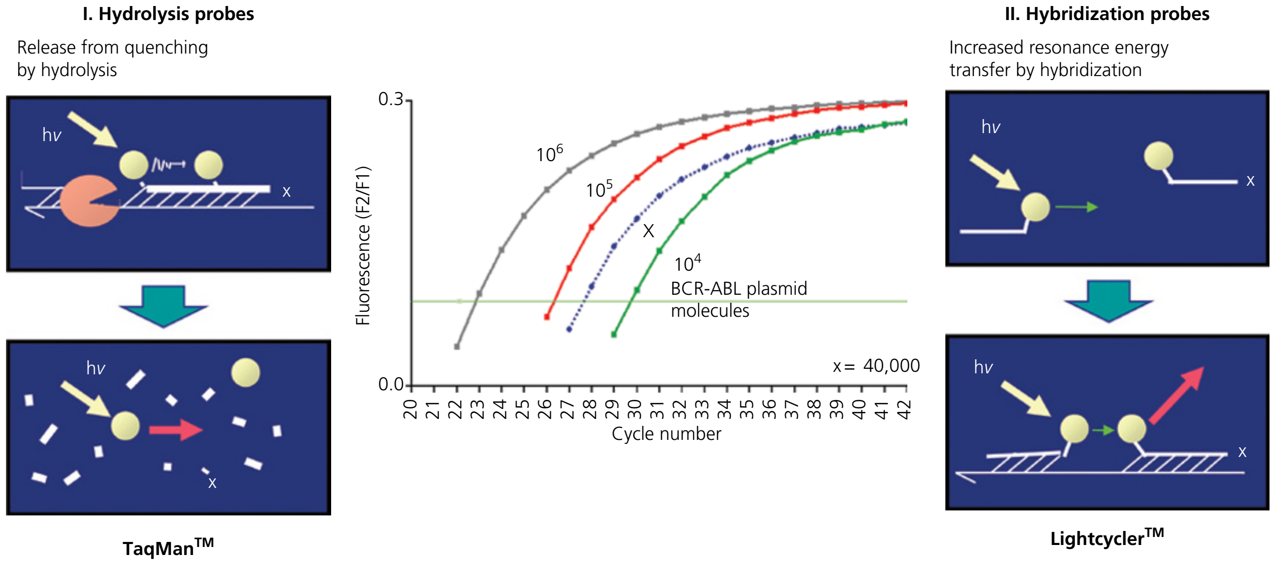

I. Hydrolysis probes
Release from quenching by hydrolysis
hν
x
hν
x
TaqMan™
0.3
0.0
Fluorescence (F2/F1)
10^6
10^5
X
10^4
BCR-ABL plasmid molecules
x = 40,000
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42
Cycle number
II. Hybridization probes
Increased resonance energy transfer by hybridization
hν
x
hν
x
Lightcycler™

Figure 25.4 Quantitative polymerase chain reaction (PCR). The cartoon shows the principle behind the two most popular quantitative approaches: the Taqman (ABI) and the LightCycler (Roche). In the Taqman reaction (I), the probe is a 5′ reporter dye and a 3′ quencher molecule. When the probe is intact, the quencher blocks laser excitation of the reporter. During PCR, the 5′ exonuclease activity of Taq polymerase releases the reporter, which, free from the quencher, is excited by the laser detection system. In the LightCycler system (II), annealing of juxtaposition probes during PCR synthesis allows for the transfer of resonance energy that can be stimulated by the laser and detected. In both systems, a standard curve is generated that plots dilutions of the standards versus the PCR cycle number at which a threshold signal is obtained (middle panel). The starting amount of target in unknown samples is thus detected by a back-calculation of cycle threshold in the unknown (Ct) against the standard curve. Source: Reproduced with permission of Dr. John Goldman.

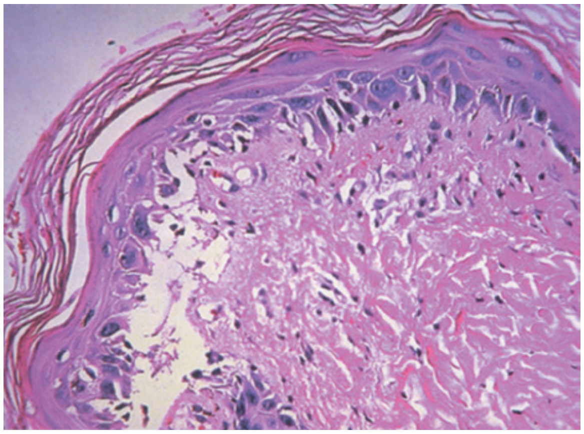


Figure 26.1 Skin biopsy from a recipient of identical twin marrow who had received a single 15 mg/kg dose of dimethylbusulfan 15 days previously. The epidermal separation, keratinocyte atypia, and scanty lymphoid dermal infiltrate can also be seen in GVHD.

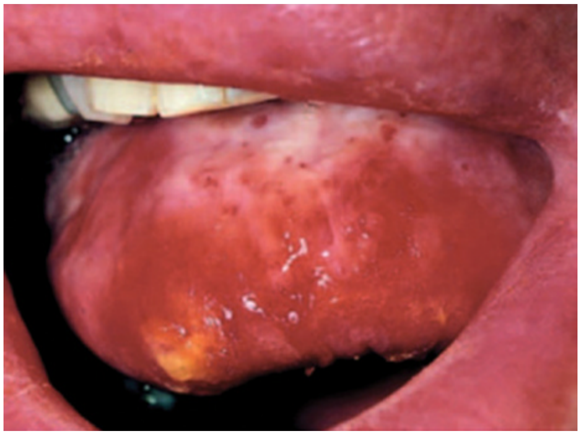


Figure 26.2 Acute glossitis 1 day post-HCT after a conditioning regime of 60 mg/kg of cyclophosphamide (CY) followed by 12 Gy of fractionated total body irradiation (TBI). There is early mucosal damage with severe erythema and focal atrophy.

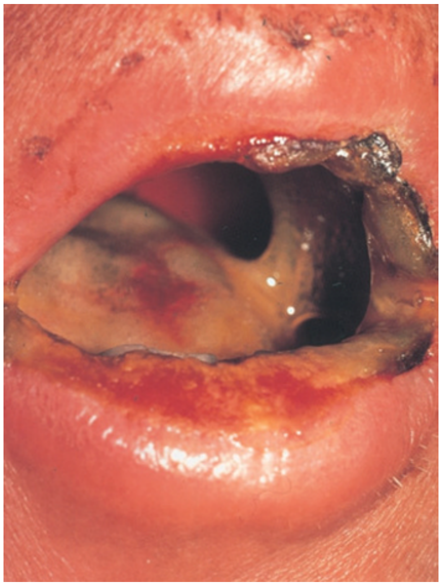


Figure 26.3 Severe mucositis of lips and tongue with pseudomembranous exudate 20 days after HCT following conditioning with TBI and chemotherapy. Similar changes may be present with severe acute GVHD or herpetic infection.

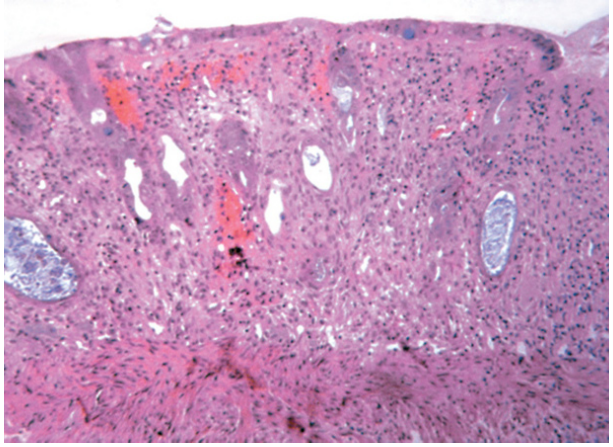


Figure 26.4 Effect of cytoreductive therapy on colonic mucosa, day 7. This biopsy specimen was taken following therapy with busulfan (BU), CY, and TBI (12 Gy). There has been obliteration of almost all epithelial cells, leaving cystic crypt remnants. (H&E with Alcian Blue counterstain.)

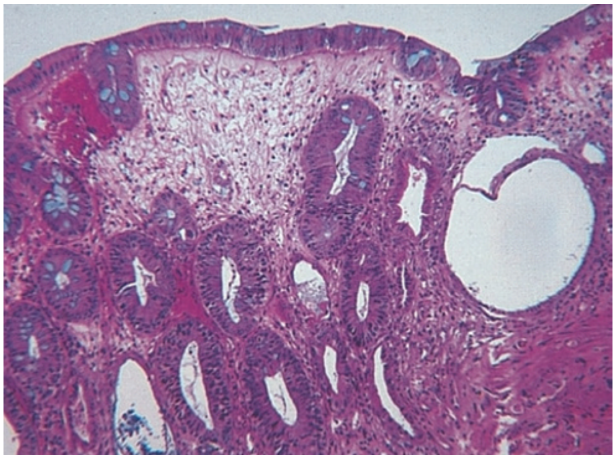


Figure 26.5 Recovery from the effects of cytoreduction therapy on colonic mucose, day 16. This repeat biopsy from the same patient as in Figure 26.4 indicates that regeneration is occurring, although some cystic crypts remain. (H&E with Alcian Blue counterstain.)

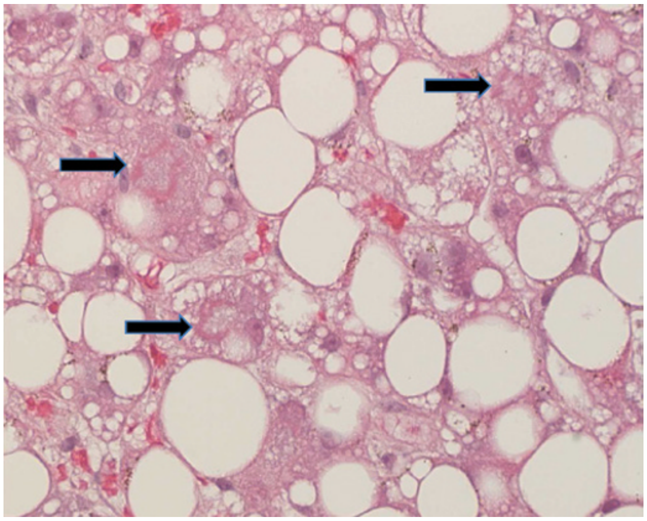


Figure 26.6 H&E-stained liver biopsy demonstrating changes of non-alcoholic steatohepatitis (NASH) with extensive macro- and lesser amounts of microsteatosis, hepatocyte ballooning, and focal Mallory body formation (arrow).

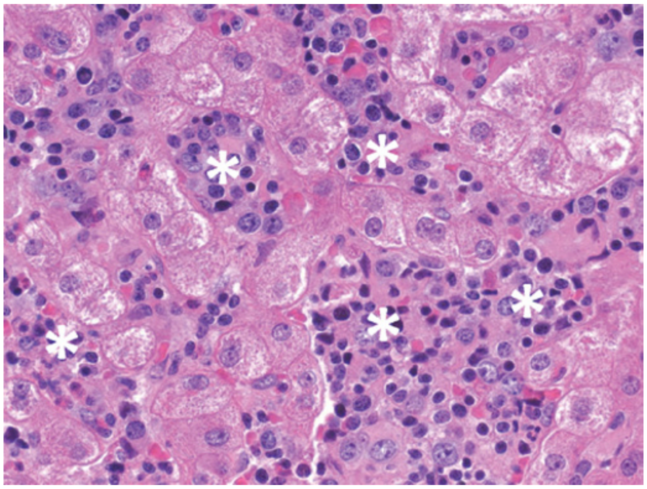


Figure 26.7 H&E-stained section of a pre-transplant liver biopsy from a patient with myelofibrosis. The hepatic sinusoids (white asterisk) are expanded by a mixed cellular infiltrate of hematopoietic precursors.

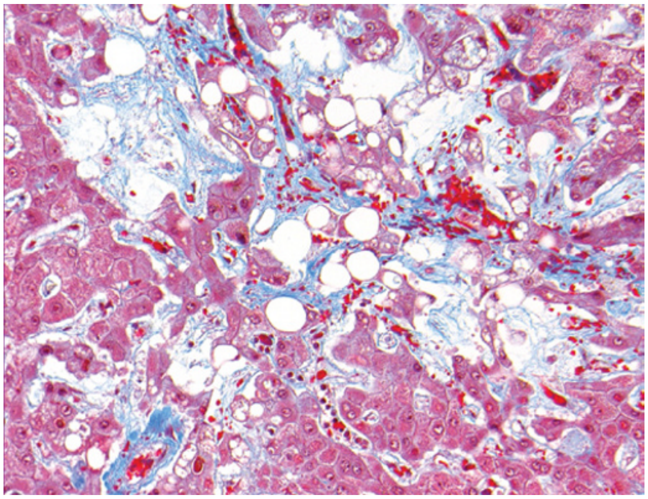


Figure 26.8 Fatal gemtuzamab ozogamicin (Mylotarg™)-associated sinusoidal obstruction syndrome. The obliterated central venule is surrounded by focal sinusoidal fibrosis. (Trichrome stain.)

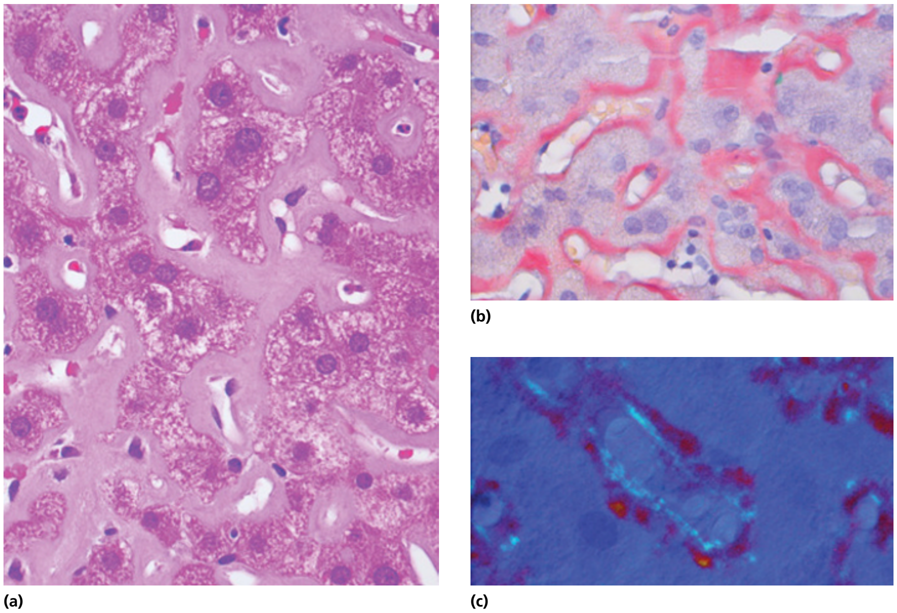


Figure 26.9 (a) H&E-stained section of a pre-transplant liver biopsy demonstrating extensive amyloid deposition in the liver sinusoids. (b) Congo Red stain of the same liver biopsy, with the sinusoidal amyloid deposit highlighted by salmon-pink staining. (c) Congo Red stain under polarized light: the sinusoidal amyloid deposit shows apple-green birefringence.

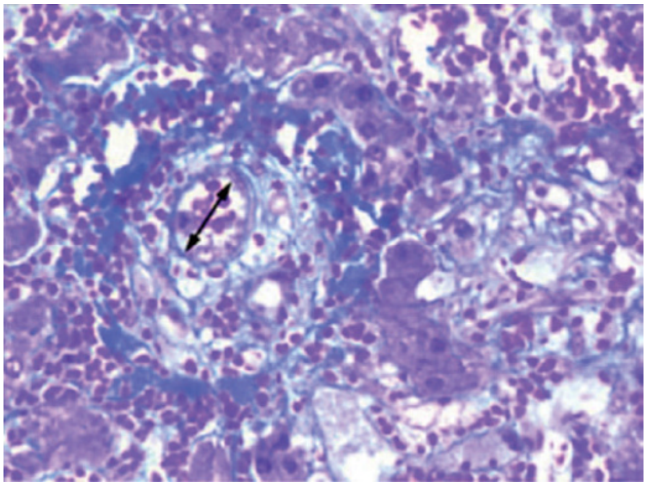


Figure 26.10 Hepatic sinusoidal obstructive syndrome (SOS) 95 days post-HCT for malignancy. The trichrome stain shows the blue outline of a hepatic venule with marked luminal narrowing (arrow shows lumen diameter) caused by subendothelial trapping of red cells. The hepatocytes of acinar zone 3 are severely disrupted. When the clinical diagnosis of hepatic SOS is uncertain, it may be confirmed by a transvenous liver biopsy with measurement of an elevated gradient between wedged and free hepatic venous pressures.

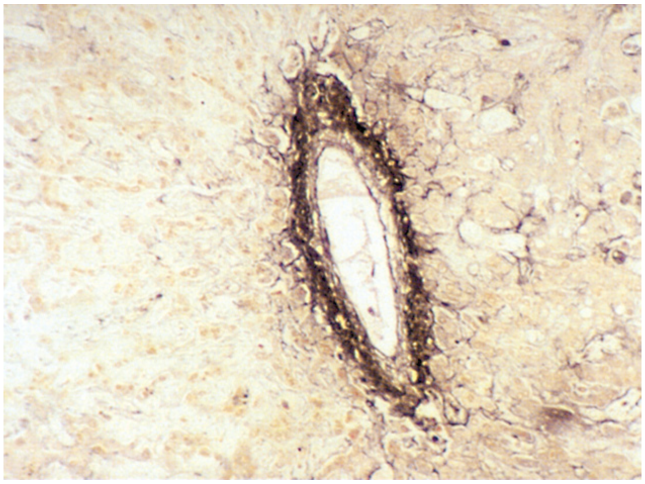


Figure 26.11 Early hepatic SOS showing perivenular staining for procoagulants. Immunohistochemical staining for factor VIII (von Willebrand) demonstrates post-sinusoidal obstruction corresponding to the endothelium-lined pores through which the sinusoids drain into the venules. Immunostaining for fibrin localizes in the same regions. These observations provide some of the rationale for using anticoagulation and anti-thrombotic therapy for hepatic SOS.

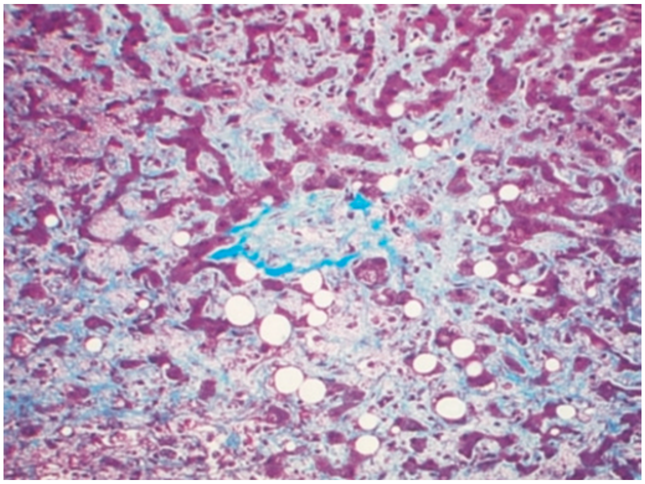


Figure 26.12 Late hepatic SOS, day 63. The trichrome stain demonstrates fibrotic obliteration of the venous lumen with extensive fibrosis in surrounding sinusoids. The resulting vascular obstruction produced intractable ascites. Immunohistochemical stains at this stage reveal extensive deposition of collagen, while fibrin and other blood procoagulants are no longer identifiable.

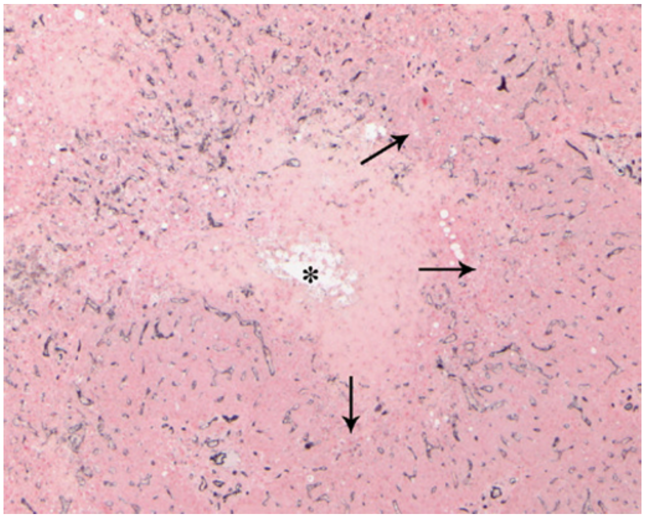


Figure 26.14 CD 31 immunostain of liver with fatal SOS. There has been partial to complete loss of sinusoidal endothelial staining of the hepatic cords in zone 3 (arrows) which surround the subtotally occluded venule lacking an endothelium (asterisk).

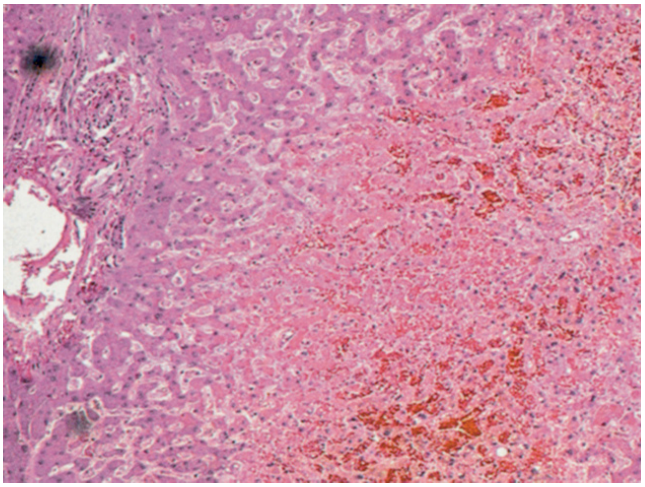


Figure 26.13 Fatal hepatic SOS at autopsy 12 days post BMT for leukemia. At this low magnification, the H&E-stained section demonstrates striking congestion, hemorrhage, and hepatocyte disruption in zone 3 (centrilobular) of the liver acinus toward the right. The portal space and surrounding acinar zone 1 on the left are well preserved. These early severe hepatic SOS lesions at times can be identified with the H&E stain after tissue fixation with B5 or methyl Carnoy's fixative. However, stains for connective tissue are more effective.

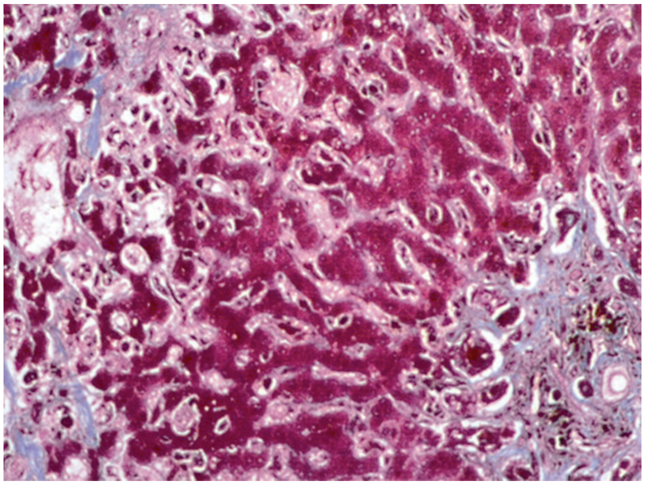


Figure 26.15 Hepatic SOS and coexisting GVHD, day 26. On this trichrome stain, the two most common liver problems after HCT can be differentiated by the zone of the liver acinus that they affect. The expanded portal space in the lower right demonstrates changes from GVHD, including fibrosis, proliferation of atypical cholangioles along the limiting plate, and destruction of small interlobular bile ducts. The SOS lesion along the left margin shows striking collections of embolized hepatocytes beneath the endothelial basement membrane.

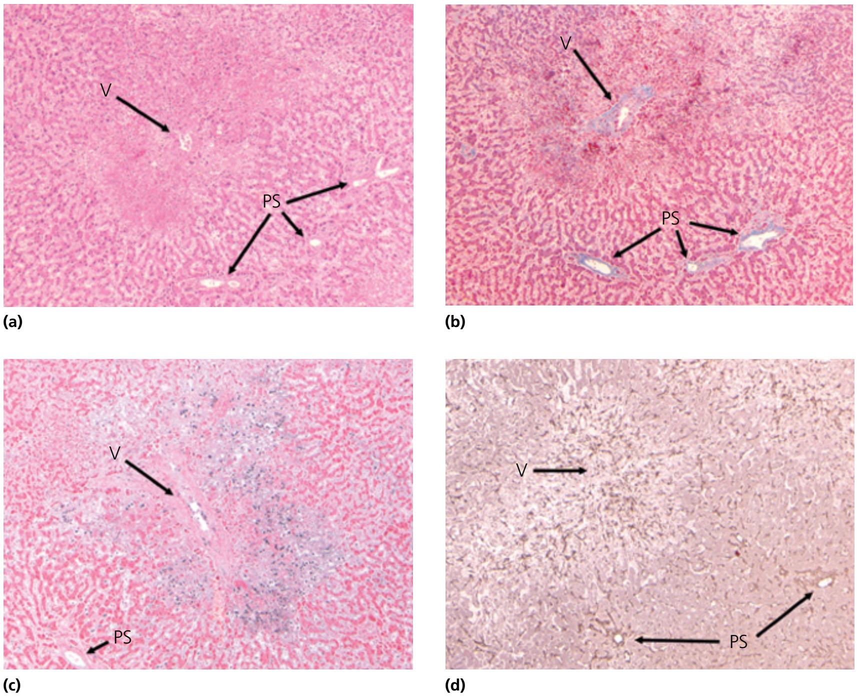


Figure 26.16 Fatal SOS. (a) H&E stain demonstrates a large perivenular zone of hemorrhagic necrosis with destruction of hepatocyte cords surrounding the damaged venule (V); the areas around the portal spaces (PS) are spared. (b) Trichrome stain demonstrates the subtotal occlusion of the venular lumen by collagen and extracellular matrix. (c) Immunostain Mac 387 for macrophage/monocytes demonstrates a zone of cells at the interface of the viable and necrotic hepatocytes. (d) Smooth muscle actin immunostain demonstrates marked expansion of hepatic stellate cell processes within the damaged sinusoids surrounding the venule.

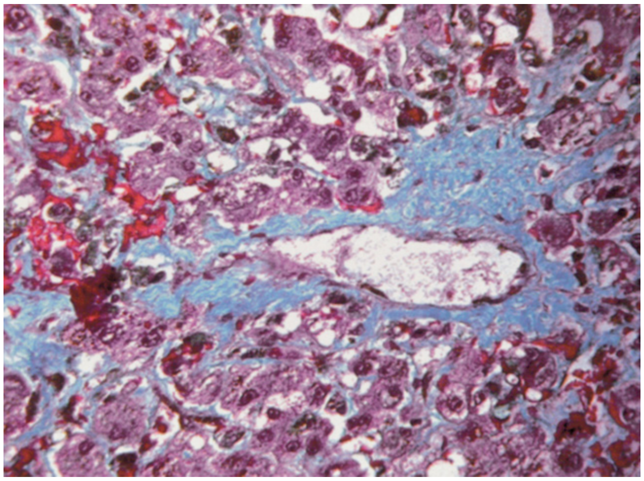


Figure 26.17 Hepatic phlebosclerosis, day 96. Eccentric thickening of the venular wall without striking luminal narrowing was associated with an early liver toxicity syndrome. The patient had received a 5-day infusion of high-dose cytosine arabinoside (Ara-C) followed by CY and TBI. Liver function abnormalities developed prior to the infusion of marrow. Changes of phlebosclerosis were widespread, but there were no identifiable hepatic SOS lesions at autopsy. (Trichrome stain.)

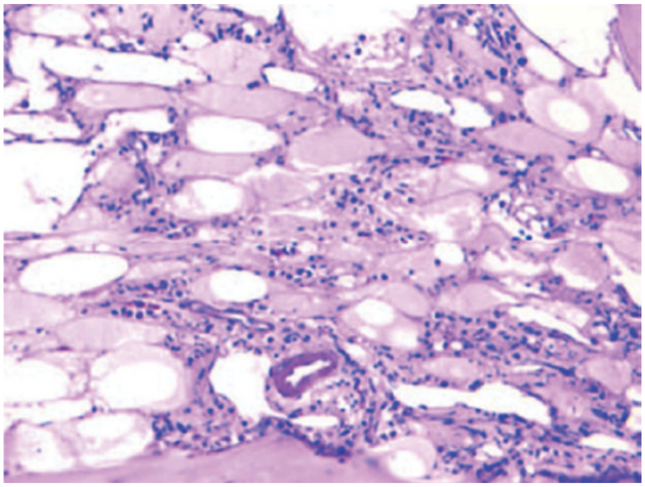


Figure 26.18 Markedly hypocellular marrow without evidence of engraftment, day 7. The interstitial fluid accumulation, fat necrosis, and iron deposition are consistent with chemoirradiation damage.

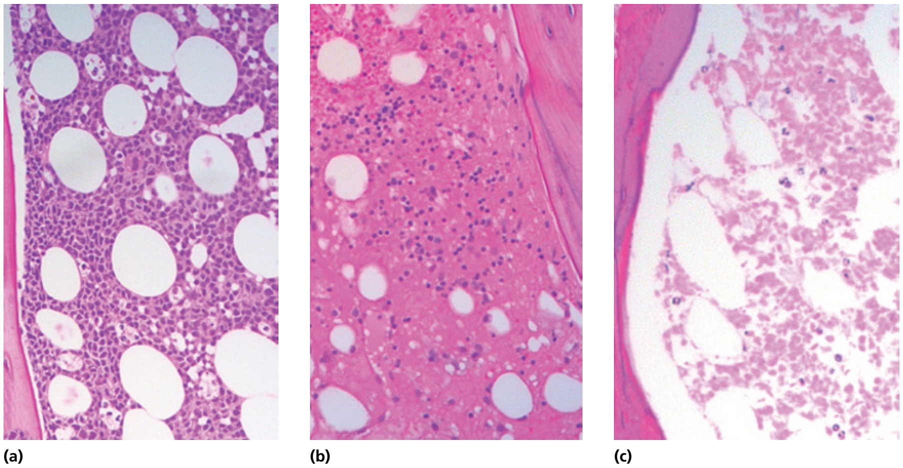


Figure 26.19 Same patient as in Figure 26.18 with acute myeloid leukemia: (a) diagnostic marrow with 96% blasts; (b) day 7 marrow after chemoreduction showing serous atrophy; (c) day 21 marrow with persistent pancytopenia showing extensive necrosis with only scattered neutrophils.

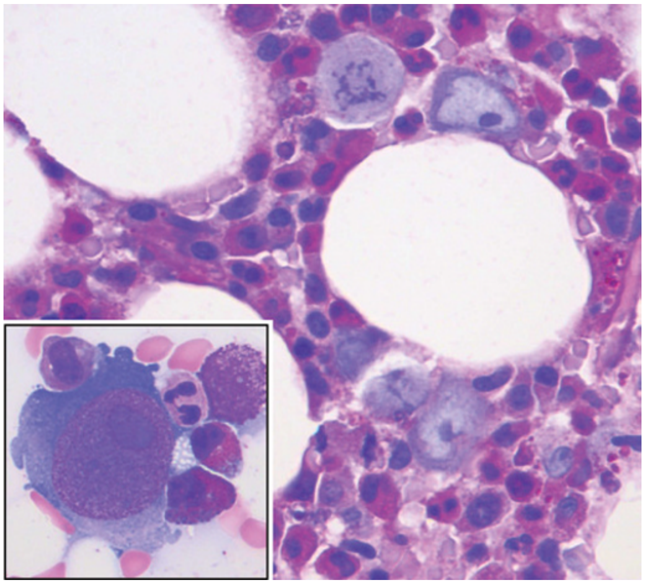


Figure 26.20 Pre-transplant marrow biopsy stained with periodic acid–Schiff (PAS) stain from a patient with diffuse large B-cell lymphoma with persistent anemia and quantitative Parvovirus virus PCR result of 13 billion. The PAS-stained marrow shows abnormally enlarged PAS negative proerythroblasts with prominent nucleoli and a marked erythroid hypoplasia. The inset at bottom left shows a Wright-stained aspirate smear with a markedly enlarged proerythroblasts with a prominent nucleoli.

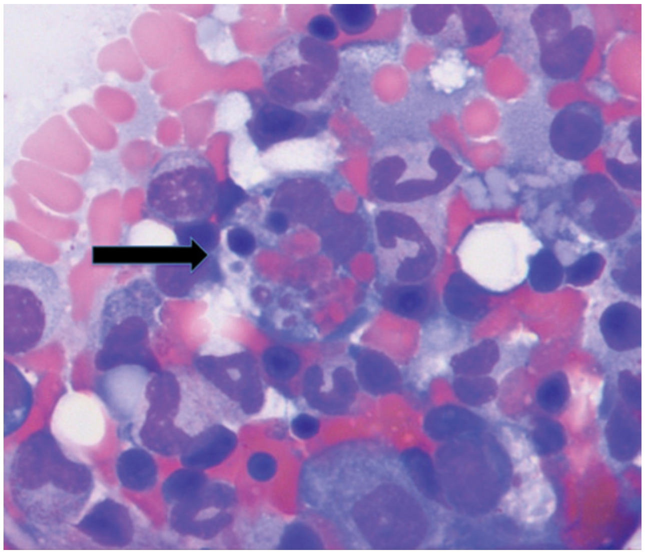


Figure 26.21 A Wright–Giemsa-stained marrow aspirate smear from a patient with large cell transformation of mycosis fungoides day 174 post-HCT with hemophagocytosis. A hematophagocytic macrophage with engulfed red blood cells and erythroid precursors in varying stages of degeneration is indicated by the back arrow.

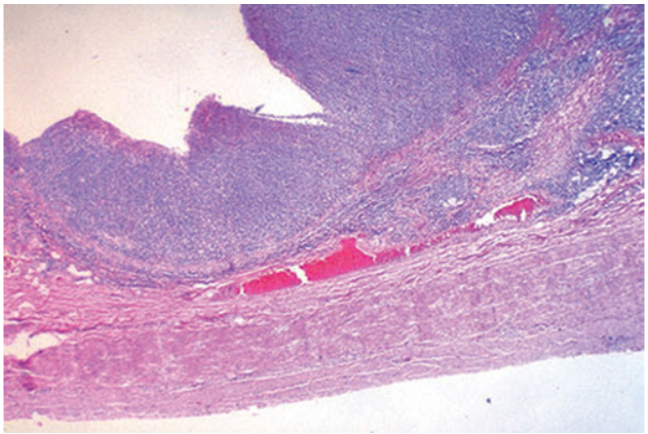


Figure 26.22 EBV-associated lymphoma infiltrating the intestinal mucosa in a boy treated for GVHD with cyclosporine and a monoclonal antibody to T-lymphocytes. Although the GVHD improved, large bizarre cells with atypical nuclei characteristic of a high-grade lymphoma rapidly formed tumor nodules in liver, spleen, lymph nodes, and mesentery.

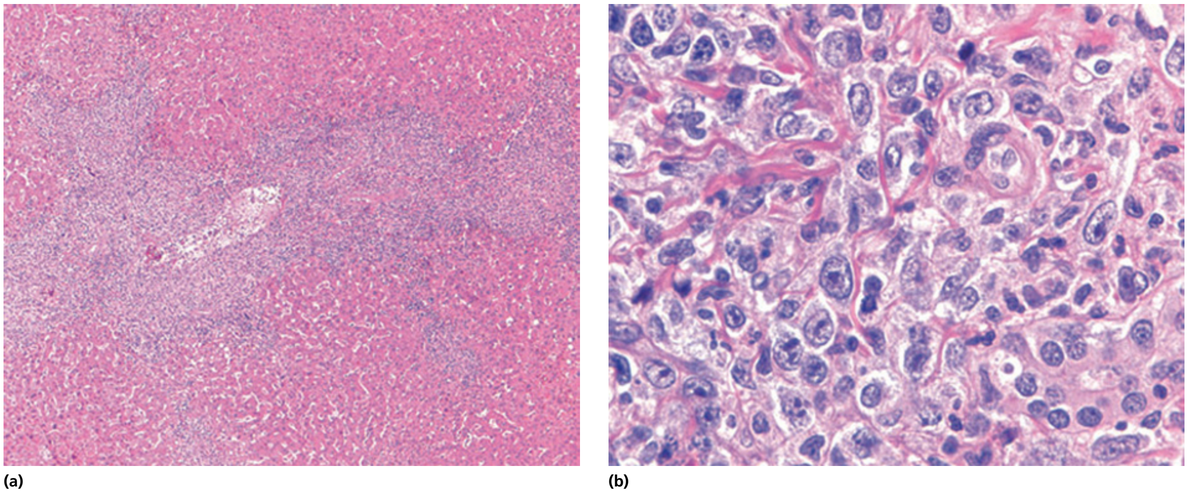


Figure 26.23 (a) Post-transplant EBV lymphoma. Following treatment for liver GVHD, the liver function abnormalities recurred. At low power, the portal space contains a prominent infiltrate that extends outwards into the periportal zone. (b) In the same case, higher magnification shows the large atypical immunoblastic cells that were positive for CD20 and EBER staining.

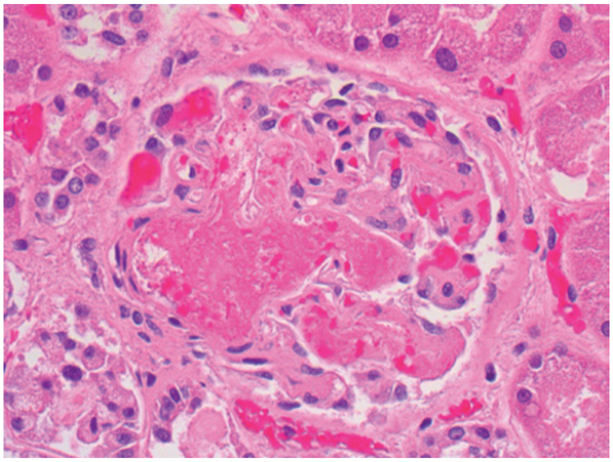


Figure 26.24 Kidney specimen stained with H&E showing transplant-associated microangiopathic/hemolytic (TAM) syndrome in a patient with AML progressed from MDS after HCT and died on day 21. There is thrombi formation in the capillary loops of the glomerulus, and characteristically showing involvement of the afferent vessel.

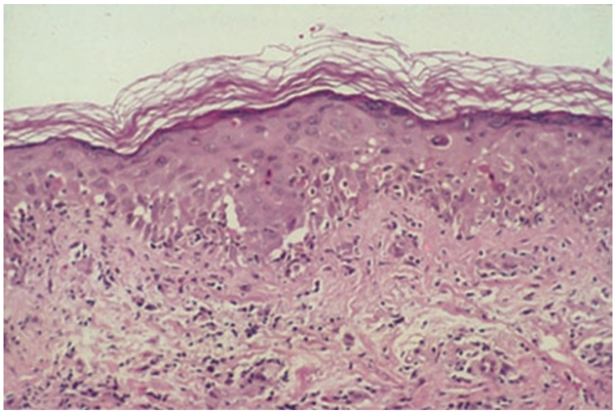


Figure 26.25 Acute skin GVHD, day 28 after mismatched allogeneic HCT. The epidermis demonstrates a prominent lichenoid reaction involving the tip of the rete ridge in the center of the specimen, a location of epithelial stem cells within the epidermis.

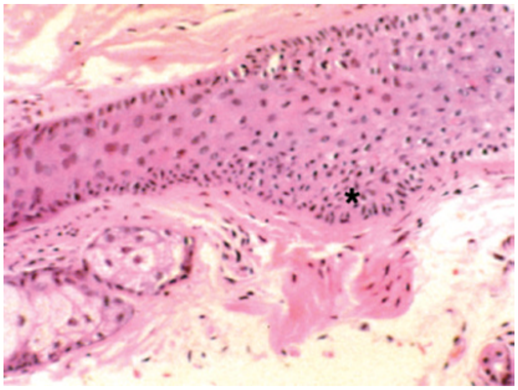


Figure 26.26 Early skin GVHD involving the parafollicular bulge region of the pilar unit. Note the small numbers of mononuclear inflammatory cells which infiltrate the parafollicular bulge area (*) near the arrector pilorum muscle in association with apoptotic bodies.

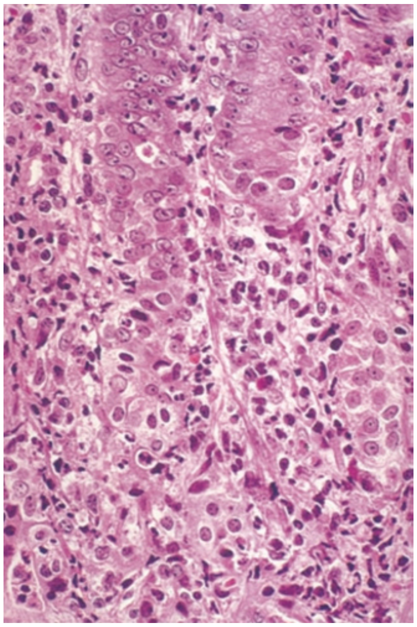


Figure 26.27 Gastric biopsy with GVHD, day 35. Several studies have shown that of all upper endoscopic biopsy sites, the stomach most frequently shows histologic changes of GVHD. In the area shown here, large numbers of lymphocytes infiltrate the lamina propria and the basilar portions of the gastric crypts. There is apoptosis of crypt epithelial cells and frank early crypt destruction. Inflammation and apoptosis of this intensity are not required for the diagnosis of gastric GVHD. More subtle yet still diagnostic alterations are illustrated in Figures 26.50 and 26.51.

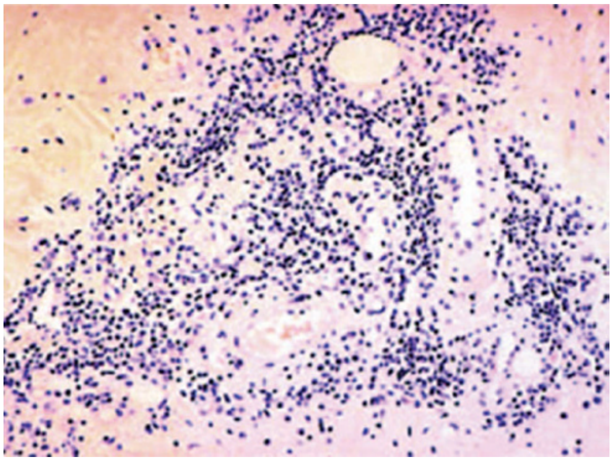


Figure 26.28 Atrophy and fibrosis of thymus associated with chronic GVHD in a 13-year-old, day 350. The patient had not received immunosuppressive drugs, so the destruction was presumably the result of chronic GVHD activity.

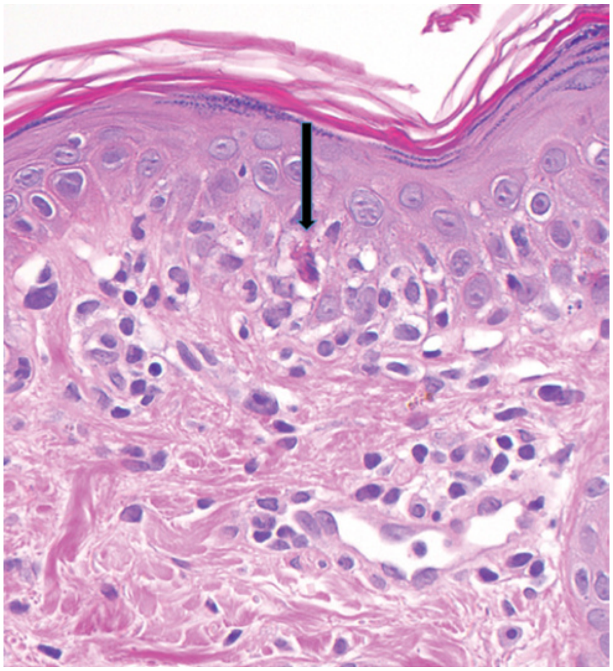


Figure 26.30 H&E stain of a skin biopsy from a, HCT patient day 25 with GVHD. There is mild spongiosis, basal vacuolization, lymphocytic inflammatory cells, and a few scattered apoptotic cells (arrow).

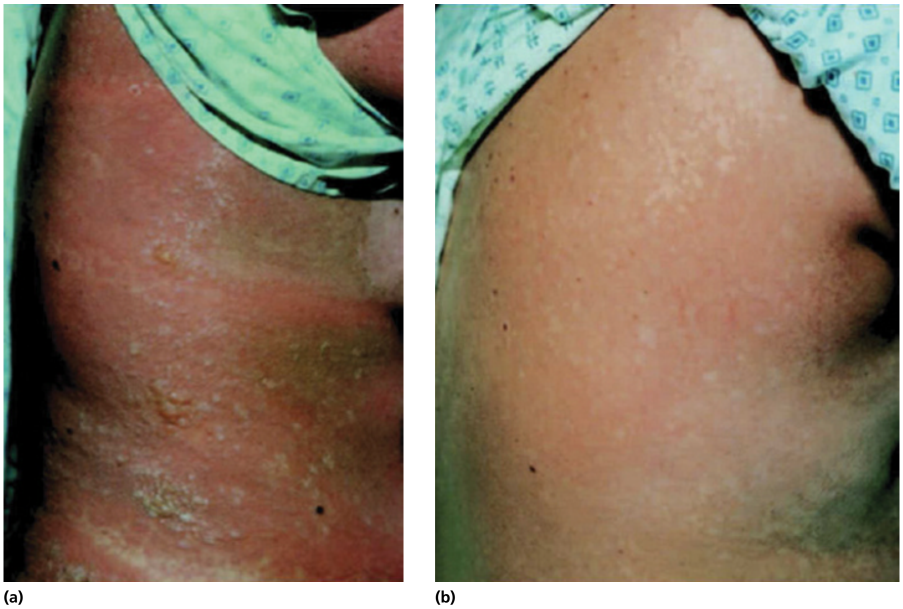


Figure 26.32 Before and after therapy photographs of the right lateral torso from the two different time points in an HCT patient: (a) erythema and desquamation before treatment for acute GVHD, day 27; (b) virtually normal skin with focal depigmentation after treatment for acute GVHD, day 74.

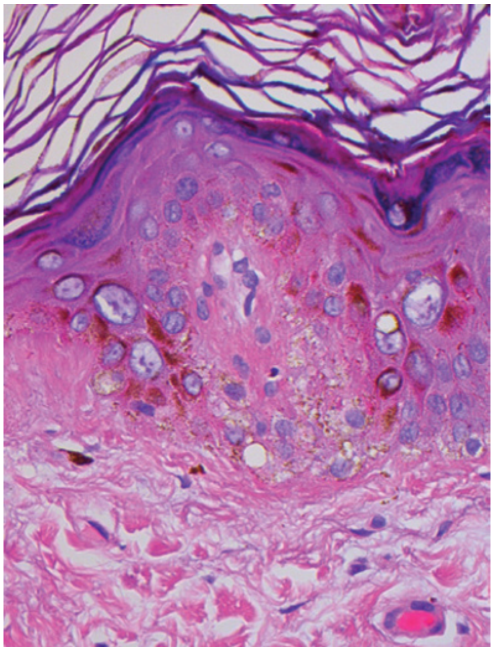


Figure 26.33 Severe keratinocyte dysplasia in a patient with myelodysplastic syndrome who is day 76 after myeloablative HCT with busulfan-based chemoreduction therapy.

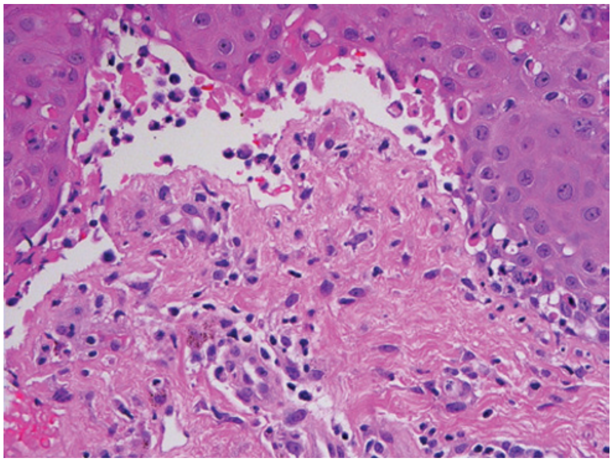


Figure 26.34 Skin biopsy from a patient who is day 36 after HCT with an erythematous non-pruritic rash involving 40% of the total body surface including the upper trunk, arms, head, and a few spots on the legs. This biopsy shows confluent basal cell necrosis with separation at the dermal epidermal junction, a histologic pattern more consistent with Stevens–Johnson type of drug eruption.

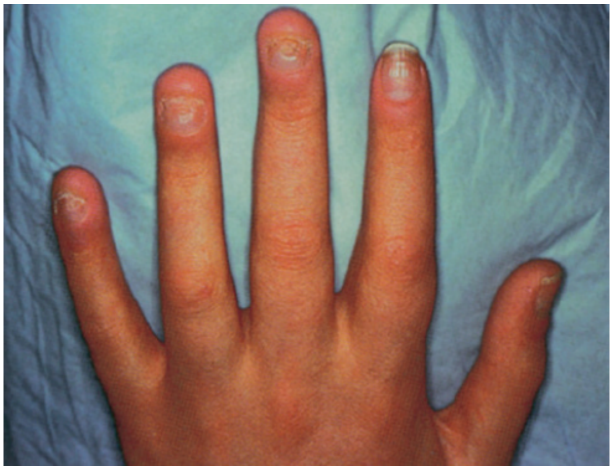
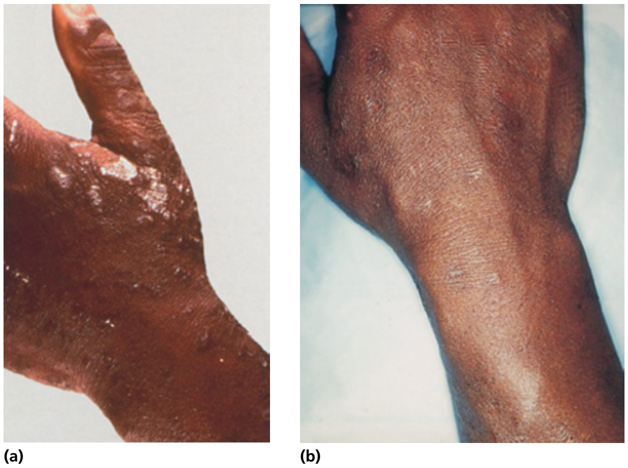


Figure 26.35 Periungual erythema and onychodystrophy 100 days after allogeneic BMT in a patient with ocular sicca and oral lesions of chronic GVHD.



(a)

(b)

Figure 26.36 Photographs from a 24-year-old African American male who received an human leukocyte antigen (HLA)-identical HCT: (a) hand with lichen planus-like chronic GVHD day 220 post-HCT; (b) forearm after treatment, day 770 post-HCT.

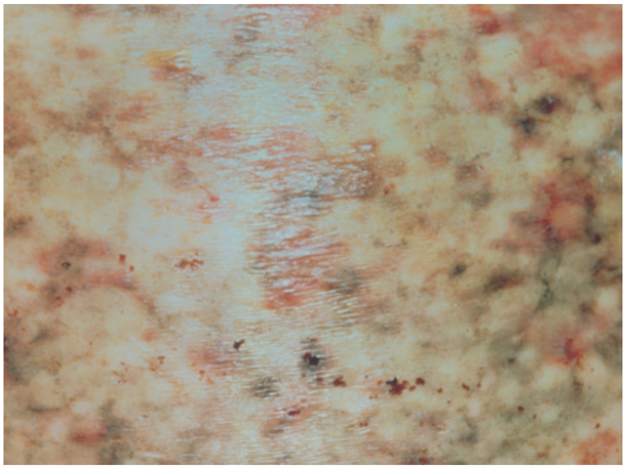


Figure 26.37 Poikiloderma in late chronic GVHD in a 2-year-old girl, day 450. Thinning of the epidermis and dermis is present along with telangiectasia and reticulated pigmentation.

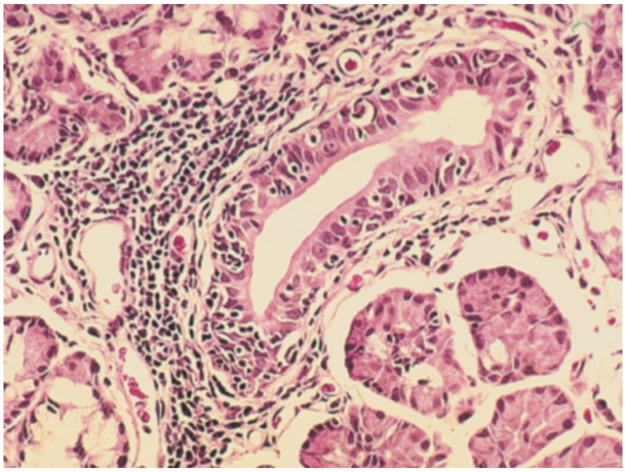


Figure 26.40 Oral labial biopsy with early chronic GVHD, day 92. The minor salivary gland exhibits a periductal mononuclear inflammatory infiltrate involving the duct epithelium, in which there is focal epithelial apoptosis. There is inflammation of adjacent acini. This process leads to fibrosis with acinar atrophy in lobules drained by the affected ducts. The squamous surface epithelium (not present here) showed grade II lesions similar to those previously illustrated in skin (see Figure 26.25). The lip biopsy is valuable in the diagnosis and staging of chronic GVHD because it frequently mirrors destructive inflammatory changes in lacrimal, tracheal, and esophageal glands as well as bile ducts.

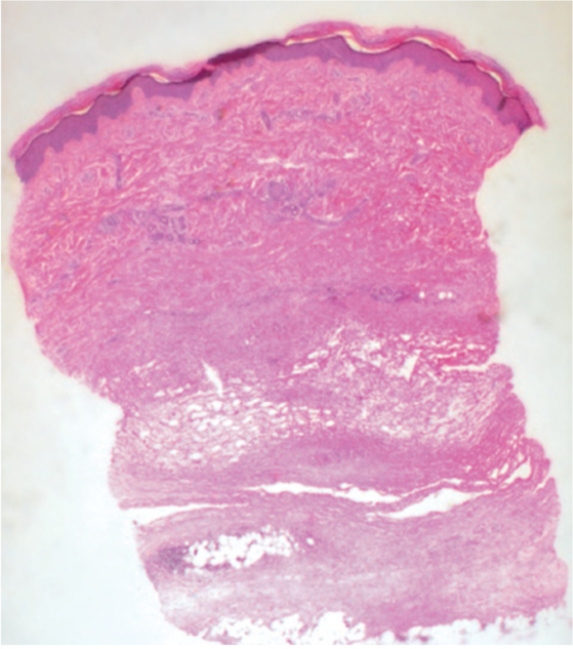


Figure 26.41 Late chronic GVHD of skin, day 959. As this patient's GVHD progressed, the epidermis became atrophic, with straightening of the dermal–epidermal junction. All dermal appendages were destroyed. The reticular dermal collagen became increasingly sclerotic. A panniculitis seen at the base of the specimen resulted in fibrosis of the subcutaneous fat and large vessels. This histologic picture corresponds to the dense hidebound and sclerodermatous changes of late chronic GVHD.

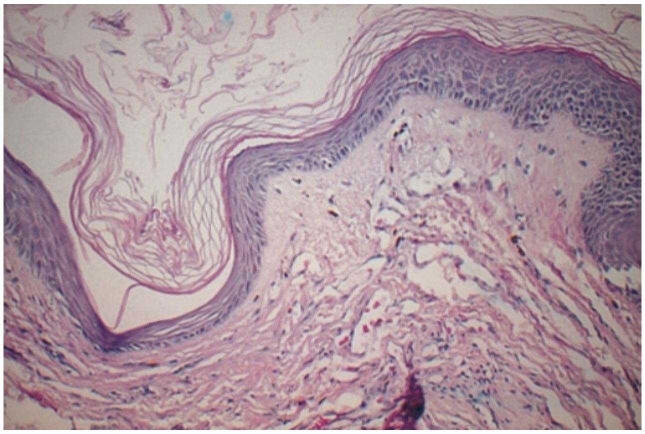


Figure 26.43 Poikiloderma, day 719. The patient was treated vigorously for extensive chronic GVHD with corticosteroids plus several other immunosuppressive agents. Although he had extensive dyspigmentation, telangiectasia, and atrophy of the skin, there were no contractures or scleroderma. Histologically, the epidermis is atrophic, with loss of rete ridges. The fibrotic papillary dermis contains many telangiectatic vessels, and there is intracellular melanin pigment. The deep dermal sweat glands remain despite earlier inflammatory involvement, and the reticular dermal collagen is normal.

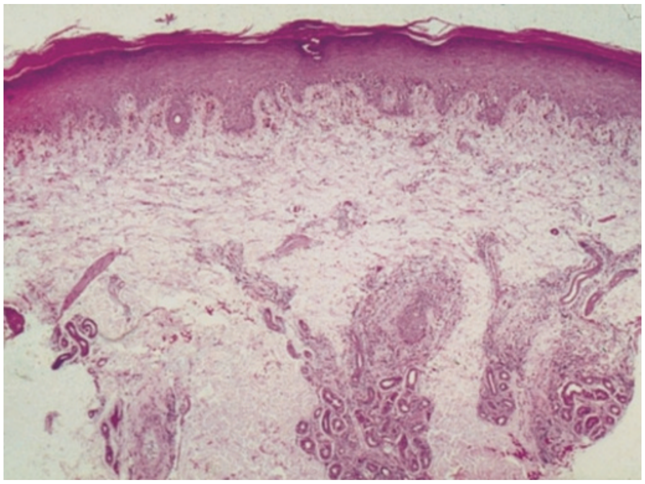


Figure 26.44 Lichenoid early chronic GVHD of skin, day 233. Biopsy of a papulosquamous lesion from the posterior neck demonstrates the characteristic features of the lichen planus-like type of chronic cutaneous GVHD. The thickened epidermis displays hyper- and parakeratosis, hypergranulosis, and acanthosis. The extensive destruction along the dermal–epidermal junction results in sawtooth-like changes of the rete ridges, mimicking lichen planus. The papillary dermis has considerable perivascular inflammation. The lichenoid reaction also involves the hair follicles and eccrine glands seen deep in the reticular dermis. At this stage, treatment can prevent progression to fibrosis (see Figure 26.41).

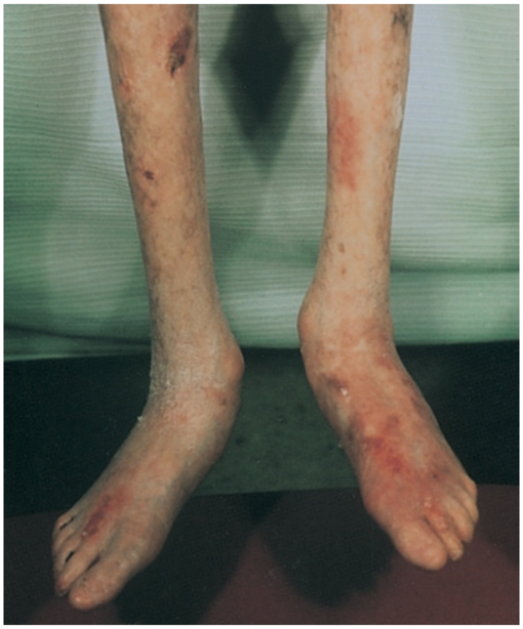


Figure 26.45 Generalized scleroderma with atrophy, sclerodactyly, and joint contracture, day 540 after HLA-identical BMT.

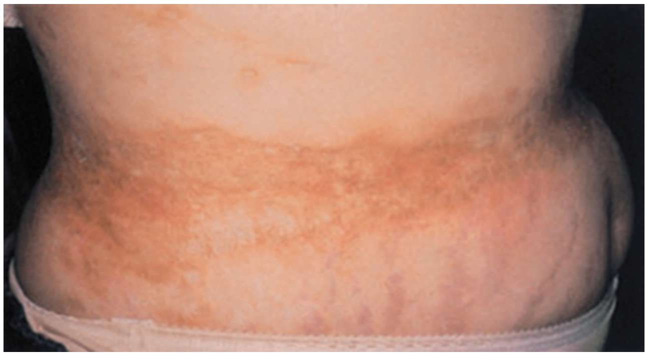


Figure 26.46 Localized late phase of chronic GVHD resembling morphea with atrophic plaque formation, induration, and peripheral hyperpigmentation, day 684.

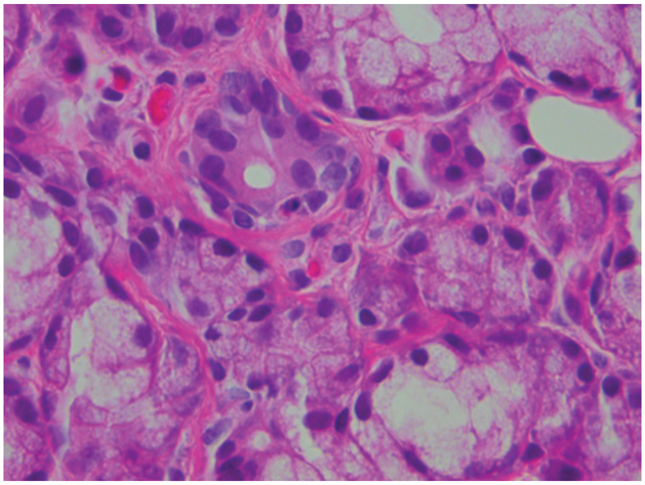


Figure 26.48 GVHD in a salivary gland biopsy from a patient with aplastic anemia day 81 after HCT on with apoptotic cells in the intercalated ducts and architectural distortion with nuclei disarray.

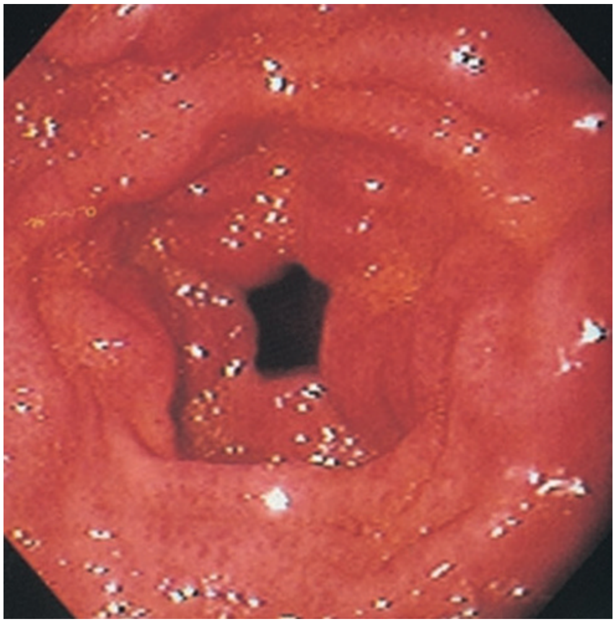


Figure 26.49 Endoscopic appearance of acute GVHD in the stomach, day 32. The pyloric channel is in the center of the picture. The mucosa of the gastric antrum is edematous, reddened, and friable.

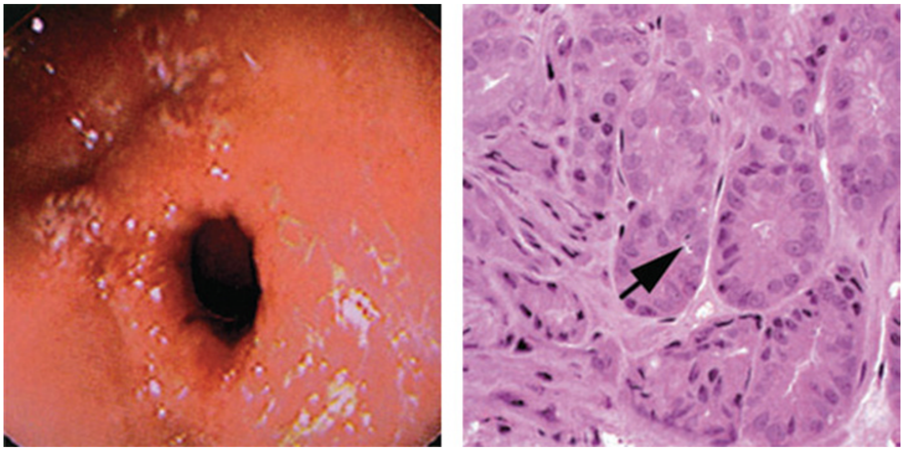
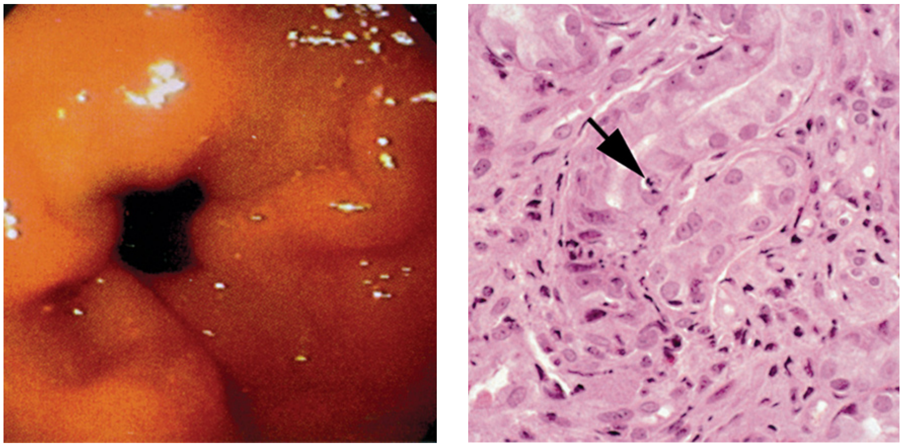


Figure 26.50 Gastric GVHD in which the severe mucosal erythema, edema, and erosion seen endoscopically on the left are more striking that the focal, mild, epithelial cell apoptosis (arrow) in the histological section on the right. Although a lymphocytic infiltrate is absent, apoptosis in multiple crypts is consistent with graft-versus-host activity. Inflammation may have been partially controlled by immunosuppressive therapy. Source: Ponec et al. 1999 [118]. Reproduced with permission of Elsevier.



(a) (b)

Figure 26.51 Gastric GVHD in which the antral mucosa is endoscopically normal aside from edema (left). With air insufflation through the endoscope the antrum did not distend and there was no motor activity. Biopsy of such a bland area can be diagnostic. In this case, as shown in the photomicrograph on the right, there was epithelial cell apoptosis (arrow) and moderate lymphocytic infiltration diagnostic for GVHD. Source: Ponec et al. 1999 [118]. Reproduced with permission of Elsevier.

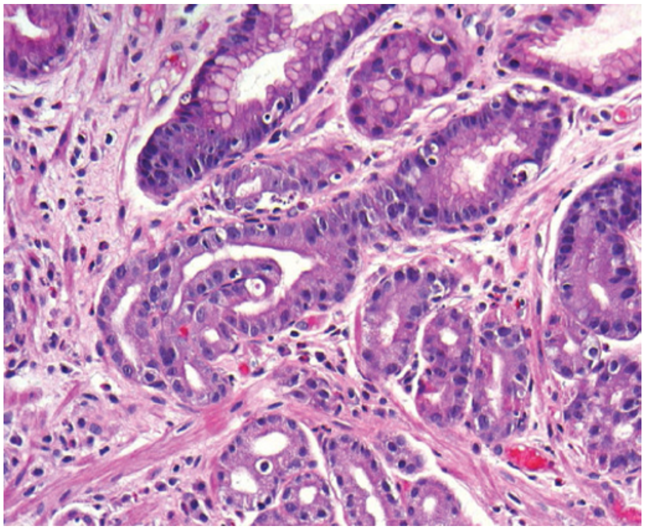


Figure 26.53 Gastric GVHD with architectural disarray and edematous antral mucosa. There are numerous intraepithelial lymphocytes infiltrating glands which also demonstrate reactive atypia. Apoptosis is widespread along with segmental disruption of the glands.

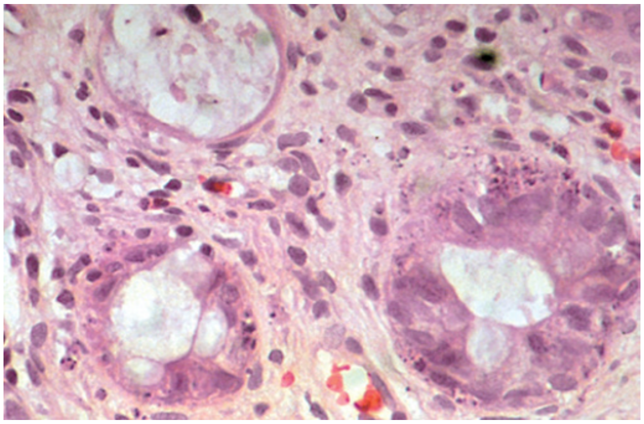


Figure 26.55 Rectal biopsy with GVHD, day 53. Three mucosal crypts display varying degrees of damage. The crypt on the right shows many apoptotic cells. More extensive cell damage is present in the crypt on the lower left. The upper crypt has lost all enterocytes. Note infiltrating lymphocytes in the lower left crypt associated with nuclear debris characteristic of apoptosis. Source: reproduced with permission of Dr. Robert C. Hackman.

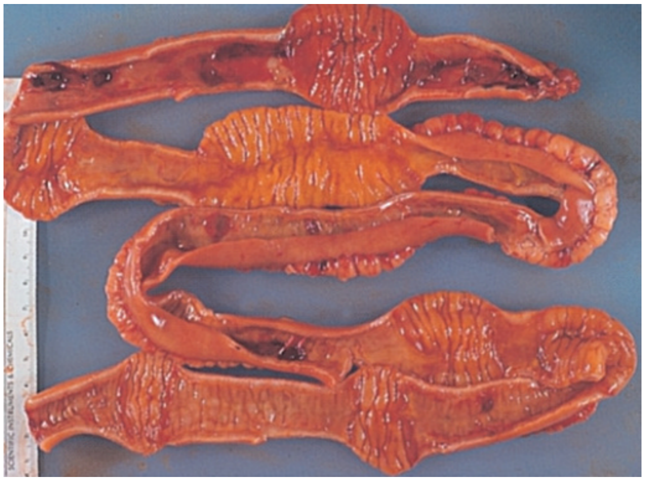


Figure 26.57 Surgical resection of severe long-standing intestinal GVHD, day 207. Scarred, ulcerated mucosa alternates with dilated areas of mucosal regeneration. The ulcerated portions demonstrate complete loss of epithelium and severe submucosal fibrosis. There is very high risk of invasive fungal or bacterial infection. Some patients have responded well to intestinal resection for localized GVHD.

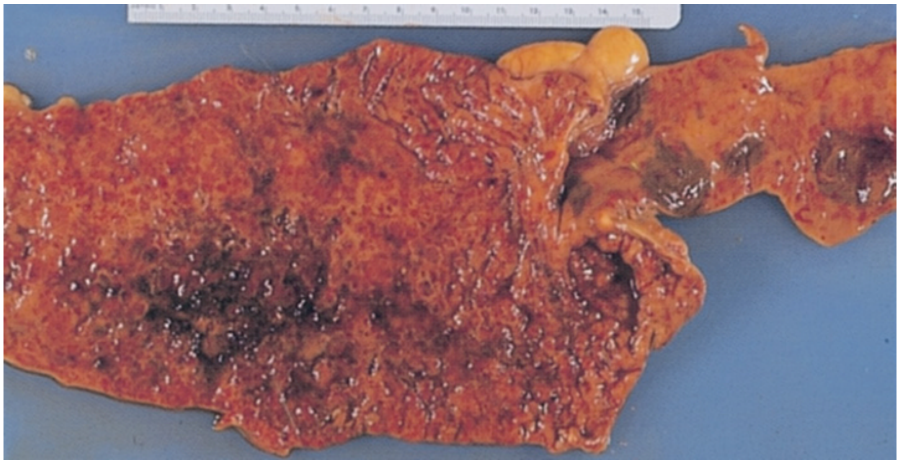


Figure 26.58 Severe GVHD of ileum and cecum, day 80. The mucosal lining and folds are replaced by a friable, beefy red, diffusely ulcerated lamina propria. The numerous exposed capillaries ooze blood and serum, a major cause of morbidity from intestinal GVHD. The endothelial cells and fibroblasts within the lamina propria are often infected with CMV, as was true in this patient.

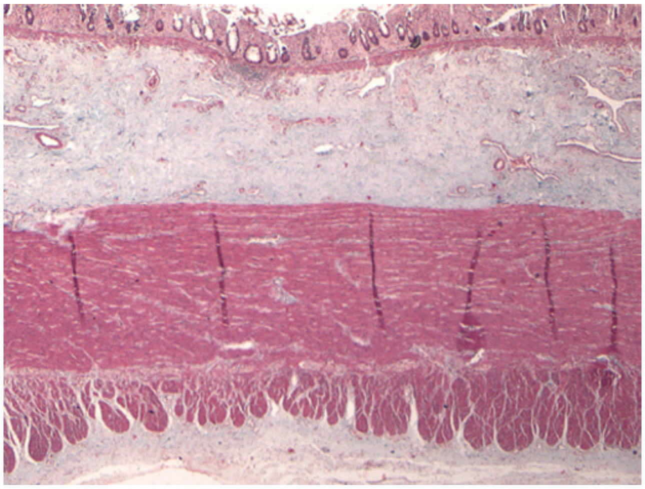


Figure 26.59 Collagenous colitis with long-standing refractory extensive chronic GVHD. The thickened bowel has extensive deposition of collagen in the submucosa, serosa as well the lamina propria.

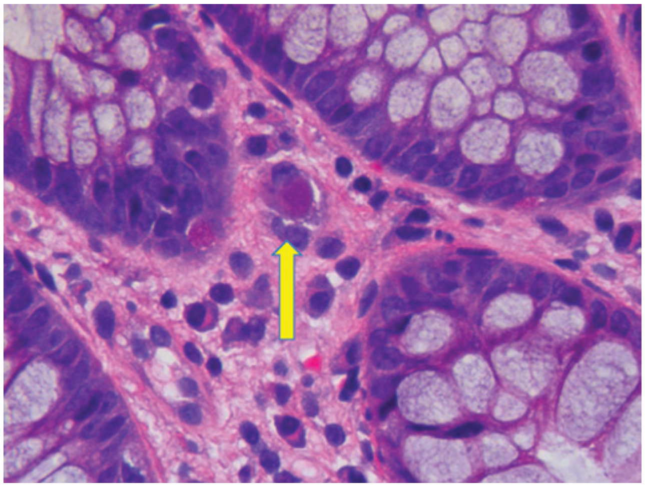


Figure 26.60 CMV infection in a patient with T-cell prolymphocytic leukemia from day 516 after HCT with chronic diarrhea. The yellow arrow indicates the classic large eosinophilic inclusion in a stromal cell.

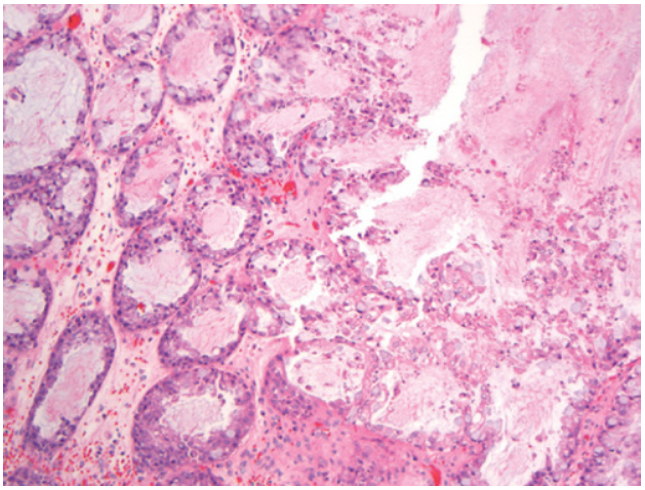


Figure 26.61 Fatal pseudomembranous colitis. An acute abdomen developed 15 days post-transplant. The degenerative colonic crypts were covered with a cap of mucin admixed with sloughed cellular debris containing large colonies of bacteria consistent with *Clostridium* sp.

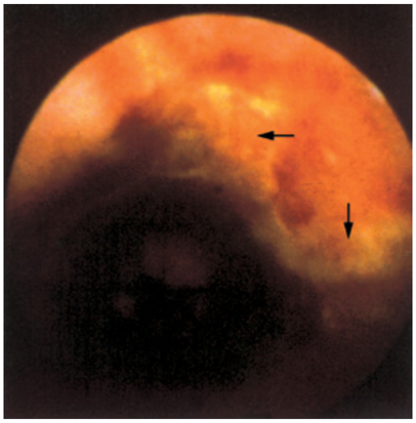


Figure 26.62 Endoscopic appearance of CMV esophagitis, day 61. A large, shallow ulcer with serpiginous, reddened borders is seen in the distal esophagus. The ulcer base (arrows) has a yellow, reticulated appearance.

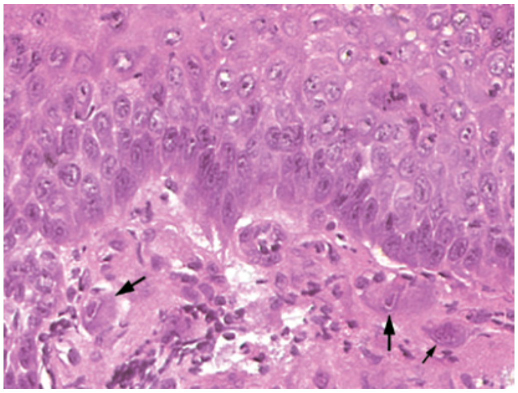


Figure 26.63 Esophageal biopsy showing CMV infection, day 61. Beneath the epithelium are two cytomegalic cells (large arrows), each containing a single, large, oval intranuclear inclusion surrounded by a clear halo (Cowdry type A). A third cell (small arrow) is probably also infected but is morphologically non-diagnostic. It would presumably be positive by immunocytochemistry and in situ hybridization techniques. Studies of cell morphology and also viral DNA and antigen expression indicate that the esophageal squamous epithelium is never infected with CMV.

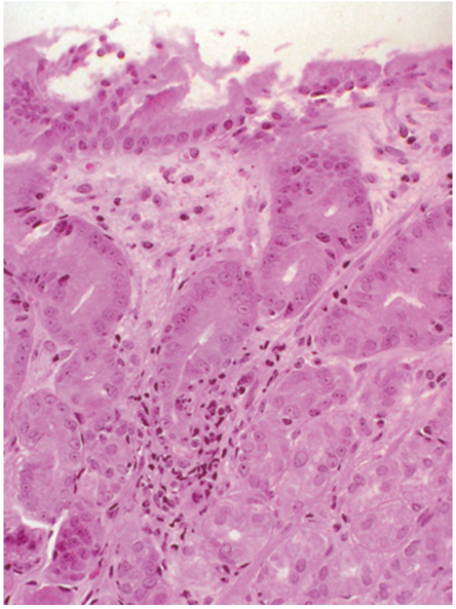


Figure 26.64 Autologous gastric GVHD or lymphocytic gastritis. The focal mononuclear inflammatory cell infiltration of the lamina propria and mucosal crypt associated with crypt epithelial cell apoptosis is histologically indistinguishable from GVHD in an allograft recipient. This inflammation may develop after autologous or syngeneic BMT if tolerance to self-MHC antigens is not re-established and autoreactive cells are allowed to persist.

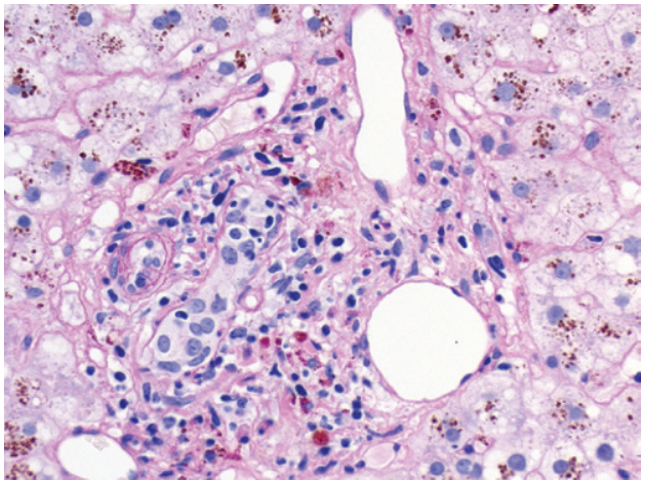


Figure 26.66 Hepatic GVHD. The PAS-stained section highlights a damaged interlobular bile duct that is infiltrated by lymphocytes. The nuclei vary in size and are irregularly arranged. The syncytium-like segment reflects nuclear loss.

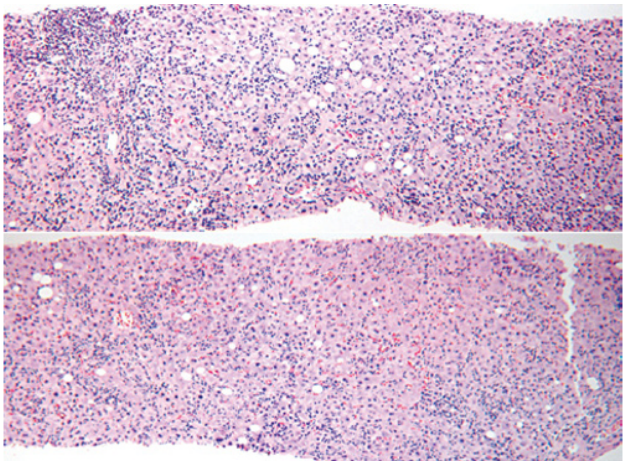


Figure 26.67 Acute hepatitic onset of GVHD at 10 months post-transplant. Three months after tapering off immunosuppression, the aminotransferases rose to 2000 u and alkaline phosphatase three times normal with normal bilirubin. This biopsy 1 was performed 26 days from the onset and shows an extensive lobular hepatitis with intense lymphocytic inflammation of sinusoids and portal spaces and hepatocellular necrosis.

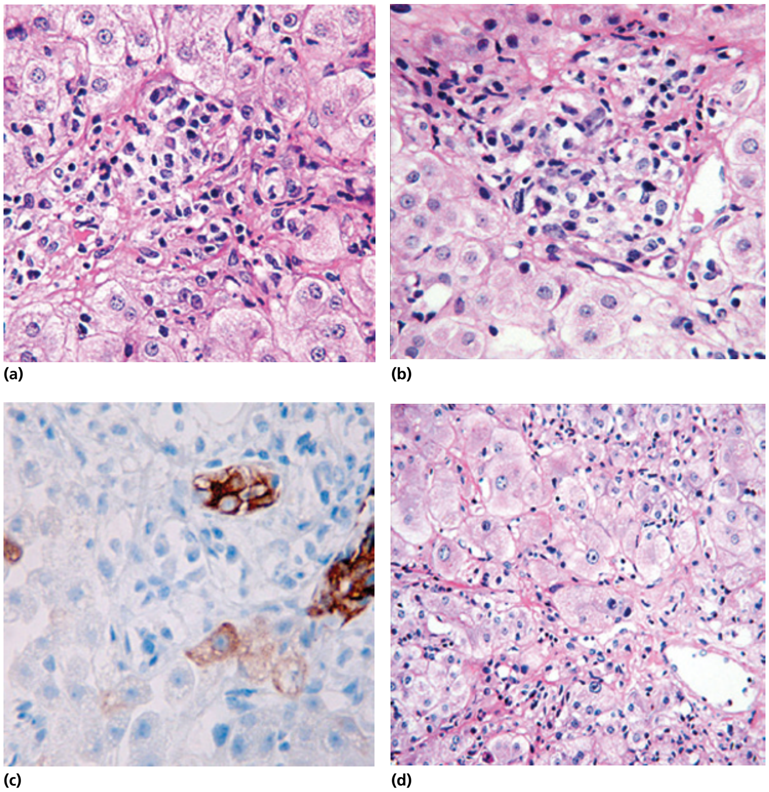


Figure 26.68 This biopsy 2 was done 1 month later (see Figure 26.67) when the total bilirubin was 30 mg/dL. Images (a) and (b) show a lymphoplasmacytic portal infiltrate which obscures the damaged interlobular bile ducts. In (c), immunostaining with cytokeratin 7 demonstrates that damaged bile ducts are still present. In (d), there is perivenular (zone 3) cholestasis. Hepatocytes show ballooning, and there is focal dropout associated with a patchy lymphocytic infiltrate. The patient made a full recovery after immunosuppressive treatment for GVHD was initiated.

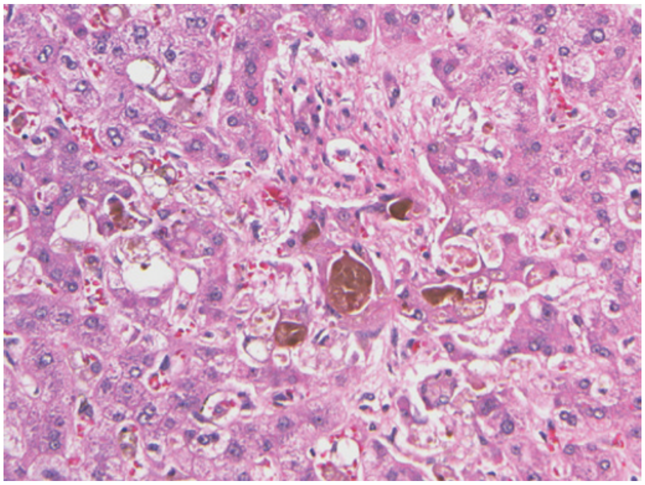


Figure 26.70 Hepatic GVHD with severe cholangitis lenta-like cholangiolar hepatocellular cholestasis. The interlobular bile duct is no longer recognizable. A ductular reaction with large bile-filled cholangioles lies along the periphery of the enlarged irregular portal space. The adjacent hepatocyte cords display marked unrest, disarray, and cytoplasmic ballooning.

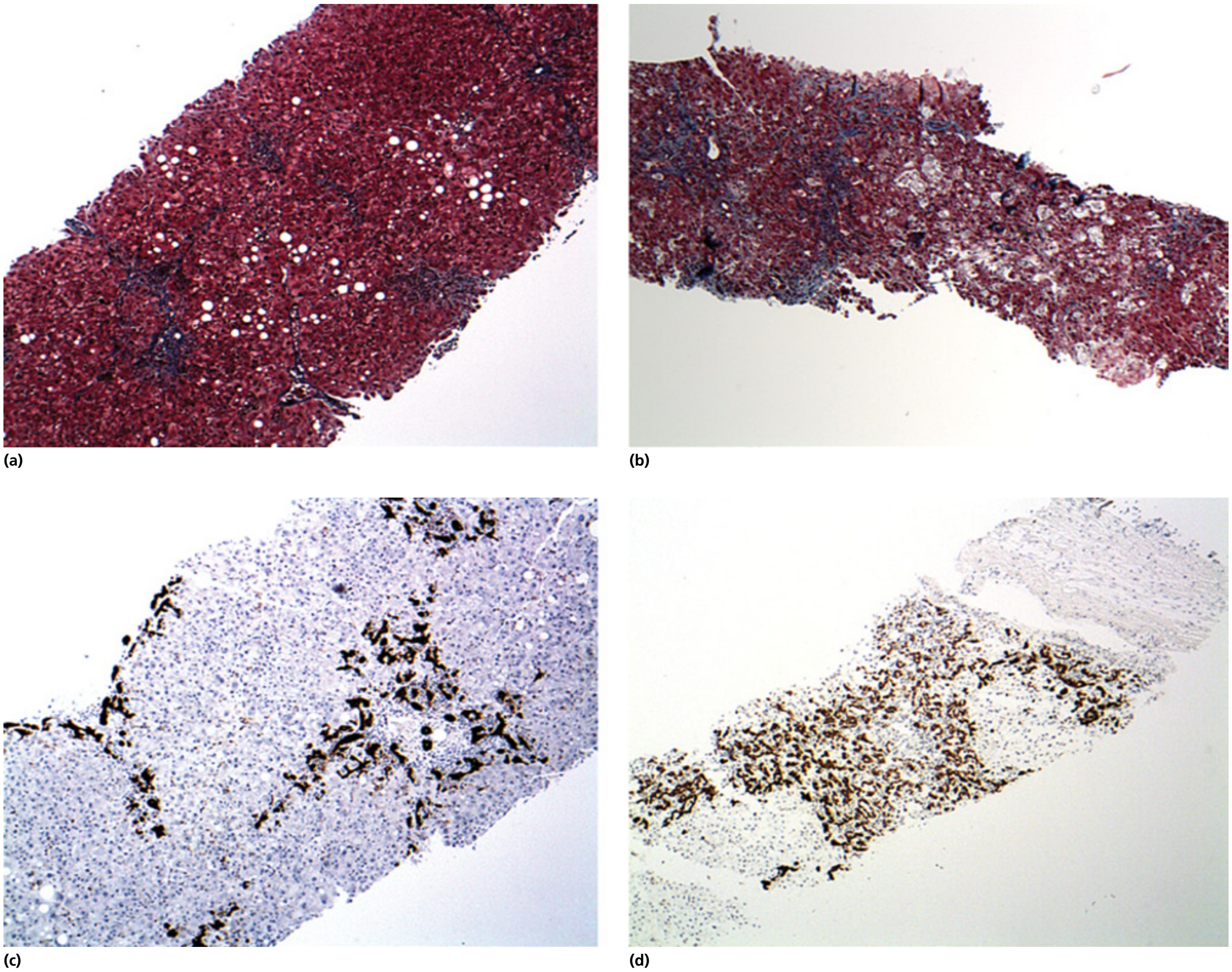


Figure 26.71 Rapidly evolving fibrosing cholestatic hepatitis C. Donor was positive for hepatitis C. Liver biopsies from day 70 [(a) and (c)] and day 88 [(b) and (d)] showing expanding portal fibrosis, hepatocyte necrosis and dropout, and marked bile ductule proliferation seen with cytokeratin 19 IHC [(c) and (d)].

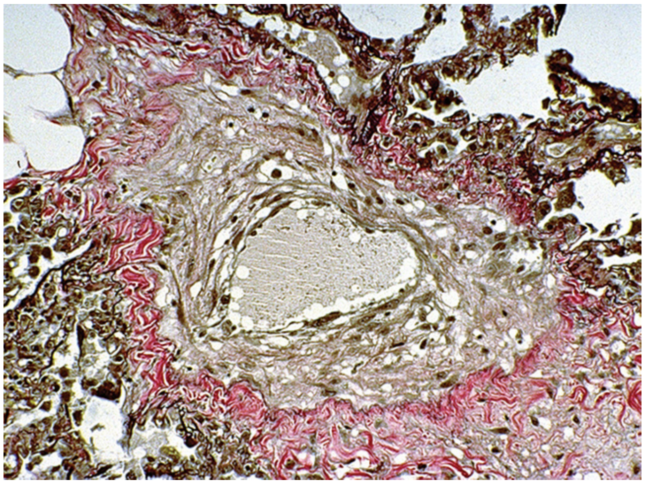


Figure 26.72 Pulmonary veno-occlusive disease (VOD) in a lung biopsy from a patient with pulmonary hypertension 48 days after second allogeneic BMT for acute lymphoblastic leukemia. Loose intimal fibrosis partially occludes the interlobular vein. This complication probably results from chemotherapy and may be more frequent than previously suspected. (Verhoeff–van Gieson elastin stain.)

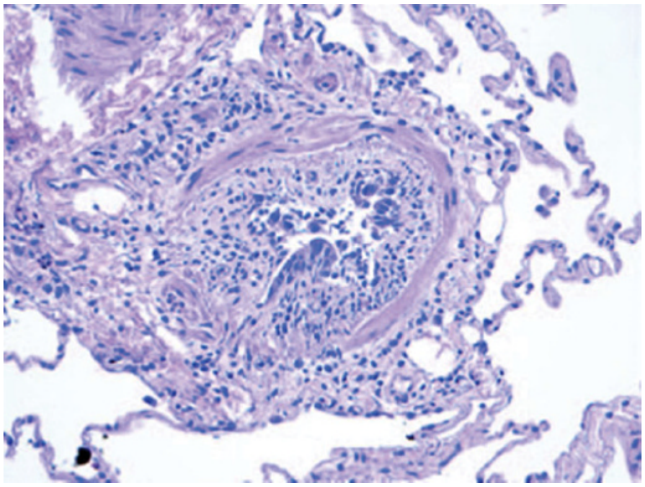


Figure 26.75 Bronchiolitis with evolving subepithelial fibrosis and lymphohistiocytic infiltration. There is marked epithelial damage. In this patient with chronic GVHD and obstructive pulmonary function abnormalities, the alterations are consistent with pulmonary GVHD. Similar changes could be seen in respiratory virus infection, which was absent in this biopsy. The ongoing inflammation and subtotal constrictive obliteration of this airway suggest that immunosuppressive therapy may be beneficial.

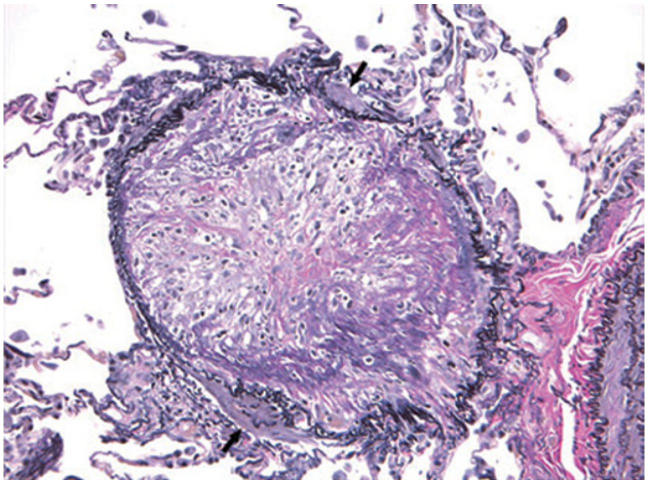


Figure 26.76 In a patient with a history similar to the preceding case (see Figure 26.75), the bronchiolitis obliterans of chronic pulmonary GVHD has progressed. This bronchiole is completely occluded by granulation tissue, and scattered mononuclear inflammatory cells are present. Without successful intervention with immunosuppressive therapy, this airway will finally probably be replaced by permanent scar tissue. The arrows indicate residual smooth muscle. The elastin fibers are black with this Verhoeff–van Gieson stain.

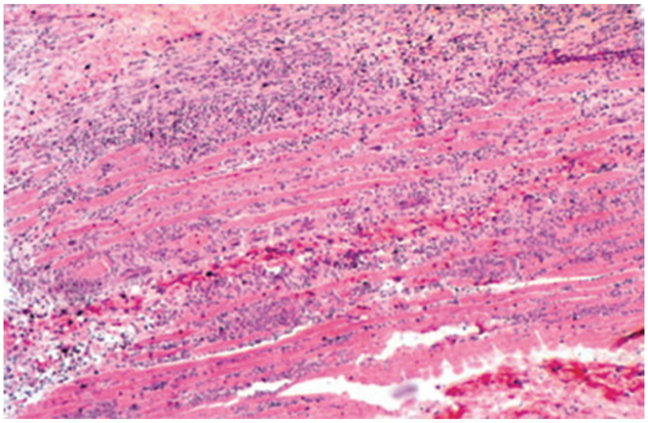


Figure 26.77 Polymyositis in deltoid muscle biopsy associated with chronic GVHD, day 1129. There is extensive endomysial chronic inflammation associated with myocyte destruction. The condition responded to immunosuppressive therapy.

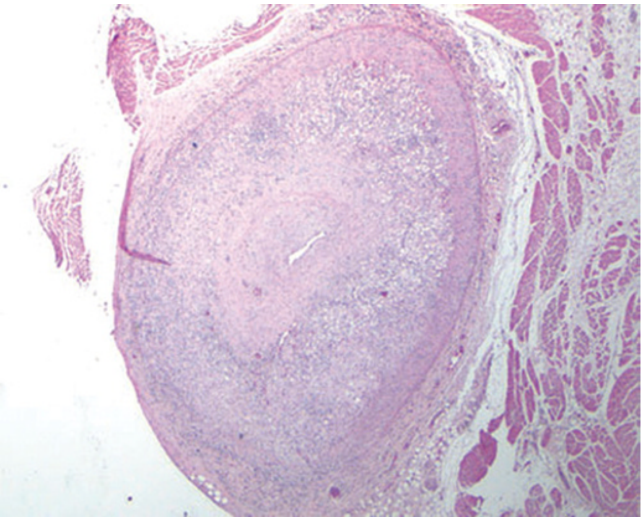


Figure 26.78 Coronary arteriopathy associated with chronic GVHD in a 15-year-old who died of myocardial infarction preceded by severe anginal symptoms. The lumen and muscular wall of all the major coronary arteries were subtotally to completely obliterated by a cellular infiltrate of lymphoid and monocytoid cells admixed with extracellular matrix.

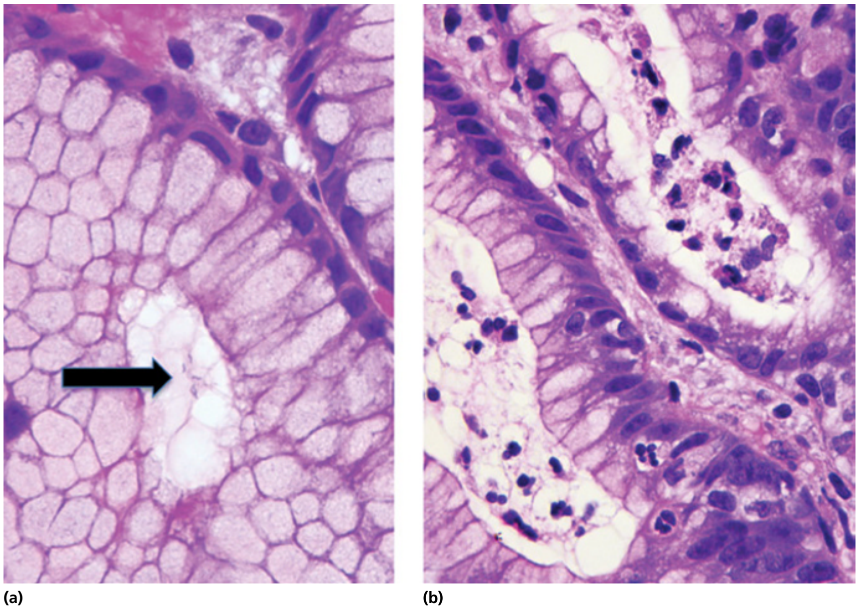


Figure 26.79 Gastric biopsy stained with H&E in a patient with chronic myeloid leukemia, day 37 post-HCT with persistent nausea and erythematous gastric lining by endoscopy non-responsive to steroids. (a) Gastric glands with a group of *Helicobacter pylori* organisms (black arrow) confirmed by immunohistochemistry. (b) Adjacent glands showing acute inflammation and a few *H. pylori* organisms.

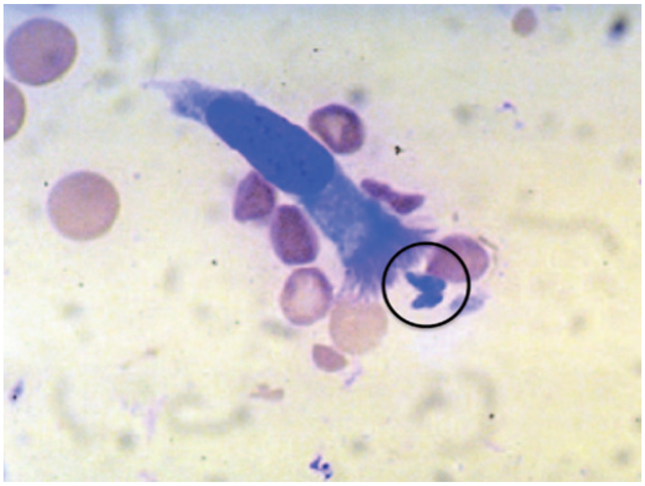


Figure 26.80 Two *Toxoplasma* tachyzoites (inside the circle) are present near the ciliated surface of a bronchial epithelial cell. Diagnosis of *Toxoplasma* pneumonia was established in less than 2 hours after bronchoalveolar lavage. Wright–Giemsa stain of cytospin preparation.

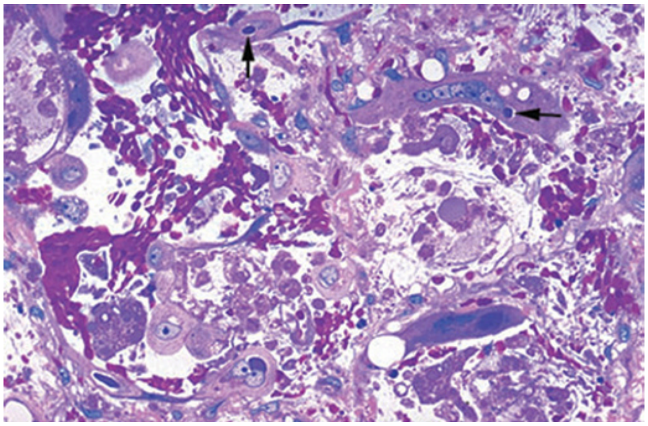


Figure 26.83 Respiratory syncytial virus (RSV) pneumonia in a lung biopsy, day 48. The presence of dark purple cytoplasmic inclusions in multinucleated epithelial cells (arrows) suggests the diagnosis but may be found with other viruses such as parainfluenza. During severe community outbreaks, infections may spread to hospitalized marrow recipients.

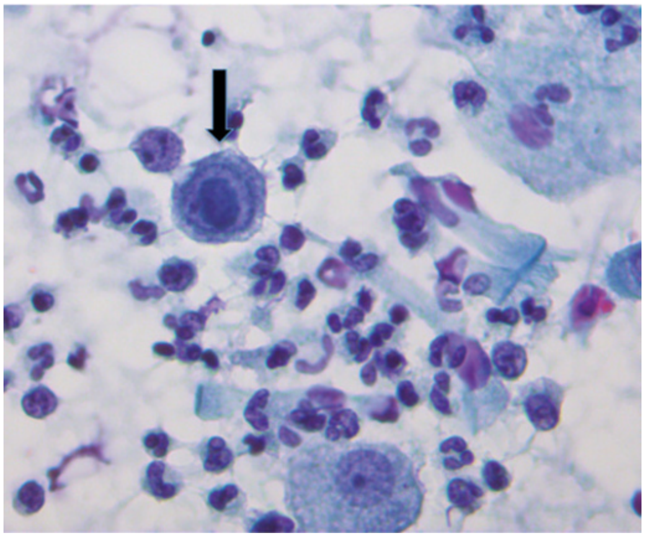


Figure 26.82 Cytomegalovirus cytopathic changes seen in a cytospin preparation of a bronchoalveolar lavage in a patient with B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma. Among a neutrophilic background is an infected alveolar macrophage (arrow) which demonstrates some cytoplasmic inclusion and a markedly enlarged nuclei with a single prominent enlarged central nucleoli. There is a relatively normal alveolar macrophage at the bottom middle of the picture for comparison.

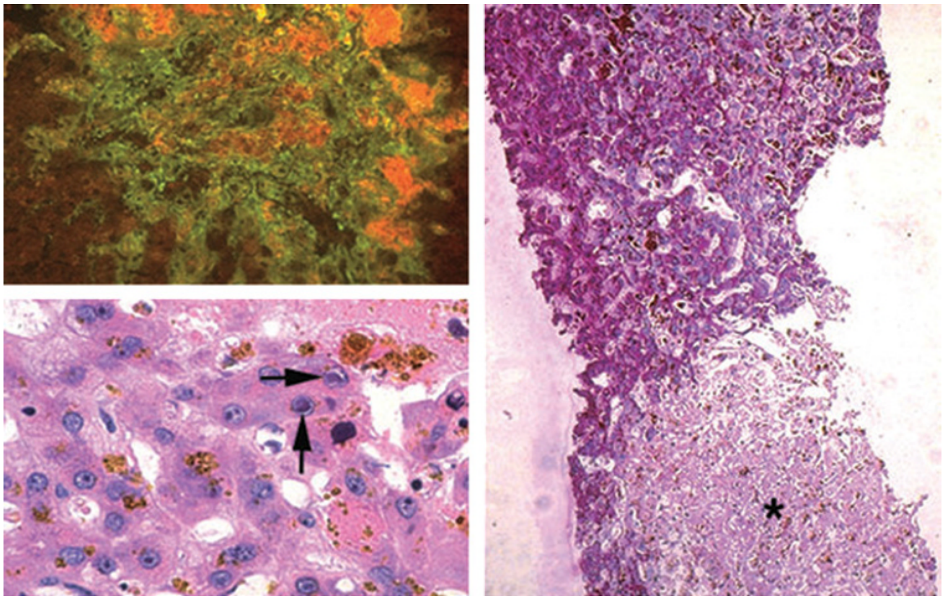


Figure 26.84 Varicella zoster virus (VZV) hepatitis in a percutaneous needle biopsy, day 79. On the right, a PAS stain demonstrates a pale necrotic area (*) bordered by cells containing nuclear inclusions (large arrows) visible by H&E stain on the lower left. Immunofluorescence confirmed the presence of VZV (upper left), and intravenous acyclovir was begun within hours of the biopsy.

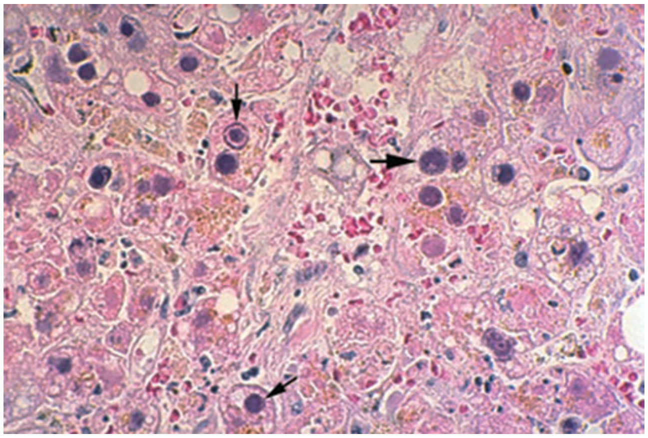


Figure 26.85 Overwhelming adenovirus hepatitis at autopsy 92 days after allogeneic BMT for AML. Dark basophilic nuclear inclusions produce "smudge cells" (large arrows) with blurred nuclear margins. Occasional cells with a halo around the inclusion (small arrow) suggest a combined infection with CMV, which was confirmed by isolation of both viruses.

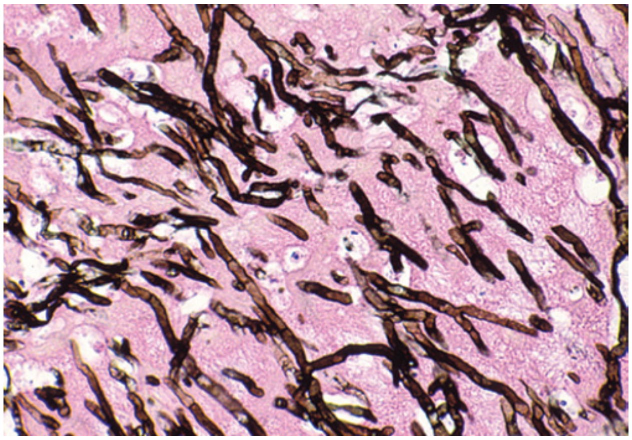


Figure 26.87 Mahan's methenamine silver stain of a lung biopsy showing a central necrotic cavity with infiltration by septate fungal 45° branching hyphae morphologically consistent with *Aspergillus*, which was subsequently confirmed with PCR. Source: reproduced with permission of Dr. Robert C. Hackman.

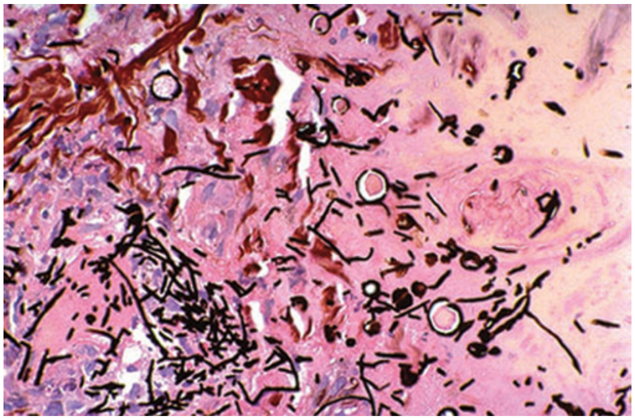


Figure 26.88 Mahan's methenamine silver stain showing that *Fusarium* hyphae invade the dermis in pre-transplant skin biopsy.

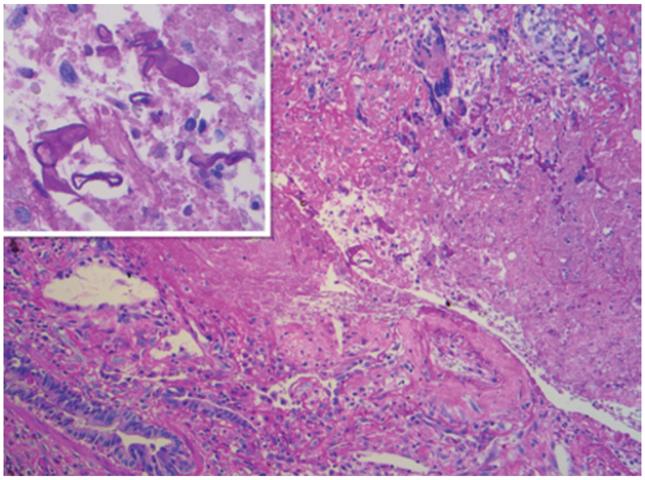


Figure 26.89 Lung biopsy specimen stained with PAS treated with diastase demonstrates Zygomycetes infection in a patient with sickle cell disease day 294 after HCT. There is extensive necrosis and giant cell reaction associated with the fungal organisms. Ribbon-like haphazardly arranged fungal hyphae are shown at high power in the inset.

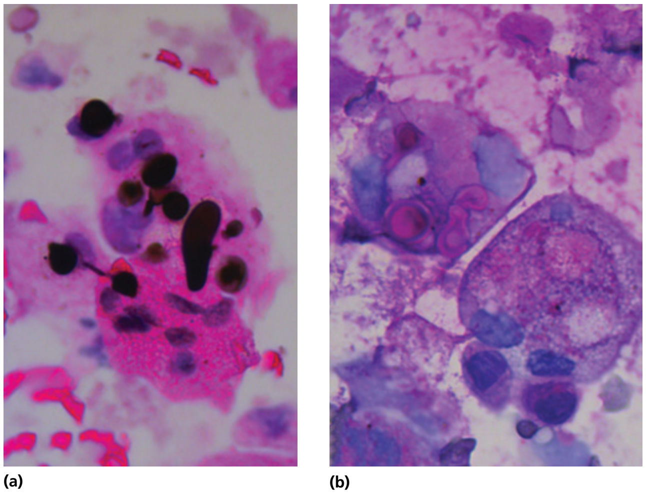


Figure 26.90 (a) A cytospin preparation of a bronchoalveolar lavage stained with Mahan's methenamine silver showing intracellular fungal organisms in an alveolar macrophage, later shown to be *Candida albicans* by fungal culture. (b) Same specimen stained with PAS showing the same intracellular *C. albicans* organism including a budding yeast form.

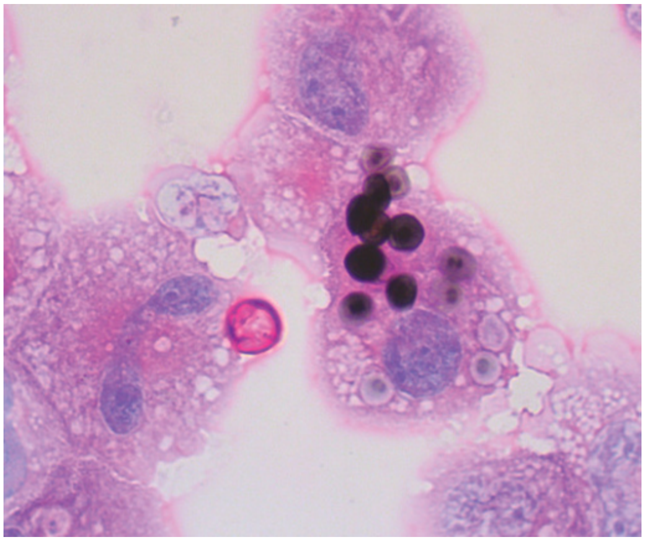


Figure 26.91 A cytospin preparation of a bronchoalveolar lavage stained with Mahan's methenamine silver showing intracellular pneumocystis organisms in an alveolar macrophage.

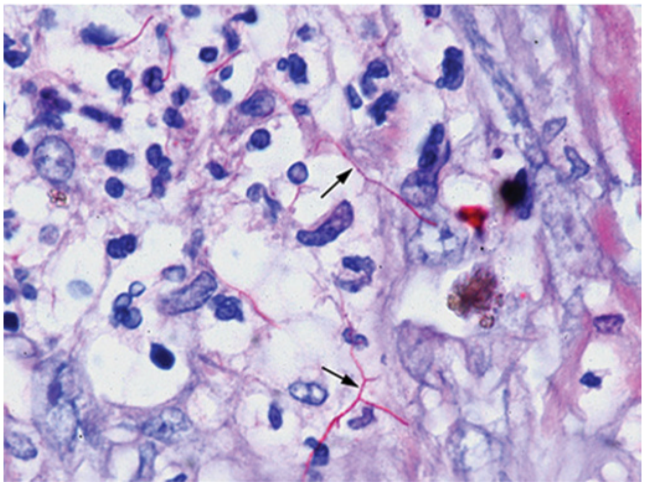


Figure 26.92 Fite stain of a lung biopsy showing thin filamentous branched *Nocardia* (arrow). Source: reproduced with permission of Dr. Robert C. Hackman.

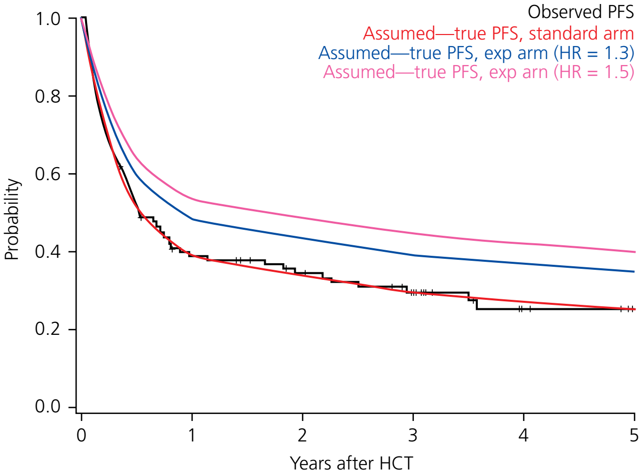


Figure 27.5 Piecewise exponential progression-free survival fit to observed data (assumed-true PFS, standard arm) with assumed-true PFS for two hypothetical experimental arms.

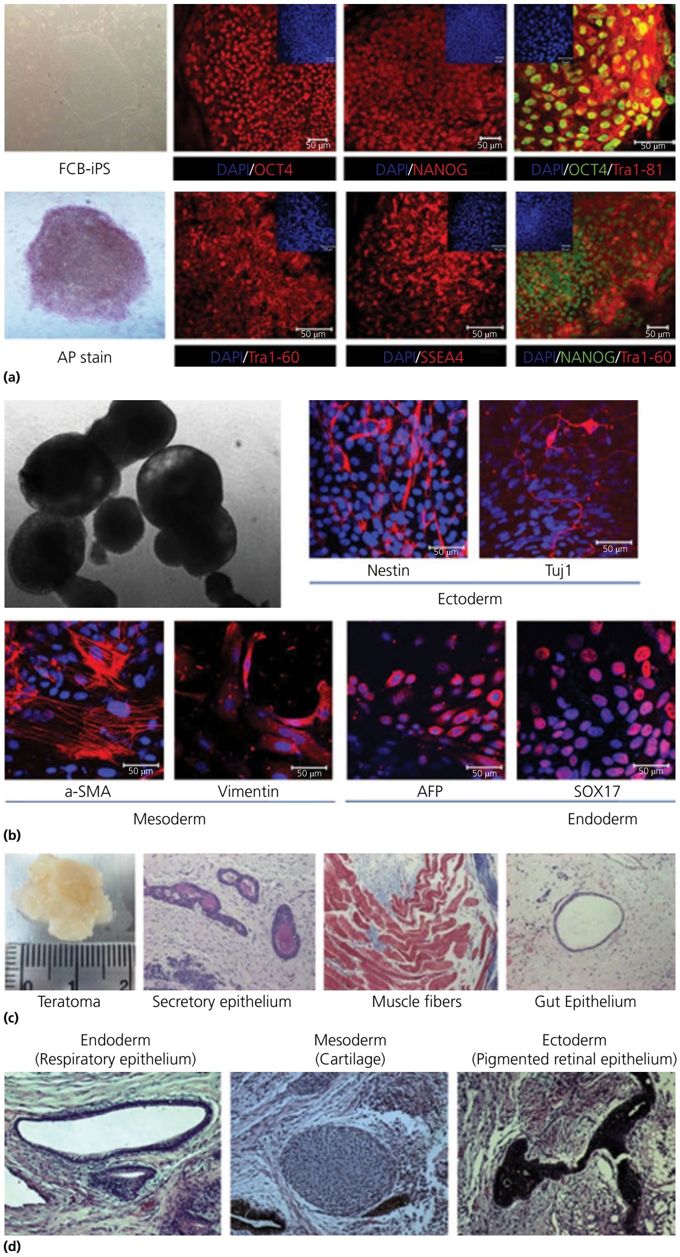
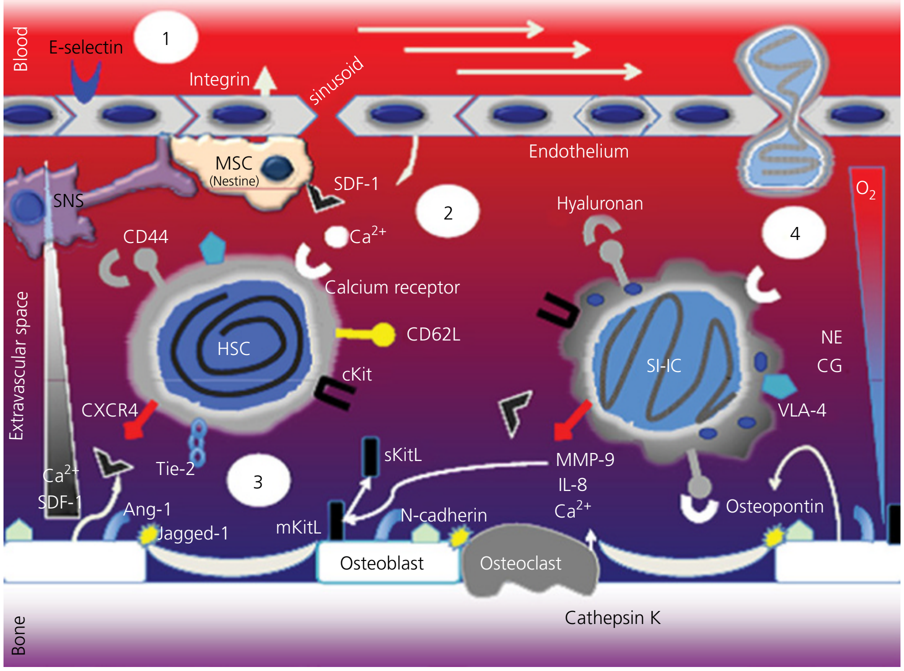

FCB-iPS
DAPI/OCT4
DAPI/NANOG
DAPI/OCT4/Tra1-81
AP stain
DAPI/Tra1-60
DAPI/SSEA4
DAPI/NANOG/Tra1-60
(a)
Nestin
Tuj1
Ectoderm
a-SMA
Vimentin
AFP
SOX17
Mesoderm
Endoderm
(b)
Teratoma
Secretory epithelium
Muscle fibers
Gut Epithelium
(c)
Endoderm
(Respiratory epithelium)
Mesoderm
(Cartilage)
Ectoderm
(Pigmented retinal epithelium)
(d)

Figure 39.5 Reprogramming 21-year-old frozen human CB CD34^{+} cells to iPS cells. (a) Immunohistochemistry for pluripotency markers Oct4, NANOG, TRA-1-60, SSEA4, and alkaline phosphatase (AP). (b) Morphology of 10-day embryoid body under phase contrast microscopy (top left), and immunostaining of iPS cell differentiated cells for ectoderm, mesoderm, and endoderm markers as noted. (c) Terotoma derived from immunodeficient mice injected with iPS cells (top left) with tissue sections representing all three embryonic germ layers. (d) Tissue sections from terotoma, as in C, but from different thawed and isolated CD34^{+} cells. Source: Broxmeyer et al. 2011 [23]. Reproduced with permission of the American Society of Hematology.


Blood
E-selectin
1
Integrin
sinusoid
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MSC
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SDF-1
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4
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Calcium receptor
HSC
CD62L
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Osteoclast
Cathepsin K
Bone

Figure 40.2 Cartoon depicting normal and leukemic cell interactions with the marrow microenvironment. Source: Schroeder and DiPersio 2012 [18]. Reproduced with permission of Society of Leukocyte Biology and John Wiley & Sons Ltd.

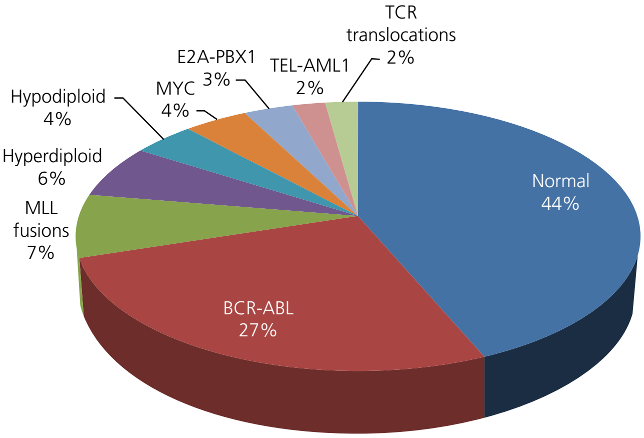
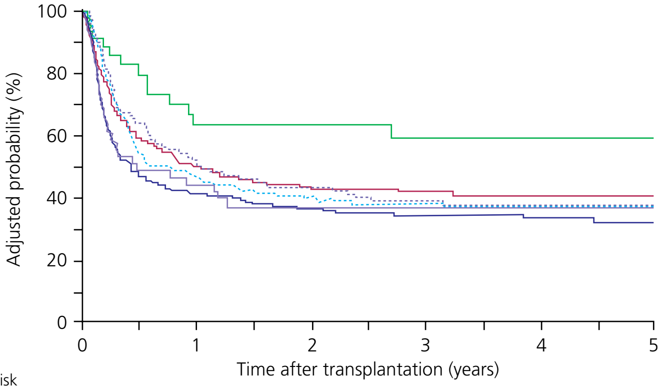


Figure 52.1 Frequency and distribution of cytogenetic abnormalities in adult patients with ALL. Dark blue includes normal cytogenetics (no recurrent chromosomal abnormalities), rust is **BCR-ABL** [t(9:22)], olive green is **MLL fusions** [t(4;11), t (1;11). T (11;19)], purple is hyperdiploid (>50 chromosomes), teal is hypodiploid (<45 chromosomes), orange is **MYC** [Ig translocations t(8;14), t(2;8), t(8;22)], light blue is **E2A-PBX1** [t(1;19)], pink is **TEL-AML1** [t(12;21)], and light green is **TCR translocations** (7q35/TCRβ, 14q1/TCRαδ), where the genes affected by chromosomal translocations are shown in bold face. Source: adapted and redrawn from Gutierrez et al. 2009 [1].



Numbers at risk		0	1	2	3	4	5
HLA-matched cord blood	—	35	20	17	13	11	8
HLA-matched bone marrow	⋯	116	62	45	35	29	24
One-mismatched cord blood (high cell dose)	—	157	72	55	44	32	25
HLA-mismatched bone marrow	⋯	166	77	60	53	44	30
One-mismatched cord blood (low cell dose)	—	44	19	13	12	10	6
Two-mismatched cord blood	—	267	100	67	49	34	21

Figure 53.4 Probability of leukemia-free survival after BM and UCB transplantation adjusted for disease status at transplantation.

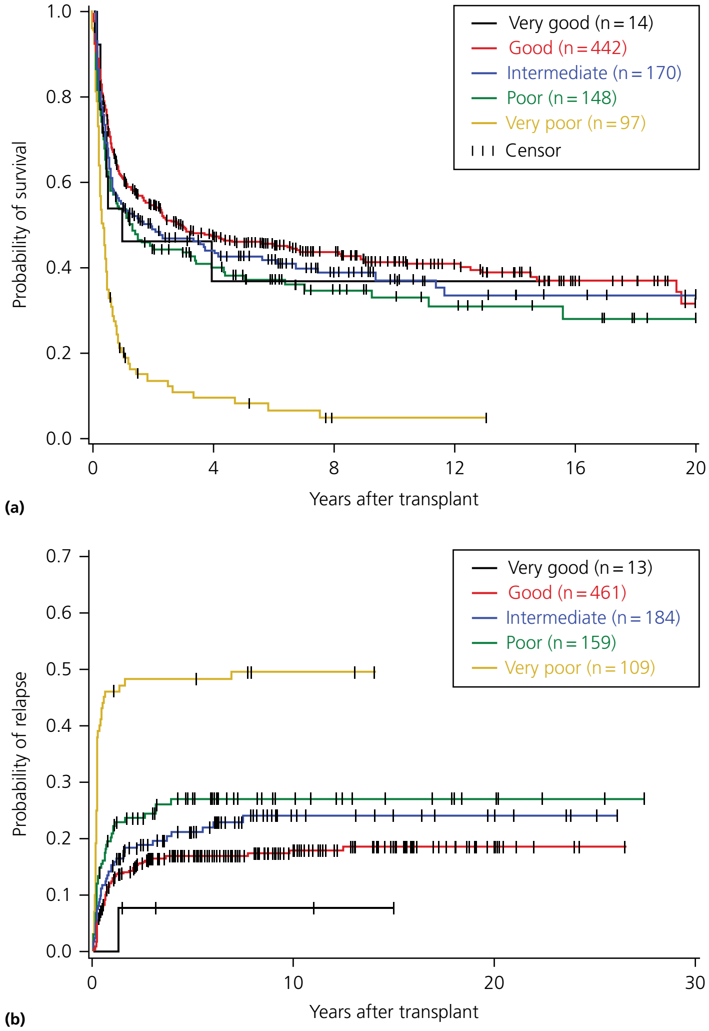


Figure 54.1 Impact of the five-group cytogenetic risk classification (incorporated into the IPSSR) [7,29] on transplant outcome. (a) Surivival. (b) Cumulative incidence of relapse. +, Censored patient.

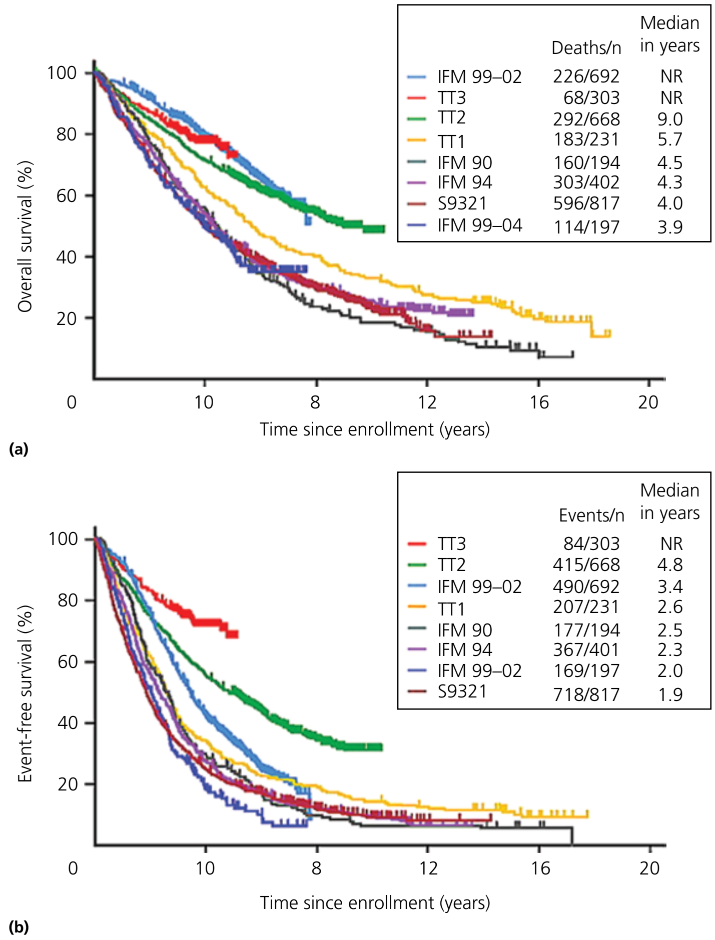


Figure 55.1 Long-term outcomes of large prospective autologous SCT trials. Source: Barlogie et al. 2010 [106]. Reproduced with permission of the American Society of Clinical Oncology.

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Abbreviations used: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CMML, juvenile (chronic) myelomonocytic leukemia *and* chronic myelomonocytic leukemia; GI, gastrointestinal; GVHD, graft-versus-host disease; GVL, graft-versus-leukemia effect; GVT response, graft-versus-tumor response; HC, hematopoietic cell; HD, Hodgkin disease; HCT, hematopoietic cell transplantation; HPC, hematopoietic progenitor cells; HSC, hematopoietic stem cell; HSPC; hematopoietic stem and progenitor cell; JMML, juvenile myelomonocytic leukemia; mAb, monoclonal antibody; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms; MSC, mesenchymal stem cell; NHL, non-Hodgkin lymphoma; PBHC, peripheral blood hematopoietic cells; PBHCT, peripheral blood hematopoietic cell transplantation; PBPC, peripheral blood progenitor cells; PBSC, peripheral blood stem cells; PTLD, post-transplant lymphoproliferative disorder; QoL, quality of life; TBI, total body irradiation.

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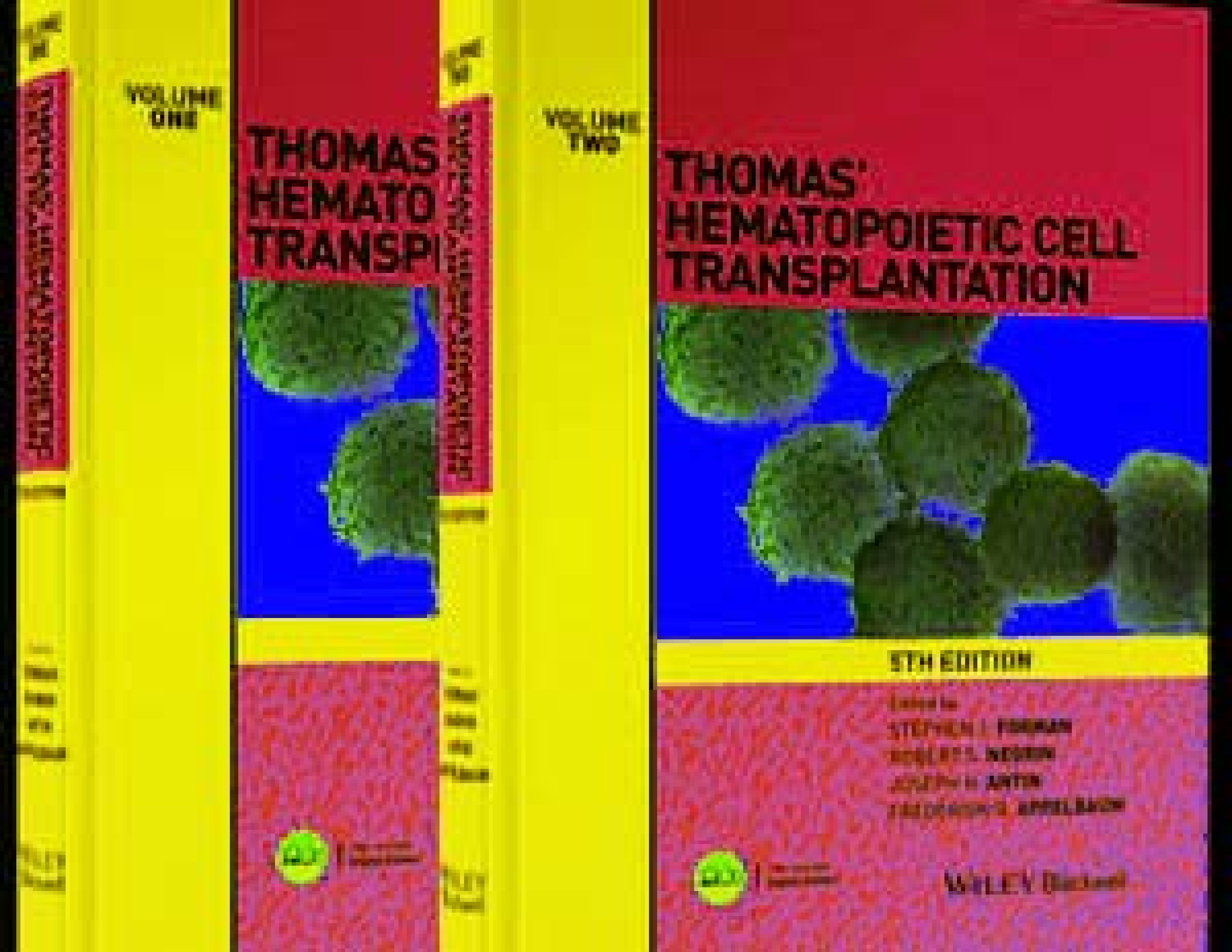
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Edited by
STEPHEN J. FORMAN
ROBERT S. NEGRIN
JOSEPH H. ANTIN
FREDERICK R. APPELBAUM
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Thomas' Hematopoietic Cell Transplantation

This book is dedicated to our patients and their families whose courage and trust enabled them to choose a difficult, dangerous, and sometimes unproven therapy which offered the only chance in their fight against a fatal disease. Today, many thousands of patients are alive after a successful hematopoietic cell transplantation, which would have been impossible without the fortitude of our patients, who took risks and thereby paved the way for the increasing application of this therapy. The progress in the area of clinical transplantation of hematopoietic cells would not have been possible without the dedicated, tireless work of the nurses and the supporting staff in the many transplant units around the world.

Thomas' Hematopoietic Cell Transplantation

Stem Cell Transplantation

VOLUME II

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Preface to the First Edition

The widespread application of bone marrow transplantation (BMT) to the treatment of a steadily increasing number of life-threatening hematological, oncological, hereditary, and immunological disorders is the culmination of more than four decades of research by many investigators. Early attempts in the 1950s to transplant living cells from one individual to another were carried out in the face of considerable skepticism. It was generally accepted as axiomatic that the immunological barrier to "foreign tissue" could never be overcome.

The horrors of Nagasaki and Hiroshima spurred interest in studies of the lethal effects of irradiation. It was discovered that mice given total body irradiation in doses lethal to the marrow could be protected from death by shielding the spleen or by an infusion of marrow, and that the marrow of such animals contained living cells of donor origin. These observations suggested that patients with leukemia might be given a lethal exposure of total body irradiation, which would destroy the malignant cells along with remaining normal marrow. The exposure would also destroy the immune system, making it possible to protect against lethality by a transplant of normal marrow cells.

The theory was correct, but results were disappointing. Because the procedure was both unproved and dangerous, only those patients who had no other options were considered. Except for a few patients with an identical twin donor, there were no survivors beyond a few months. Understanding of the human leukocyte antigen (HLA) system was not yet available, and little was known about the complication we now call graft-versus-host disease (GVHD). Thus, after a brief period of enthusiasm, most investigators abandoned this seemingly hopeless pursuit. Fortunately, work in animal models continued. Studies in inbred rodents defined the genetics of the major histocompatibility system and the fundamental rules of transplantation biology. Immunosuppressive drugs were developed to limit the severity of the immune reactions between donor and host. Demonstration of successful marrow transplants in the canine model using littermates matched for the major histocompatibility complex set the stage for successful transplantation of marrow between human siblings. Thus, it is clear that a long series of experimental studies in animals ultimately made human marrow transplantation possible.

By the late 1960s, much was known about the HLA system, more effective antibiotics were available, and platelet transfusions were becoming routine. Thus began the modern era of human BMT. The past 25 years have witnessed an almost exponential growth in the number of transplants being performed and the number of diseases being considered for BMT. Initially, most grafts employed marrow from an HLA-identical sibling. Autologous marrow, long known to be effective in animal systems, is now being used with increasing frequency following intensive cancer chemotherapy. Hematopoietic progenitor cells from the peripheral blood are now being used for BMT, either alone or to supplement marrow. As a result of increasing national and international cooperation, large panels of volunteer marrow donors of known HLA type are becoming available to patients whose own marrow cannot be used or who do not have a family donor.

Currently, thousands of transplants are being performed each year world-wide. With the demonstration that marrow could be transplanted and that the cure rate would be substantial, the logical step was taken to treat patients early during the course of their respective disease (i.e. in leukemia when the burden of malignant cells was relatively low and when the patient was in excellent clinical condition). With improved patient selection, development of improved tissue typing methods, availability of potent antimicrobial agents, advances in supportive care, and improved prevention of GVHD, the results of BMT have continued to improve.

Marrow transplantation is now being applied to a long list of diseases with a wide range of results depending on the disease, the type of transplant, and the stage of the disease. For some of the diseases, BMT has already proven to be the most effective therapy (e.g., some leukemias and severe aplastic anemia), whereas for others it is the only available curative treatment (e.g., thalassemia). In very rare genetic disorders, one successful BMT may establish the success of the treatment. For other more common disorders, controlled trials are necessary to define the proper role of allogeneic or autologous BMT, or therapy not involving BMT.

Only through rigorous study and long-term follow-up can novel approaches be confirmed as effective (or ineffective). For those working in the field of marrow transplantation, a source of intellectual satisfaction has been the interdisciplinary nature of the studies. A view of the wide-ranging disciplines involved can be gleaned by reading the chapter titles for this book. A successful BMT program is always a team effort. There must be cooperation between blood banks, referring physicians, radiation oncologists, immunologists, and physicians from many subspecialties. A dedicated support staff of technicians, data managers, and, above all, nurses, is crucial. The nursing team in particular is responsible for the day-to-day care of patients. Nurses not only provide the bedside management of complex protocol studies, but also bear the burden of emotional support through the difficult hospital period. They are the most readily available source of information for the patients and families day and night. Without a strong nursing team, the entire BMT program is jeopardized.

Most important are the patients who come to the transplant center with the courage to accept days, weeks, and sometimes months of discomfort in the hope of surviving a fatal disease. We must ensure that we acknowledge and respect the dignity and individuality of each patient, that we provide adequate information for informed decision making and then include patients and families in the decision process. The greatest reward for clinical investigators is to see patients reintegrated into their personal, social, and professional lives, free of their disease and its complications.

Stephen J. Forman
Karl G. Blume
E. Donnall Thomas
Summer, 1993

Preface to the Fifth Edition

Twenty-one years ago after the first edition and six years following the fourth edition of this textbook, the editors and publisher, Wiley-Blackwell, are proud to present the fifth edition of *Thomas' Hematopoietic Cell Transplantation*. The inspiration for the development of this fifth edition came from the rapid expansion of knowledge underlying transplantation biology, increased understanding of the complications of treatment, and new information related to the pathogenesis and treatment of diseases for which transplantation is utilized. The book also covers the expansion of stem-cell donor options and the genetic manipulation of cells of the hematopoietic and immune system.

In comparing this edition with the previous one, readers will find that there are new chapters focused on contributions to the field provided from basic science experiments in stem-cell biology, immunology, and tolerance. Along with these new chapters, previous contributions have been revised to reflect the deepening of our understanding of the benefits and challenges of transplantation and the impact of new therapies in the treatment of diseases for which transplant is a potentially curative therapy. In order to expand the information in each chapter, we have limited references to include only those that are most relevant.

New to the fifth edition is a companion digital edition replacing the CD ROM. The digital edition contains the entire text of the book in a searchable form and includes all the figures in a convenient downloadable format. The availability of the digital edition reflects changes in the way readers, including basic science and clinical investigators, clinicians, and nurses, access new information for both their research work and clinical care of patients.

We, the editors, also greatly appreciate the assistance of the staff at Wiley-Blackwell, who have been extremely helpful in the process of creating this new edition, now in two volumes, with a very different and modern cover presentation. The editors feel very proud to have assembled such a talented group of authors representing our colleagues who are experts in the clinical and basic science of transplantation. In addition, this edition would not have been possible without the dedicated work of our assistants, Sara E. Clark-Fuentes, Tanya Chiatovich, and, most importantly, Kimberlie Laramie. Finally, this edition honors the memory of Dr. Karl G. Blume and Dr. E. Donnall Thomas, who passed away in 2012. Their laboratory and clinical contributions helped begin and expand the field of transplantation as a curative therapy for patients suffering from leukemia and, over time, many other disorders. Both Dr. Thomas and Dr. Blume were the first two editors of the book and this edition is dedicated to them, and also to our patients and their families whose trusting partnership makes it possible to make progress and extend hope. We also honor the memory of Dr. Thomas' wife, Dottie, who we warmly consider to be the "mother" of hematopoietic cell transplantation, for her own contributions in helping Dr. Thomas and the field and for her support and editing of the first four editions of the book. They were our colleagues, mentors, and friends, and their impact will be felt in the field for many years to come.

Stephen J. Forman, MD
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Tribute

Our textbook honors Dr. E. Donnall Thomas, the single individual most responsible for creating the subject of this book, and Karl G. Blume, the Founder of the Transplant Programs both at City of Hope and Stanford University. Don and Karl were the first editors of this textbook and both passed away in the years following the publication of the fourth edition. We include this tribute as a remembrance of each of them, given the work that they did in their rich, professional lives to develop stem cell transplantation as a curative therapy for so many patients around the world.

E. DONNALL THOMAS, M.D.
March 15, 1920–October 20, 2012

Don was born on March 15, 1920, and was the son of a solo general practitioner in a small Texas village. As a child he often recalled accompanying his father to his small office and to patients' homes and between he and his father, they spanned the period from horse and buggy house calls to our modern high-tech medicine. Don received his B.A. and M.D. from the University of Texas where he met his wife, Dottie (September 18, 1922–January 9, 2015). Besides raising three children and eight grandchildren together, Dottie was Don's partner in every aspect of his professional life, from working in the laboratory to editing manuscripts and administering grants. Anyone who has been fortunate enough to work with Don knows that if he was the father of marrow transplantation, then Dottie was, without question, the mother, and we honor her memory as our friend and colleague in this tribute, too.

Don graduated from Harvard Medical School in 1947 and completed his internship and residency at Peter Bent Brigham Hospital in Boston. It was while in medical school that Don first became interested in normal and malignant hematopoiesis. During those years, Sidney Farber was initiating his first studies of the use of antifolates to treat children with acute leukemia, and Don witnessed the very first patient to achieve a remission with this approach. He was exposed to the pioneering work of Allan Erslev and his search for erythropoietin, and most importantly, he learned from Leon Jacobson and his studies showing that shielding the spleen protected mice from the otherwise lethal effects of total body irradiation. As data emerged that a similar irradiation protection effect could be achieved by transferring bone marrow from a nonirradiated to an irradiated mouse, Don became convinced of the clinical potential of marrow transplantation.

In 1955, Don moved to Cooperstown, New York, and the Mary Imogene Bassett Hospital, a Columbia University affiliate, where he began working on marrow transplantation both in the canine model and in humans with Dr. Joseph Ferrebee. In 1957, he published the first report on human patients, showing that complete remissions of leukemia could be achieved using total body irradiation followed by infusion of marrow from an identical twin. At that time, there was little understanding of the principles of human histocompatibility and so attempts to expand these studies to patients without identical twins were uniformly unsuccessful. These failures were the stimulus for a long series of experiments conducted by Don in the canine model, showing that it was possible to expose dogs to supralethal doses of irradiation and rescue them by reinfusing their own marrow, that the marrow could be cryopreserved, and that large doses of peripheral blood could substitute for marrow. However attempts at allogeneic transplantation in this outbred species continued to fail because of graft-versus-host disease or graft rejection.

In 1963, Don moved to Seattle and the University of Washington to become the first head of the Division of Oncology. There he developed techniques for rudimentary histocompatibility typing in the dog and, by the mid-1960s, showed that by selecting matched donors and using methotrexate posttransplant, it was possible to successfully transplant marrow between matched littermates in almost every case. At the same time, based on the work of Dausset, Payne, Amos, and others, understanding of human histocompatibility dramatically increased and so, in the late 1960s, Don made the decision to return to the subject of allogeneic transplantation in humans. He began to assemble a team of physicians, nurses, and support personnel (many of whom are still part of the current Seattle Transplant Program) and obtained a program project grant from the National Cancer Institute. In November 1968, Dr. Robert Good and his colleagues carried out the first marrow transplant from a matched sibling for an infant with immunodeficiency, and in March 1969, Don performed the first matched sibling donor transplant for a patient with leukemia.

The Seattle Transplant Program was originally housed at the Seattle Public Health Hospital, but in 1972, when the hospital was faced with closure by the federal government, the Program moved to Providence Hospital. In 1975, Don and his team moved to the newly created Fred Hutchinson Cancer Research Center, a move that provided Don with increased space, resources, and scientific collaborations. That same year, he and his colleagues published their classic *New England Journal of Medicine* paper summarizing the field of allogeneic transplantation and particularly the early Seattle experience. These results demonstrated not only the feasibility of the procedure but also that there was a plateau on the survival curves following transplantation, suggesting that some of these patients were cured with this novel technique. Don continued to lead the Clinical Research Division of the Center and its transplant program until his partial retirement in 1989. He continued writing manuscripts, delivering lectures, and participating in research discussions at the Center.

Appropriately, Don received almost every possible award for his work, including the American Society of Hematology's Henry M. Stratton Award, the General Motor's Kettering Prize, the American Society of Oncology's Karnofsky Award, the Presidential Medal of Science, and, of course, the 1990 Nobel Prize in Medicine which he shared with Joseph Murray. With each award, Don always emphasized how much of his work was a team effort. He invariably mentioned the contributions of Rainer Storb, Dean Buckner, Reg Clift, Paul Neiman, Alex Fefer, and Bob Epstein, who helped form the original Seattle Transplant Team, and of Ted Graham, who moved with Don from Cooperstown to help in the animal research. Don never failed to credit the nursing and support staff who played such a critical role in these efforts and, like Karl, always acknowledged the patients and their families who were true partners in his work.

KARL G. BLUME, M.D.
April 10, 1937–January 9, 2013

Karl G. Blume was born in Germany on April 10, 1937; he received his medical education under the mentorship of Professor Georg W. Löhr at the University of Freiburg and graduated in 1963. After graduation he undertook his residency at Marburg and then held two fellowships: one at Freiburg and the other at City of Hope in 1970 and 1971, respectively. His early work was in red cell biochemistry, and he pursued his postdoctoral fellowship under Dr. Ernest Beutler.

In 1975, after discussions with Dr. E. Donnall Thomas in Seattle and with his encouragement, Dr. Beutler decided to develop an allogeneic bone marrow transplantation (BMT) program at City of Hope and recruited Karl to return to City of Hope to establish the program. The program at City of Hope was, in many ways, derived from work and discussions that Karl had with colleagues at the Fred Hutchinson Cancer Research Center and resulted in lifelong friends from those early days of the development of transplantation to treat diseases of bone marrow and immune system origin. The first transplantation at City of Hope was performed on May 18, 1976.

Karl's clinical and research work focused on the problems that confront patients undergoing transplantation, namely, prevention of relapse, understanding the process of immune reconstitution, the treatment and prevention of graft-versus-host disease and cytomegalovirus infection management, and, among his early insights, issues related to patients' quality of life after transplantation and their long-term health. In 1981, Karl was the principal investigator for City of Hope's National Cancer Institute's first approved program project grant in transplantation, which contained projects that focused on those problems, and he organized a talented group of young laboratory and clinical scientists to work together in understanding and solving these challenges. He published the first results from City of Hope on the transplants for acute leukemia in the *New England Journal of Medicine* in 1981, showing the impact of remission status on outcome.

Based on his success at City of Hope, in 1987 Karl was recruited by Stanley Schrier and Ron Levy to begin a new transplant program at Stanford University. Thus, he again developed from scratch his second successful transplantation program, mentoring numerous young physicians and inspiring colleagues and staff to work with him in a common quest to cure patients with hematologic malignancy and return them to their normal lives. Just as he had done at City of Hope, he developed and led another successful program project grant application. He also established the Division of Blood and Marrow Transplantation at Stanford University, taking a personal interest in the lives of each of his patients, incorporating all aspects of the care team into their treatment, including nursing, social work, dietary, and physical therapy, and confirming again a model for patient care and research in use at transplant programs around the country. During that time, he trained both Rob Negrin and Nelson Chao, who went on to direct transplantation programs at Stanford University and at Duke University, respectively. Therefore, Karl was responsible for at least three of the major BMT programs in the United States, not to mention the numerous fellows he trained and mentored at City of Hope and Stanford University.

In 2000, Karl attained emeritus status at Stanford University and stepped down from the role of chief of the BMT division to develop the Stanford Cancer Institute. He continued to serve Stanford University as associate director of research in the Department of Medicine. In 2003, he directed his energies toward developing and achieving the coveted National Cancer Institute-designated cancer center status for the Stanford Cancer Institute, a first for the university.

Karl was also a driving force in 1994 in the founding of the American Society of Blood and Marrow Transplantation, now an international organization for physicians and researchers committed to serving patients with a variety of blood diseases. He was also a leader in the American Society of Hematology and, for many years, was in charge of the organization's Career Development Program. Early on, he recognized that our responsibilities were not only to our patients now but also to those who come to us in the future. He became the first honorary member of ASBMT and, with Richard O'Reilly, was the first coeditor of *The Biology of Blood and Marrow Transplantation*, the major journal in the field. Karl worked tirelessly to make it the central journal in the field, and when there were questions about the cost of the first issues, he made a personal financial donation to ensure the journal's successful launch.

Karl won many honors, both in the United States and Germany, but his major legacy is the impact he had on the thousands of patients he treated and the others he inspired to follow in his footsteps. All of us who knew and loved him knew that his highest priorities remained the quality of life of his patients and their families and the well-being of his transplantation team and those of his colleagues around the country and world. He held us all to high standards of excellence, but he maintained it with wonderful warmth, humor, and sensitivity over the decades and across continents. All of us who worked with him benefited from his respect and could always count on him to extend a helping hand and open ears, as he wanted nothing more than to help, to give of himself, and to be a force for good in this world.

In addition to his interest in music, sports – especially Stanford athletics – and the politics of the world and its impact on all of us, he was an exemplary family man. His children, Philipp and Caroline, could always rely on him for advice, affection, and guidance. In his last years, the lives and activities of his grandchildren, Adrian, Katie, Laura, Kevin, and David, were his focus and, of course, they adored him. Vera, his wife of 45 years, was his partner in his life and his work, as Dottie was to Don, and, at difficult times, always his source of strength and calm.

Although Don and Karl are most noted for their leadership and pioneering scientific achievements, for those of us who have been fortunate enough to work with them, they are equally admired for the way in which they achieved their success. They were always focused, hardworking, and uncompromising in their laboratories and especially in clinical research. They could both be demanding critics and held others accountable for their actions, yet at the same time, they were always generous with their ideas, loyal to their colleagues, and quick to deflect praise to their coworkers and help younger colleagues who chose this career.

For many of us in the field who had the privilege of knowing and working with both Don and Karl and for the many patients who are alive because of their work and dedication, the sentiment of a Hebrew proverb seems appropriate for each of them: "Say not in grief he is no more, but live in thankfulness that he was."

List of Abbreviations

AA	Amyloid A
AA	Aplastic anemia
AABB	American Association of Blood Banks
aaIPI	Age-adjusted International Prognostic Index
AAV	Adeno-associated virus
ABC	Activated B-cell-like
ABL	Abelson leukemia virus
aBMT	Autologous bone marrow transplantation
ABMTR	Autologous Blood and Marrow Transplant Registry – North America
ABVD	Doxorubicin, bleomycin, vinblastine, dacarbazine
ABW	Actual body weight
AC	Adriamycin (doxorubicin), cyclophosphamide
ACE	Adriamycin (doxorubicin), cyclophosphamide, etoposide
ACEI	Angiotensin-converting enzyme inhibitor
ACIF	Anticomplement immunofluorescence
ACIP	Advisory Committee on Immunization Practices
ACOR	Association of Online Cancer Resources
ACP	Advanced care planning
ACR	Albumin-to-urine creatinine ratio
ACS	Acute chest syndrome
ACTH	Adrenocorticotropic hormone
ACVBP	Adriamycin (doxorubicin), cyclophosphamide, vindesine, bleomycin, prednisone
AD	Autoimmune disease
ADA	Adenosine deaminase
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADH	Antidiuretic hormone
ADL	Activities of daily living
AdV	Adenovirus
AEL	Acute erythroleukemia
AFP	α-Fetoprotein
AGM	Aorta–gonad–mesonephros (region)
aGVHD	Acute graft-versus-host disease
AH	Ancestral haplotype
aHCT	Autologous hematopoietic cell transplantation
AHNMD	Associated clonal hematologic non-mast-cell lineage disease
AHPCS	Autologous hematopoietic progenitor-cell support
aHR	Adjusted hazard ratio
AHRQ	Agency for Health Care Research and Quality
aHUS	Atypical hemolytic uremic syndrome
AIDS	Acquired immune deficiency syndrome
AIF	Apoptosis-inducing factor
AIHA	Autoimmune hemolytic anemia
AIRE	Autoimmune regulator
AITD	Autoimmune thyroid disease
AITL	Angioimmunoblastic T-cell lymphoma
AKI	Acute kidney injury
AKIN	Acute Kidney Injury Network
aKIR	Activating killer immunoglobulin-like receptor
AL	Amyloid light-chain
ALA	α-Lipoic acid
ALC	Absolute lymphocyte count
ALCL	Anaplastic large-cell lymphoma
ALDH	Aldehyde dehydrogenase
ALG	Antilymphocyte globulin
ALK	Anaplastic lymphoma kinase
ALL	Acute lymphoblastic leukemia
AlloHCT	Allogeneic hematopoietic cell transplantation
AlloSCT	Allogeneic stem-cell transplantation
ALP	Alkaline phosphatase
ALPS	Autoimmune lymphoproliferative syndrome
ALT	Alanine aminotransferase
AMA	Antimitochondrial antibody
AMKL	Acute megakaryoblastic leukemia
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
ANKL	Aggressive natural killer cell leukemia
AOTONJ	Antiosteoclastic therapy-associated osteonecrosis of the jaws
AP	Accelerated phase
AP-1	Activator protein-1
APACHE	Acute Physiology, Age, Chronic Health Evaluation
APBMT	Asia Pacific Blood and Marrow Transplantation
APC	Antigen-presenting cell
APECED	Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy
API	4′,6-Diamidino-2-phenylindole
APL	Acute promyelocytic leukemia
APMF	Acute panmyelosis with myelofibrosis
Ara-C	Cytosine arabinoside
ARB	Angiotensin receptor blocker
ARDS	Acute or adult respiratory distress syndrome
ARF	Acute renal failure
ARL	AIDS-related lymphoma
ARS	Acute radiation syndrome
ARSA	Arylsulfatase A
ART	Assisted reproductive technology
ASBMT	American Society for Blood and Marrow Transplantation
ASCT	Autologous stem-cell transplantation
ASO	Allele-specific oligonucleotide
ASO-PCR	Allele-specific oligonucleotide polymerase chain reaction
AST	Aspartate aminotransferase
AT	Alkyltransferase
ATG	Antithymocyte globulin
ATL	Adult T-cell leukemia
ATLL	Adult T-cell leukemia/lymphoma
ATM	Ataxia Telangiectasia Mutated
ATRA	All-*trans*-retinoic acid
AUC	Area under the curve
AutoHCT	Autologous hematopoietic cell transplantation
AVP	Arginine vasopressin
AYA	Adolescent and young adult
AZT	Zidovudine
BAEP	Brainstem auditory evoked potentials
BAFF	B-cell-activating factor
BAIAP3	Brain-specific angiogenesis inhibitor 1-associated protein 3
BAL	Bronchoalveolar lavage
BC	Blast crisis

BCC	Basal cell carcinoma
BCMA	B-cell maturation antigen
BCNA	Bicyclic nucleoside analog
BCNU	1,3-Bis(2-chloroethyl)-1-nitrosourea (carmustine)
BCR	B-cell receptor
BCR	Breakpoint cluster region
BD	Bortezomib, dexamethasone
BDP	Beclomethasone 17,21-dipropionate
BEAC	BCNU, etoposide, Ara-C (cytosine arabinoside), cyclophosphamide
BEAM	BCNU, etoposide, Ara-C (cytosine arabinoside), melphalan
BED	Biologically effective dose
BEP	Bleomycin, etoposide, cisplatin
BER	Base excision repair
BFI	Brief Fatigue Inventory
BFM	Berlin–Frankfurt–Münster
BFU-E	Burst-forming units – erythroid
BIW	Twice weekly
BL	Burkitt lymphoma
BLA	Biologics license application
BL-CFC	Blast colony-forming cell
BLS	Bare lymphocyte syndrome
BLyS	B-lymphocyte stimulator
BM	Bone marrow
BMC	Bone marrow cell
BMD	Bone mineral density
BMDW	Bone Marrow Donors Worldwide
BMFS	Bone marrow failure syndromes
BMI	Body mass index
BMSC	Bone marrow stem cell
BMSCT	Blood and Marrow Stem Cell Transplant
BMT CTN	Blood and Marrow Transplant Clinical Trials Network
BMT	Bone marrow transplantation
BMTCN	Blood and Marrow Transplantation Certified Nurse
BMTinfonet	Blood and Marrow Transplant Information Network
BMTSS	Bone Marrow Transplant Survivor Study
BNLI	British National Lymphoma Investigation
BNP	Brain natriuretic peptide
BO	Bronchiolitis obliterans
BOOP	Bronchiolitis obliterans organizing pneumonia
BPDCN	Blastic plasmacytoid dendritic cell neoplasm
BPI	Bactericidal/permeability-increasing
BPI	Brief Pain Inventory
BR	Bendamustine, rituximab
Breg	Regulatory B (cell)
BSE	Bovine spongiform encephalopathy
BSI	Brief symptom inventory
BTK	Bruton's tyrosine kinase
BTLA	B- and T-lymphocyte attenuator
BU	Busulfan
BuMel	Busulfan, melphalan
BUN	Blood urea nitrogen
C1P	Ceramide-1 phosphate
CABSI	Catheter-associated bloodstream infection
CAEBV	Chronic active Epstein–Barr virus
CALGB	Cancer and Acute Leukemia Group B
CALLA	Common acute lymphoblastic leukemia antigen
CAR	Chimeric antigen receptor
CAR	Cocksackie and adenovirus receptor
CAR	CXCL12 adventitial reticular cell
CARES	Cancer Rehabilitation Evaluation System
cART	Combination antiretroviral therapy
CB	Cord blood
CBC	Cord blood cell
CBF	Core binding factor
CBG	Corticosteroid-binding globulin
CBHC	Cord blood hemapoietic cell
CBSC	Cord blood stem cell
CBT	Cognitive–behavioral therapy
CBT	Cord blood transplantation
CBV	Cyclophosphamide, BCNU, etoposide
CC	Complete chimerism
CCG	Children's Cancer Group
CCI	Charlson Comorbidity Index
CCI	Corrected count increment
CCNU	1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea
CCR	Cytologic complete remission
CCSG	Children's Cancer Study Group
CCyR	Complete cytogenetic remission
CDA	Cytidine deaminase
CDAD	*Clostridium difficile*-associated diarrhea
CDAI	Crohn Disease Activity Index
CDC	Centers for Disease Control and Prevention
CDC	Complement-dependent cytotoxicity
CDF	Cumulative distribution function
CDK	Cyclin/cyclin-dependent kinase
CE	Carboplatin, etoposide
CEA	Carcinoembryonic antigen
CEACAM	Carcinoembryonic-antigen-related cell-adhesion molecule
CEC	Carboplatin, etoposide, cyclophosphamide
CEL-NOS	Chronic eosinophilic leukemia-not otherwise specified
CEM	Carboplatin, etoposide, melphalan
CESD	Center for Epidemiologic Studies – Depression
CFAR	Cyclophosphamide, fludarabine, alemtuzumab, rituximab
CFH	Complement factor H
CFU	Colony-forming unit
CFU-B	Colony-forming unit – B-lymphocyte
CFU-BLAST	Colony-forming unit – blast
CFU-E	Colony-forming unit – erythroid
CFU-F	Colony-forming unit – fibrolast
CFU-GEMM	Colony-forming unit – granulocyte/erythroid/macrophage/megakaryocyte
CFU-GM	Colony-forming unit – granulocyte–macrophage
CFU-MEG	Colony-forming unit – megakaryocytic
CFU-Mix	Colony-forming unit – mixed
CFU-S	Colony-forming unit – spleen
CFU-T	Colony-forming unit – T-lymphocyte
CGD	Chronic granulomatous disease
CGH	Comparative genomic hybridization
cGVHD	Chronic graft-versus-host disease
CHD	Coronary heart disease
CHF	Congestive heart failure
CHOEP-14	Doxorubicin, vincristine, etoposide, prednisone
CHOP	Cyclophosphamide, hydroxydaunomycin, vincristine (Oncovin), prednisone
CHR	Complete hematologic response
CHRIs	Child Health Ratings Inventory
CHS	Chediak–Higashi syndrome
CI	Comorbidity index
CI	Confidence interval
CI	Cumulative incidence
CIA	Collagen-induced arthritis
CIBMTR	Center for International Blood and Marrow Transplant Research
CID	Combined immunodeficiency syndrome
CIDP	Chronic inflammatory demyelinating polyneuropathy
CIF	Cumulative incidence function
CIHHV-6	Chromosomal integration of human herpesvirus 6
CIPN	Critical illness polyneuropathy
CJD	Creutzfeldt–Jakob disease
CKD	Chronic kidney disease
CLIA	Clinical Laboratory Approved Amendments
CLL	Chronic lymphoid/lymphocytic leukemia
CLO	Clofarabine

CLP	Common lymphoid progenitor
CLR	C-type lectin receptor
CMF	Cyclophosphamide, methotrexate, fluorouracil
CMI	Cellular mediated immunity
CML	Chronic myeloid/myelogenous leukemia
CMML	Chronic myelomonocytic leukemia
CMN	Chronic myeloid neoplasm
CMP	Common myeloid progenitor
CMR	Complete molecular response
CMV	Cytomegalovirus
CMV-Ig	Cytomegalovirus antibody-enriched intravenous immunoglobulin
CMV-IP	Cytomegalovirus-associated interstitial pneumonia
CNI	Calcineurin inhibitor
CNL	Chronic neutrophilic leukemia
CNS	Central nervous system
COG	Children's Oncology Group
ConA	Concanavalin A
COP	Cryptogenic organizing pneumonia
COPE	Coping Orientations to Problems Experienced Scale
COX-2	Cyclooxygenase-2 (inhibitor)
CP	Chronic phase
CR	Complete remission
CRBSI	Catheter-related bloodstream infection
CREG	Cross-reactive group
CRM	Continual reassessment method
CRS	Cytokine release syndrome
CRVT	Catheter-related venous thrombosis
CSA	Cyclosporine A
CSF	Cerebrospinal fluid
CSF	Colony-stimulating factor
CSP	Cyclosporine
CT	Computed tomography
CT	Cyclophosphamide, thioTEPA
CTC	Cyclophosphamide, thioTEPA, carboplatin
CTC	Cytotoxic T cell
CTCL	Cutaneous T-cell lymphoma
CTD	Cyclophosphamide, thalidomide, dexamethsone
CTL	Cytotoxic T-lymphocyte
CTLA	Cytotoxic T-lymphocyte antigen
CTLP	Cytotoxic T-lymphocyte precursor
CTN	Clinical Trials Network
CTX	Cyclophosphamide
CTXD	Cancer and Treatment Distress
CTZ	Chemotactic trigger zone
CVAD	Fractionated cyclophosphamide, vincristine, adriamycin, dexamethasone
CVC	Central venous catheter
CVD	Cardiovascular disease
CVID	Common variable immune deficiency
CVRF	Cardiovascular risk factor
CVVH	Continuous veno-venous hemofiltration
CVVHDF	Continuous veno-venous hemodiafiltration
CY	Cyclophosphamide
CyBorD	Cyclophosphamide, bortezomib, dexamethasone
CYP	Cytochrome P
DAD	Direct antigen detection
DAF	Decay-accelerating factor
DAG	Diacylglycerol
DAH	Diffuse alveolar hemorrhage
DAMP	Damage-associated molecular pattern
DAS	Dasatinib
DC	Dendritic cell
DCEP	Dexamethasone, cyclophosphamide, etoposide, cisplatin
DCK	Deoxycytidine kinase
DDR	Discoidin domain receptor
DFA	Direct fluorescent antibody (test)
DFCI	Dana–Farber Cancer Institute
DFS	Disease-free survival
DGK	Deoxyguanosine kinase
DH	Double hit
DHAP	Dexamethasone, high-dose cytarabine, cisplatin
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
DHFR	Dihydrofolate reductase
DHPG	9-(1,3-Dihydroxy-2-propoxymethyl)guanine (ganciclovir)
DIC	Disseminated intravascular coagulation
DILI	Drug-induced liver injury
DIPSS	Dynamic International Prognostic Scoring System
DKA	Diabetic ketoacidosis
DLA	Dog leukocyte antigen
DLBCL	Diffuse large B-cell lymphoma
DLCL	Diffuse large cell lymphoma
DLCO	Diffusion capacity of the lung for carbon monoxide
DLI	Donor lymphocyte infusion
DLT	Dose-limiting toxicity
DM	Diabetes mellitus
DMSO	Dimethyl sulfoxide
DN	Double negative
DNA	Deoxyribonucleic acid
DNAX	DNA accessory molecule
DNR	Do not resuscitate
DOTA	1,4,7,10-Tetraazacyclododecane-*N*,*N*′,*N*′′,*N*′′′-tetraacetic acid
DOTMP	1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid
DPC	Days post-coitum
DPTS	Delayed pulmonary toxicity syndrome
DR	Death receptor
DRG	Dorsal root ganglion
DSA	Donor-specific antibody
DSB	Double-strand break
DSII	Disease-Specific Impairment Inventory
DSRCT	Desmoplastic small round cell tumor
dsRNA	Double-stranded RNA
DST	Donor-specific transfusion
DTH	Delayed type hypersensitivity
DTI	Diffusion-tensor MRI
DTPA	Diethylenetriaminepentaacetic acid
DVT	Deep venous thrombosis
DXA	Dual-energy X-ray absorptiometry
EAE	Experimental allergic encephalomyelitis
EAE	Experimental autoimmune encephalomyelitis
EATL	Enteropathy-associated T-cell lymphoma
EB	Embryoid body
EBMT	European Group for Blood and Marrow Transplantation
EBMTR	European Bone Marrow Transplant Registry
EBNA	Epstein–Barr (virus) nuclear antigen
EBV	Epstein–Barr virus
EBV-LDP	Epstein–Barr virus-associated lymphoproliferative disorder
EC	Endothelial cell
EC	Epirubicin, cyclophosphamide
ECAT	Extracorporal adsorption therapy
ECFC	Endothelial colony-forming cells
ECG	Electrocardiogram
ECIL	European Conference on Infections in Leukaemia
ECM	Extracellular matrix
ECP	Extracorporeal photopheresis
ED	Erectile dysfunction
EDSS	Expanded Disability Status Scale
EDTMP	Ethylenediaminetetramethylenephosphonic acid
EEG	Electroencephalogram
EFS	Event-free survival
EGBMT	European Group for Blood and Marrow Transplantation
ELISA	Enzyme-linked immunosorbent assay

ELN	European LeukemiaNet
EM	Erythema multiforme
EMLA	Eutectic Mixture of Local Anesthetic
EN	Enteral nutrition
ENKL	Extranodal NK/T-cell nasal type leukemia
ENSG	European Neuroblastoma Study Group
ENU	Ethyl-*N*-nitrosourea
EORTC	European Organization for Research and Treatment of Cancer
EPC	Endothelial progenitor cell
EPO	Erythropoietin
EPOCH	Etoposide, prednisone, vincristine (Oncovin), doxorubicin hydrochloride
ER	Endoplasmic reticulum
ER	Estrogen receptor
ERIC	European Research Initiative in chronic lymphocytic leukemia
ERT	Enzyme replacement therapy
ESBL	Extended-spectrum β-lactamase
ESC	Embryonic stem cell
ESFT	Ewing sarcoma family of tumors
ET	Essential thrombocythemia
ETP	Early thymic progenitor
ETS	Euthyroid sick syndrome
EWOG	European Working Group
EWOG-MDS	European Working Group of Myelodysplastic Syndromes in Childhood
FA	Fanconi anemia
FAB	French–American–British Classification
FACIT-SP	Functional Assessment of Chronic Illness Therapy – Spirituality and Wellbeing Scale
FACS	Fluorescence-activated cell sorter
FACT	Foundation for the Accreditation of Cellular Therapy
FACT	Functional Assessment of Chronic Illness Therapies
FACT-BMT	Functional Assessment of Cancer Therapy – BMT Module
FAMA	Fluorescent-antibody staining of membrane antigen
FasL	Fas ligand
FC	Facilitator cell
FCH	Fibrosing cholestatic hepatitis
FCR	Fludarabine, cyclophosphamide, rituximab (RTX)
FDA	Food and Drug Administration
FDG	[^{18}F]Fluorodeoxyglucose
FDR	False discovery rate
FEC	Fluorouracil, epirubicin, cyclophosphamide
$\mathbf{FEV_1}$	Forced expiratory ventilation in 1 second
FFP	Fresh frozen plasma
FFS	Failure-free survival
FHCRC	Fred Hutchinson Cancer Research Center
FISH	Fluorescence in situ hybridization
FKBP	Tacrolimus (FK)-binding protein
FL	Follicular lymphoma
FLACC	Face, Legs, Activity, Cry, Consolability (pain assessment tool)
FLAIR	Fluid attenuated inversion recovery
FLC	Free light chain
FLIPI	Follicular Lymphoma International Prognostic Index
FLU	Fludarabine
FN	Febrile neutropenia
FNA	Fine-needle aspiration
FNH	Focal nodular hyperplasia
FoxP3	Forkhead box P3
FSH	Follicle-stimulating hormone
FSI	Fatigue Symptom Inventory
FTBI	Fractionated total body irradiation
FTBI	Full total body irradiation
FTI	Farnesyltransferase inhibitor
FTOC	Fetal thymic organ culture
5-FU	5-Fluorouracil
FVC	Forced vital capacity
GA	Glatiramer acetate
GABG	German Autologous Bone Marrow Transplant Group
GALT	Gut-associated lymphoid tissue
GAP	GTPase-activating protein
GBM	Glioblastoma multiforme
G-BM	G-PBHC and/or G-CSF primed bone marrow
gc	Genome copies
GCB	Germinal center B-cell-like
G-CSF	Granulocyte colony-stimulating factor
GCT	Germ-cell tumor
GDP	Guanosine diphosphate
GEBDIS	German Breast Cancer Dose Intensity Study
GEE	Generalized estimating equation
GEL/TAMO	Grupo Español de Linfomas/Trasplante Autólogo de Médula Ósea
GEM	Gemcitabine
GEM	Grid Enabled Measures
GEP	Gene expression profiling
GF	Graft failure
GFP	Green fluorescent protein
GFR	Glomerular filtration rate
GGT	γ-Glutamyl transferase
GH	Growth hormone
GHRH	Growth hormone-releasing hormone
GHSG	German Hodgkin Study Group
GI	Gastrointestinal (tract)
GICAT	Gruppo Italiano Collaborativo AIDS e Tumori (Italian Cooperative Group on AIDS and Tumors)
GISL	Gruppo Italiano per lo Studio dei Linfomi (Italian Lymphoma Study Group)
GITMO	Gruppo Italiano per il Trapianto di Midollo Osseo
GM	Galactomannan
GM-CSF	Granulocyte–macrophage colony-stimulating factor
GMP	Granulocyte macrophage progenitor (cell)
GMP	Guanosine monophosphate
GNB	Gram-negative bacilli
GnRH	Gonadotropin-releasing hormone
gp	Glycoprotein
G-PBHC	Granulocyte colony-stimulating factor-mobilized peripheral blood hematopoietic cell
GPI	Glycosylphosphatidylinositol
GPI-AP	Glycosylphosphatidylinositol-anchored protein
GRE	Glucocorticoid response element
GSH	Glutathione (reduced)
GST	Glutathione-*S*-transferase
GTP	Good tissue practice
GTP	Guanosine triphosphate
GVD	Gemcitabine, vinorelbine, liposomal doxorubicin
GVH	Graft-versus-host
GVHD	Graft-versus-host disease
GVHL	Graft-versus-Hodgkin disease (lymphoma)
GVL	Graft-versus-leukemia
GVLE	Graft-versus-leukemia effect
GVT	Graft-versus-tumor
GWAS	Genome-wide association study
H&E	Hematoxylin and eosin (stain)
HA	Hemagglutinins
HAART	Highly active antiretroviral therapy
HADS	Hospital Anxiety and Depression Scale
HAMA	Human antimouse antibody
HA-MRSA	Healthcare-associated MRSA
HAV	Hepatitis A virus
HBoV	Human bocavirus
HBV	Hepatitis B virus
HC	Hematopoietic cell
HC	Hemorrhagic cystitis
HC	Histocompatibility complex

4-HC	4-Hydroperoxycyclophosphamide
HCG	Human chorion gonadotropin
HCoV	Human coronavirus
HCT	Hematopoietic cell transplantation
HCT-CI	HCT-Comorbidity Index
HCV	Hepatitis C virus
HD	Hodgkin disease
HDAC	High-dose Ara-C
HDC	High-dose chemotherapy
HDIT	High-dose immunosuppressive therapy
HDT	High-dose therapy
HEPA	High-efficiency particulate air
HES	Hydroxyethyl starch
HES	Hypereosinophilic syndrome
hESC	Human embryonic stem cell
HEV	Hepatitis E virus
HFTBI	Hyperfractionated total body irradiation
HGF	Hepatocyte growth factor
HGNC	HUGO Genome Nomenclature Committee
HHS	Human and Health Services
HHV	Human herpesvirus
HI	Hemagglutination inhibition
HIB	*Haemophilus influenzae* type B
HIF	Hypoxia-inducible transcription factor
HIV	Human immunodeficiency virus
HL	Hodgkin lymphoma
HLA	Human leukocyte antigen
HLH	Hemophagocytic lymphohistiocytosis
hMPV	Human metapneumovirus
HOVON	Hemato-Oncologie voor Volwassenen Nederland
HPA	Hypothalamic–piuitary–adrenal
HPC	Hematopoietic progenitor cell
HPI	Helicase–primase inhibitor
HPV	Human papillomavirus
HR	Hazard ratio
HR	Homologous recombination
HRCT	High-resolution chest tomography
HRE	Hypoxia-inducible transcription factor response element
HRhV	Human rhinovirus
HRPBC	High-risk primary breast cancer
HRQOL	Health-related quality of life
HRSA	Health Resources and Services Administration
HRT	Hormone replacement therapy
HSC	Hematopoietic stem cell
HSC	Hepatic stellate cell
HSCT	Hematopoietic stem-cell transplantation
HSDD	Hypoactive sexual desire disorder
HSPC	Hematopoietic stem/progenitor cell
HSTCL	Hepatosplenic T-cell lymphoma
HSV	Herpes simplex virus
HSV-TK	Herpes simplex virus thymidine kinase
HT	Histologic transformation
5-HT	5-Hydroxytryptamine (serotonin)
hTERT	Human telomerase reverse transcriptase
HTLV	Human T-cell lymphotropic virus
HTS	High-throughput sequencing
HU	Hydroxyurea
HUS	Hemolytic uremic syndrome
HVEM	Herpes virus entry mediator
HVG	Host-versus-graft (reaction)
IA	Invasive aspergillosis (infection)
IBCSG	International Breast Cancer Study Group
IBDIS	International Breast Cancer Dose Intensity Study
IBMTR	International Bone Marrow Transplant Registry
IBW	Ideal body weight
ICAM	Intracellular adhesion molecule
ICE	Ifosfamide, carboplatin, etoposide

ICOS	Inducible costimulator (protein)
ICOS-L	Inducible costimulator ligand
ICSI	Intracytoplasmic sperm injection
ICU	Intensive care unit
ID	Intermediate dose
iDC	Immature dendritic cells
IDO	Indoleamine 2,3-dioxygenase
IDOX	4-Iodo-4-deoxydoxorubicin
IDSA	Infectious Diseases Society of America
IE	Immediate early (antigen expression)
IEM	Inborn errors of metabolism
IFI	Invasive fungal infection
IFN	Interferon
IFRT	Involved field radiotherapy
Ig	Immunoglobulin
IGCCCG	International Germ Cell Cancer Collaborative Group
IGEV	Ifosfamide, gemcitabine, vinorelbine, prednisone
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
IgG	Immunoglobulin G
IgH	Immunoglobulin heavy chain
IGRT	Image-guided radiation therapy
IHC	Immunohistochemistry
IHWG in HCT	International Histocompatibility Working Group in Hematopoietic Cell Transplantation
iKIR	Inhibitory killer immunoglobulin-like receptor
IL	Interleukin
IM	Imatinib
IMGT	International ImMunoGenetics
IMID	Immunomodulatory drug
IMPDH	Inosine monophosphate dehydrogenase
IMRT	Intensity-modulated radiation therapy
IMWG	International Myeloma Working Group
IND	Improvised nuclear device
IND	Investigational new drug
iNK-T	Invariant natural killer T (cell)
iNOS	Inducible NO synthase
INR	International normalized ratio
INRG	International Neuroblastoma Risk Group
INRGSS	International Neuroblastoma Risk Group Staging System
INSS	International Neuroblastoma Staging System
INSTI	Integrase strand transfer inhibitor
IORT	Intraoperative radiotherapy
IP	Interstitial pneumonia
IP_3	Inositol-1,4,5-triphosphate
IPD	Invasive pneumococcal disease
IPEX	Immunodysregulation polyendocrinopathy enteropathy X-linked (syndrome)
IPFSG	International Prognostic Factor Study Group
IPI	International Prognostic Index
iPS	Induced pluripotent stem
IPS	Idiopathic pneumonia syndrome
IPS	Interstitial pneumonia syndrome
IPSS	International Prognostic Scoring System
IPV	Inactivated polio vaccine
IRB	Institutional Review Board
IRF	Interferon regulatory factor
IRSG	Intergroup Rhabdomyosarcoma Study Group
IS	Immune synapse
IS	Immunosuppressive
IS	International Scale
ISC	Intestinal stem cell
ISO	Isohemagglutinin
IST	Immunosuppressive therapy
ISW	Immune suppression withdrawal
ITAM	Immunoreceptor tyrosine-binding activation motif
ITCC	Innovative Therapies for Children with Cancer

ITD	Internal tandem duplication
ITIM	Immunoreceptor tyrosine-binding inhibition motif
iTreg	Induced regulatory T (cell)
ITT	Intent-to-treat
IUPM	Infectious units per million
IV	Intravenous
IVF	In vitro fertilization
IVF	Intravenous fluid
IVIg	Intravenous immunoglobulin
IκB	Inhibitor I kappa B
JACIE	Joint Accreditation Committee of ISCT-Europe and EBMT
JAK	Janus kinase
JALSG	Japan Adult Leukemia Study Group
JCBBN	Japan Cord Blood Bank Network
JCV	JC virus
JIA	Juvenile idiopathic arthritis
JMDP	Japan Marrow Donor Program
JMML	Juvenile myelomonocytic leukemia
K15	Cytokeratin 15
KD	Kinase domain
KGF	Keratinocyte growth factor
KIR	Killer immunoglobulin-like receptor
KM	Kaplan–Meier
KPC	*Klebsiella pneumoniae* carbapenemase
KPS	Karnofsky Performance Score
KREC	Kappa-deleting recombination excision circle
LAIV	Live attenuated influenza vaccine
LAMP	Loop-mediated isothermal amplification
LAVV	Live attenuated varicella vaccine
LBL	Lymphoblastic lymphoma
LC	Langerhans cell
LCH	Langerhans cell histiocytosis
LCL	Lymphoblastic cell lines
LD	Linkage disequilibrium
LDH	Lactate dehydrogenase
LEDGF	Lens epithelium-derived growth factor
LenDex	Lenalidomide, dexamethasone
LET	Linear energy transfer
LFA	Leukocyte function-associated antigen
LFR	Limited field radiation
LFS	Leukemia-free survival
LFS	Lung function score
LGL	Large granular lymphocyte
LH	Luteinizing hormone
LHRH	Luteinizing hormone-releasing hormone
LIF	Leukemia inhibitory factor
LMD	Laser microdissection
LMP	Latency membrane protein
LMWH	Low molecular weight heparin
LN	Lymph node
LOH	Loss of heterozygosity
LPHD	Lymphocyte-predominant Hodgkin disease
LPS	Lipopolysaccharide
LQ	Linear quadratic
LRD	Lower respiratory tract disease
LRP	Lipoprotein receptor-related protein
LRP	Lung resistance protein
LRR	Leucine-rich-repeat
LSD	Lyosomal storage disorders
LTC-IC	Long-term culture initiating cell
LTFU	Long-term follow-up
LT-HSC	Long-term hematopoietic stem cell
LUTS	Lower urinary tract syndrome
LV	Lenti virus
LVEF	Left ventricular ejection fraction
M protein	Monoclonal protein
MA	Myeloablative
mAb	Monoclonal antibody
MAC	Membrane attack complex
MAC	Myeloablative conditioning
MAFIA	Macrophage Fas-induced apoptosis
MALDI-TOFMS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MAP	High-dose methotrexate, cisplatin, and doxorubicin
MAPK	Mitogen-activated protein kinase
MBC	Metastatic breast cancer
MBL	Monoclonal B-cell lymphocytosis
MBP	Myelin basic protein
MCD	Minimal change disease
MCL	Mantle cell lymphoma
MCL	Mast cell leukemia
mCMV	Murine cytomegalovirus
M-CSF	Macrophage colony-stimulating factor
MCyR	Major cytogenetic response
MDASI	M.D. Anderson Symptom Inventory
mDC	Mature dendritic cells
MDex	Melphalan, dexamethasone
MDRD	Modification of Diet in Renal Disease (Study)
MDS	Myelodysplastic syndrome
MDSC	Myeloid-derived suppressor cell
MEAC	Minimum effective analgesic concentration
MEL	Melphalan
MEP	Megakaryocyte erythrocyte progenitor
mESC	Mouse embryonic stem cell
mesna	Sodium 2-mercaptoethanesulfonate
MetaEICESS	Meta European Intergroup Cooperative Ewing Sarcoma Study
MF	Mycosis fungoides
MF	Myelofibrosis
MFC	“Multiparametric” flow cytometric
MFC	Multidimensional flow cytometry
MFD	Matched family donor
MG	Myasthenia gravis
MGDF	Megakaryocyte growth and development factor
MGH	Massachusetts General Hospital
MGMT	Methylguanine methyltransferase
MGUS	Monoclonal gammopathy of undetermined significance
mHA	Minor histocompatibility antigen
mHAQ	Modified Health Assessment Questionnaire
MHC	Major histocompatibility complex
MHV	Mouse hepatitis virus
MIC	Minimum inhibitory concentration
MICA	MHC class I chain-related gene A
MIP-1	Macrophage inflammatory protein 1
MIPI	Mantle Cell Prognostic Index
MIRD	Medical internal radiation dose
MIRL	Membrane inhibitor of reactive lysis
miRNA	micro-RNA
mITT	Modified intent-to-treat
MJM	Muscle and Joint Measure
MKP	Megakaryocyte progenitor
MLC	Mixed leukocyte culture
MLD	Metachromatic leukodystrophy
MLL	Mixed leukemia lymphoma gene
MLL	Mixed lineage leukemia
MLR	Mixed leukocyte reaction
MLS	Maroteaux–Lamy syndrome
MM	Multiple myeloma
MMF	Mycophenolate mofetil
MMR	Major molecular response
MMR	Measles/mumps/rubella
MMRD	Mismatched related donor
MMUD	Mismatched unrelated donor

MNC	Mononuclear cell
MODS	Multiple-organ dysfunction syndrome
MOG	Myelin oligodendrocyte glycoprotein
MOPP	Mechlorethamine, vincristine (Oncovin), procarbazine, prednisone
MP	Melphalan, prednisone
MP	Methylprednisolone
MPA	Mycophenolic acid
MPAG	7-*O*-Mycophenolic acid glucuronide
MPAL	Mixed phenotype acute leukemia
MPB	Mobilized peripheral blood (cells)
MPFC	Multiparameter flow cytometry
MPN	Myeloproliferative neoplasm
MPP	Multipotent progenitor
MPT	Melphalan, prednisone, thalidomide
MPV	melphalan, prednisone, bortezomib (Velcade)
MRC	Medical Research Council
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
m-RNA	Messenger RNA
MRP	Multidrug resistance-associated protein
MRS	Magnetic resonance spectroscopy
MRSA	Methicillin-resistant *Staphylococcus aureus*
mRSS	Modified Rodman skin score
MS	Mass spectrometry
MS	Multiple sclerosis
MSC	Mesenchymal stem cell
MSC	Mesenchymal stromal cell
MSCH	Mouse spinal cord homogenate
MSFC	Multiple sclerosis functional composite
MSI	Microsatellite instability
MSKCC	Memorial Sloan Kettering Cancer Center
MS-LCH	Multi-system Langerhans cell histiocytosis
MSSA	Methicillin-susceptible *Staphylococcus aureus*
MTD	Maximum tolerated dose
MTOR	Mammalian target of rapamycin
Mtvs	Mouse mammary tumor virus
MTX	Methotrexate
MUD	Matched unrelated donor
MV	Measles virus
MVA	Multivariate analysis
MZ	Marginal zone
NA	Nucleoside analog
NA-DP	Nucleoside analog diphosphate
NANT	New Approaches to Neuroblastoma Therapy
NAP1	North American Pulsed Field Type 1
NASH	Non-alchoholic steatohepatitis
NAT	Nucleic acid amplification test
NA-TP	Nucleoside analog triphosphate
nbmtLINK	National Bone Marrow Transplant link
NC	Nucleated cell
NCAM	Neural cell adhesion molecule
NCCN	National Comprehensive Cancer Network
NCHS	National Center for Health Statistics
NCI	National Cancer Institute
NCIC	National Cancer Institute of Canada
NCI-WG	National Cancer Institute – Working Group
nCR	Near complete response
NCR	Natural cytotoxicity receptor
NDK	Nucleotide diphosphate kinase
NE	Norepinephrine
NEMO	Nuclear factor-kappa B essential modulator
NER	Nucleotide excision repair
NF-AT	Nuclear factor of activated T cells
NFκB	Nuclear factor-kappa B
NGS	Next-generation sequencing
NHANES	National Health and Nutrition Examination Surveys
NHEJ	Non-homologous end-joining
NHL	Non-Hodgkin lymphoma
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institutes of Health
NIL	Nilotinib
NIMA	Non-inherited maternal antigen
NIPA	Non-inherited paternal antigen
NK	Natural killer (cell)
NK	Normal karyotype
NK-T	Natural killer T (cell)
NLR	Nucleotide-binding oligomerization domain leucine-rich-repeat-containing receptor
NM	Non-myeloablative
NMA	Non-myeloablative
NMDA	*N*-Methyl-D-aspartate
NMDP	National Marrow Donor Program
NMHCT	Non-myeloablative hematopoietic cell transplantation
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NO	Nitric oxide
NOD	Non-obese diabetic (mouse)
NOD	Nucleotide-binding oligomerization domain
NPC	Nasopharyngeal carcinoma
NRM	Non-relapse mortality
NRS	Neurological Rating Scale
NRTI	Nucleoside reverse transcriptase inhibitor
NS	Nephrotic syndrome
NS	Noonan syndrome
NSAID	Non-steroidal anti-inflammatory drug
N-SH2	N-Terminal src-homology 2
NST	Non-myeloablative stem-cell transplantation
NT-proBNP	N-Terminal fragment of pro-brain natriuretic peptide
nTreg	Naturally occurring regulatory T (cell)
NWTS	National Wilms Tumor Study
NZB	New Zealand black
NZW	New Zealand white
OATP	Organic anion-transporting polypeptide
OCTP	Organic cation-transporting polypeptide
OFAR	Oxaliplatin, fludarabine, cytarabine, rituximab
OLD	Obstructive lung disease
ONJ	Osteonecrosis of the jaw
ONS	Oncology Nursing Society
OPA	Office of Patient Advocacy
OPG	Osteoprotegerin
OPM	Oropharyngeal mucositis
O-PRISM	Oncologic Pediatric Risk of Mortality
OR	Odds ratio
OS	Overall survival
PACT	Psychological Assessment of Candidates for Transplantation Scale
PAD	Bortezomib, adriamycin, dexamethasone
PALE	Post-transplant acute limbic encephalitis
PAM	Pretransplant assessment of mortality
PAMP	Pathogen-associated molecular pattern
PAS	Periodic acid–Schiff
PB	Peripheral blood
PBHC	Peripheral blood hematopoietic cell
PBHCT	Peripheral blood hematopoietic cell transplantation
PBL	Peripheral blood lymphocyte
PBMC	Peripheral blood mononuclear cell
PBMTC	Pediatric Blood and Marrow Transplant Consortium
PBPC	Peripheral blood progenitor cell
PBSC	Peripheral blood stem cell
PCA	Patient-controlled analgesia
PCNSL	Primary central nervous system lymphoma
PCORI	Patient-Centered Outcomes Research Institute
PCR	Polymerase chain reaction
PCR	Programmed cell renewal

PCT	Pulmonary cytolytic thrombi
PCV	Pneumococcal conjugate vaccine
PCV	Protein conjugate vaccine
PD	Programmed death
PD	Progressive disease
PDC	Plasmacytoid dendritic cell
PDE	Phosphodiesterase
PDGFA	Platelet-derived growth factor A chain
PDGFR	Platelet-derived growth factor receptor
pDLI	Preemptive donor lymphocyte infusion
PDQ	Physician Data Query
PedsQL	Pediatric Quality of Life Inventory
PEGS	Platinum, etoposide, gemcitabine, methylprednisolone
PERDS	Peri-engraftment respiratory distress syndrome
PET	Positron emission tomography
PETHEMA	Programa para el Tratamiento de Hemopatías Malignas
PFS	Progression-free survival
PFT	Pulmonary function test
PGD	Pre-implantation genetic diagnosis
P-gp	P-glycoprotein
Ph	Philadelphia (chromosome)
PH	Pulmonary hypertension
PHA	Phytohemagglutinin A
PHN	Post-herpetic neuralgia
PHQ-9	Patient Health Questionnaire-9
PI	Protease inhibitor
PI3K	Phosphatidylinositol 3-kinase
PICC	Peripherally inserted central venous catheter
PID	Primary immunodeficiency disease
PIG-A	Phosphatidylinositol glycan-A
PIP$_2$	Phosphatidylinositol 4,5-diphosphate
PIP$_3$	Phosphatidylinositol 3,4,5-triphosphate
PIQ	Performance IQ
PJP	*Pneumocystis jirovecii* pneumonia
PK	Pharmacokinetic
PKC	Protein kinase C
PlGF	Placental growth factor
PLL	Prolymphocytic leukemia
PLP	Proteolipid protein
PLPHA	Postlumbar puncture headache
PMC	Persistent mixed chimerism
PMF	Primary myelofibrosis
PMGCT	Primary mediastinal germ-cell tumor
PML	Progressive multifocal leukoencephalopathy
PMN	Polymorphonuclear neutrophil
PN	Parenteral nutrition
PN	Peripheral neuropathy
PNET	Primitive neuroectodermal tumor
PNH	Paroxysmal nocturnal hemoglobinuria
PNH-sc	Subclinical paroxysmal nocturnal hemoglobinuria
POEMS	Polyneuropathy, organomegaly, endocrinopathy, M protein, skin changes
POF	Premature ovarian failure
POG	Pediatric Oncology Group
POLST	Physician Orders for Life Sustaining Treatment
PomDex	Pomalidomide, dexamethasone
PORN	Progressive outer retinal necrosis
PORT	Patient Outcomes Assessment Research Team
PPI	Proton pump inhibitor
PR	Partial remission or partial response
PR	Progesterone receptor
PR3	Proteinase 3
PRCA	Pure red cell aplasia
PRES	Posterior reversible encephalopathy syndrome
PRO	Patient-reported outcome
PROMIS	Patient-Reported Outcomes Measurement Information System

PRR	Pathogen recognition receptor
PRST	Pediatric Registry for Stem Cell Transplantations
PSA	Prostate-specific antigen
PSC	Pluripotent stem cell
PSE	Prednisone
PSQI	Pittsburgh Sleep Quality Index
PT	Pertussis toxoid
PTA	Peripheral tissue antigen
PTCL	Peripheral T-cell lymphoma
PTCL-nos	Peripheral T-cell lymphoma not otherwise specified
PTH	Parathyroid hormone
PTLD	Post-transplant lymphoproliferative disorder
PTPase	Protein tyrosine phosphatase
PUVA	Psoralen–ultraviolet A irradiation (treatment)
PV	Polycythemia vera
PVR	Polio virus receptor
PyVAN	Polyomavirus-associated nephropathy
PyVHC	Polyomavirus-associated hemorrhagic cystitis
QALY	Quality-adjusted life-year
QLQ	Quality of Life Questionnaire
QOL	Quality of life
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
QTL	Quantitative trait loci
QW	Once weekly
RA	Refractory anemia
RA	Retinoic acid
RA	Rheumatoid arthritis
RA	Right atrium
RAb	Radiolabeled antibody
RAEB	Refractory anemia with excess blasts
RAEB-T	Refractory anemia with excess blasts in transformation
RANKL	Receptor activator of nuclear factor **k**appa B ligand
RARS	Refractory anemia with ringed sideroblasts
RBC	Red blood cell
RCC	Ratio of costs to charges
RCC	Refractory cytopenia of childhood
RCC	Renal cell carcinoma
R-CHOP	Rituximab, cyclophosphamide, hydroxydaunomycin, vincristine (Oncovin), prednisone
RCMD	Refractory cytopenia with multilineage dysplasia
RCR	Replication competent retrovirus
RCT	Randomized controlled trial
REAL	Revised European–American Lymphoma
R-EPOCH	Rituximab, etoposide, prednisone, vincristine (Oncovin), doxorubicin hydrochloride
RES	Reticuloendothelial system
R-ESHAP	Rituximab, etoposide, cytarabine, cisplatin, methylprednisolone
RFS	Relapse-free survival
Rh	Rhesus
rHBV	Recombinant hepatitis B vaccine
RHC	Residual host cells
rhGH	Recombinant human growth hormone
r-hIL-7	Recombinant human interleukin-7
rHuEPO	Recombinant human erythropoietin
R-hyperCVAD	RTX (rituximab), vincristine, doxorubicin, dexamethasone
RI	Relapse incidence
RIC	Reduced-intensity conditioning
RICR	Reduced-intensity conditioning regimen
RIFLE	Risk, injury, failure, loss, end-stage
RISC	RNAi-induced silencing complex
RIT	Radioimmunotherapy
RLD	Restrictive lung disease
RLR	Rig-1-like receptor
RN	Registered nurse

RNAi	RNA interference
RNR	Ribonucleotide reductase
ROC	Receiver-operating characteristic
ROS	Reactive oxygen species
RR	Relapse rate
RR	Relative risk
RR	Response rate
RRE	Rev response element
RRE	Risk ratio estimate
RRMS	Relapsing–remitting multiple sclerosis
RRT	Regimen-related toxicity
RSCH	Rat spinal cord homogenate
RSCL	Rotterdam Symptom Checklist
RSV	Respiratory syncytial virus
RT	Reverse transcriptase
RTE	Recent thymic emigrant
RTID	Respiratory tract infectious disease
RT-PCR	Real-time polymerase chain reaction
RTQ	Real-time quantitative
RTX	Rituximab
RVD	Revlimid/lenalidomide, Velcade/bortezomib, dexamethasone
S1P	Sphingosine-1 phosphate
SAA	Serum amyloid A
SAA	Severe aplastic anemia
SAAG	Serum-ascites albumin gradient
SAHA	Suberoylanilide hydroxamic acid
SAP	Serum amyloid protein P
SARS	Severe acute respiratory syndrome
SAS-SR	Social Adjustment Scale – Self Report
SBT	Sequence-based typing
SCC	Squamous cell carcinoma
SCD	Sickle cell disease
SCF	Stem-cell factor
scFv	Single-chain variable fragment
SCID	Severe combined immunodeficiency syndrome
SCID-hu	Severe combined immunodeficiency syndrome/human (mouse)
SCL	Stem-cell leukemia
SCT	Stem-cell transplantation
SCTOD	Stem Cell Therapeutics Outcomes Database
SD	Stable disease
SD	Standard deviation
SDC	Standard-dose chemotherapy
SDF	Stroma cell-derived factor
SDS	Shwachman–Diamond syndrome
SE	Standard error
SEC	Sinusoidal endothelial cell
SEER	Surveillance Epidemiology and End Results
Sema	Semaphorin
SEREX	Serological identification of antigens by recombinant expression cloning
SERM	Selective estrogen response modulator
SF-36	Medical Outcomes Study Short Form 36
SFGM	Société Française de Greffe de Moelle
SFK	SRC-family kinase
SFOP	Société Française de l'Oncologie Pédiatrique, French Society of Pediatric Oncology
SFQ	Sexual Function Questionnaire
SGOT	Serum glutamic-oxaloacetic transaminase
SHBG	Sex hormone-binding globulin
SHEA	Society for Healthcare Epidemiology of America
Shh	Sonic hedgehog
SHM	Somatically hypermutated
SI	Stimulation index
SIADH	Syndrome of inappropriate antidiuretic hormone
SIG	Special Interest Group
sIgm	Cell surface immunoglobulin M
siRNA	Short interfering RNA
SJS	Stevens–Johnson syndrome
SKD	Severe keratinocyte dysplasia
SLAM	Signaling lymphocyte activation molecule
SLD	Sublethal damage
SLE	Systemic lupus erythematosus
SLEDAI	SLE Disease Activity Index
SM	Systemic mastocytosis
SMILE	Ddexamethasone, methotrexate, ifosfamide, L-asparaginase, etoposide
SMN	Subsequent malignant neoplasm
SMR	Standardized mortality ratio
SNP	Single nucleotide polymorphism
SNRI	Serotonin–norepinephrine reuptake inhibitor
SNS	Sympathetic nervous system
SOFA	Sequential (or sepsis-related) Organ Failure Score
SOP	Standard operating procedure
SOS	Sinusoidal obstruction syndrome
SOT	Solid organ transplantation
SP	Surfactant protein
SPECT	Single photon emission computed tomography
SR	Sensitive relapse
SR	Standard risk
SS	Sézary syndrome
SSA	Sex steroid ablation
SSA	Single-strand annealing
SSc	Systemic sclerosis
SSD	Source-to-skin distance
SSOP	Sequence-specific oligonucleotide probe
SSP	Sequence-specific primer
SSRI	Selective serotonin reuptake inhibitor
SST	Short Syntachten Test
STAMP-I	Cyclophosphamide, cisplatin, BCNU
STAMP-V	Cyclophosphamide, carboplatin, thioTEPA
STATs	Signal transducers and activators of transcription
STBI	Single-fraction total body irradiation
ST-HSC	Short-term hematopoietic stem cell
STING	Stimulator of interferon genes
STR	Short tandem repeat
SVC	Superior vena cava
SWOG	Southwest Oncology Group
T1DM	Type 1 diabetes mellitus
T3	Triiodothyronine
T4	Thyroxine
TAA	Tumor-associated antigen
TAAM	Time-averaged mean of the maximum
TACL	Therapeutic Advances in Childhood Leukemia
TAD	Thalidomide, adriamycin, dexamethasone
TA-GVHD	Transfusion-associated graft-versus-host disease
TAI	Thoracoabdominal irradiation
TAM	Transplant-associated microangiopathy
t-AML	Therapy-related acute myeloid leukemia
TAP	Transporter associated with antigen processing
TAR	Tat response element
TBI	Total body irradiation
TCA	Tricyclic antidepressant
TCD	T-cell depletion
TCD	Transcranial Doppler (ultrasonography)
TCE	T-cell epitope
T_{CM}	Central memory T (cells)
Tcon	Conventional T
TCR	T-cell receptor
TD	Thalidomide, dexamethasone
TDM	Therapeutic drug monitoring
TdT	Terminal deoxynucleotidyl transferase
TED	Thromboembolic disease
T_{EM}	Effector memory T (cell)
TEN	Toxic epidermal necrolysis

TEPA	*N*-Triethylenephosphoramide
TEPC	Thymic epithelial progenitor cell
TERS	Transplant Evaluation Rating Scale
TERT	Telomerase reverse transcriptase
TF	Tissue factor
T_{FH}	T follicular helper
TFIIH	Transcription factor IIH complex
TFPI	Tissue factor pathway inhibitor
TG	Triglyceride
TGF-β	Transforming growth factor beta
T-GVHD	Transfusion-associated graft-versus-host disease
TH	Helper T-lymphocytes
T_H	T helper
thioTEPA	*N*-Triethylenethiophosphoramide
TICE	Paclitaxel, ifosfamide followed by high-dose carboplatin, etoposide
TIL	Tumor-infiltrating lymphocytes
TIP	Paclitaxel, ifosfamide, cisplatin
TIV	Trivalent influenza vaccine
TK	Thymidine kinase
TKI	Tyrosine kinase inhibitor
TLC	Total lung capacity
TLI	Total lymphoid irradiation
TLR	Toll-like receptor
TLS	Tumor lysis syndrome
TM	Thalassemia major
TMA	Thrombotic microangiopathy
TMD	Temporomandibular dysfunction
t-MDS	Therapy-related myelodysplastic syndrome
TMEV	Theiler's murine encephalomyelitis virus
TMI	Total marrow irradiation
TMLI	Total marrow and lymphoid irradiation
TMP–SMX	Trimethoprim–sulfamethoxazole
TM–SLF	Trimethoprim–sulfamethoxazole
TNC	Total nucleated cell
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TNF-α	Tumor necrosis factor α
TOI	Trial Outcome Index
TPA	Tissue plasminogen activator
T-PLL	T-cell prolymphocytic leukemia
TPN	Total parenteral nutrition
TPO	Thrombopoietin
TR	Tandem repeat
TRAIL	TNF-related apoptosis-inducing ligand
TRALI	Transfusion-related acute lung injury
TREC	T-cell receptor excision circles
Treg	Regulatory T (cell)
Treo	Treosulfan
TRM	Transplant-related mortality
TRT	Testosterone replacement therapy
TRV	Tricuspid regurgitation velocity
TSH	Thyroid-stimulating hormone
TSLP	Thymic stromal lymphoietin
TT	*N*-Triethylenethiophosphoramide, thioTEPA
TTP	Thrombotic thrombocytopenic purpura
TTR	Transthyretin
UBC	Umbilical cord
UCB	Umbilical cord blood
UCB	Unrelated cord blood
UCBT	Umbilical cord blood transplant
UGT	Uridine 5′-diphosphate glucuronosyltransferase
UPD	Uniparental disomy
URD	Unrelated donor
URI	Upper respiratory tract infection
UV	Ultraviolet
VAD	Vincristine, adriamycin, dexamethasone
VATS	Video-assisted thoracoscopic surgery
VBAD	Vincristine, BCNU, doxorubicin, dexamethasone
VBMCP	Vincristine, BCNU, melphalan, cyclophosphamide, prednisone
VCD	Velcade/bortezomib, cyclophosphamide, dexamethasone
VDCR	Velcade/bortezomib, dexamethasone, cyclophosphamide, Revlimid/lenalidomide
VDR	Vitamin D receptor
VE	Vascular endothelial
VEGF	Vascular endothelial growth factor
VeIP	Vinblastine, ifosfamide, cisplatin
VF	Ventricular fibrillation
VGPR	Very good partial remission
VIC	Virus isolation by cell culture
VIP	Etoposide, ifosfamide, cisplatin
VIP	Vasoactive intestinal peptide
VISA	Vancomycin-intermediate *Staphylococcus aureus*
VLCFA	Very long-chain fatty acid
VNTR	Variable number of tandem repeat
VOD	Veno-occlusive disease
VRE	Vancomycin-resistant *Enterococcus*
VSAA	Very severe aplastic anemia
VT	Ventricular tachycardia
VTD	Velcade/bortezomib, thalidomide, dexamethasone
vWF	von Willebrand factor
VWMD	Vanishing white matter disease
VZIG	Varicella zoster immune globulin
VZV	Varicella zoster virus
WAS	Wiskott–Aldrich syndrome
WBC	White blood cell
WBM	Whole bone marrow
WBMT	Worldwide Network for Blood & Marrow Transplantation
WBRT	Whole-brain radiotherapy
WES	Whole exome sequencing
WGA	Wheat germ agglutinin
WGS	Whole genome sequencing
WMDA	World Marrow Donor Association
WNV	West Nile virus
WPSS	WHO classification-based Prognostic Scoring System
WSG	West German Study Group
wt	Wild-type
X-ALD	X-linked adrenoleukodystrophy
XRT	External-beam radiotherapy
YFC	[^{90}Y]ibritumomab tiuxetan, fludarabine, cyclophosphamide
ZAP-70	Zeta-associated protein
ZFN	Zinc finger nuclease
ZIG	Zoster immune globulin
ZIP	Zoster immune plasma

CHAPTER 56

Hematopoietic Cell Transplantation for Hodgkin Disease

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Epidemiology and etiology

Hodgkin disease (HD) is a clonal lymphoid disorder of mononuclear Hodgkin cells and multinucleated Reed–Sternberg cells, which are considered to be derived from germinal center B cells [1]. In 2013, it was estimated that there would be 9290 new cases of HD in the United States and that 1180 people would die with this diagnosis [2]. Between 2000 and 2004, the HD incidence rate in the United States was 2.7 per 100,000 [3], and this incidence rate has been stable in recent years. There is a bimodal incidence with one peak in the late teen years and early adulthood, and a second peak that is seen in adults after age 50 years. The incidence rate for HD is higher in men, and higher in Whites than in Blacks, Asians, and Hispanics.

The risk factors associated with HD are not as consistent or well understood as those identified for non-Hodgkin lymphoma. Reed–Sternberg cells often express Epstein–Barr virus (EBV)-associated antigens. The HD incidence rate is higher in patients who have had infectious mononucleosis, although links between EBV and HD are inconsistent. Patients infected with HIV are more likely to develop HD, and HD has been described following solid organ transplantation. Some studies have shown a slight familial risk associated with HD, and there appears to be a higher risk in monozygotic twins of affected individuals.

Molecular and clinical biology

The World Health Organization (WHO) classification divides Hodgkin disease into two entities that have different clinical and biologic characteristics [1]. Approximately 5% of cases are classified as nodular lymphocyte predominant Hodgkin lymphoma (nodular LPHD), which is characterized by neoplastic cells known as popcorn or L&H (lymphocytic and/or histiocytic) cells. The neoplastic cells almost always express CD20. Most patients have classical Hodgkin lymphoma, which is subdivided into nodular sclerosis, mixed cellularity, lymphocyte-rich, and lymphocyte-depleted subtypes. The neoplastic Reed–Sternberg cells within classical HD subtypes usually express CD30, and often CD15.

Hodgkin cells release a variety of cytokines, including various interleukins. This may explain the inflammatory background seen in HD biopsies and explain eosinophilia and some systemic symptoms seen in HD, and also fibrosis seen in biopsies. No consistent cytogenetic abnormalities are seen in HD, although immunoglobulin gene rearrangements are characteristic. No consistent environmental causes of HD have been identified, although some studies have identified increased risk associated with woodworking.

Clinical description

The most common HD symptom is painless lymphadenopathy. The disease spreads contiguously and patients may have localized or widespread disease. Mediastinal involvement is most common in the nodular sclerosis subtype. Extranodal disease is less common than in non-Hodgkin lymphoma (NHL). Patients may have systemic or "B" symptoms consisting of unexplained fevers, night sweats, or weight loss. Pruritus may be seen and precede the diagnosis of HD for months.

Approximately 70% of patients with nodular LPHD are males in the age range 30–50 years. Patients often have localized disease, and may have an indolent disease course. The most common subtype of classical HD is nodular sclerosis, which is more likely to be present in patients with limited-stage disease. The mixed cellularity subtype is more likely to be found in patients with immunodeficiency syndromes.

Non-transplant approaches

More than 50% of HD patients, including those with advanced disease, can be cured with a wide variety of chemotherapy regimens [4,5]. This accomplishment is one of the greatest achievements of modern medicine. There has been a dramatic decline in HD mortality and the 5-year survival rate is now approximately 85%.

In the past, limited-stage HD was usually treated with radiation therapy. Limited radiation may be used for low-stage nodular LPHD. However, concerns about late toxicity associated with extensive radiation fields have led to the common practice of using abbreviated courses of chemotherapy followed by involved-field radiation for most patients. In some cases, chemotherapy alone may be used. Patients with advanced disease or bulky masses are treated with a full course of chemotherapy and sometimes receive additional radiotherapy. A major focus of new treatment regimens involves the reduction of late toxicities of HD treatment.

Although most patients with HD are cured with initial therapy, a significant proportion still fail to attain a complete remission or

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relapse after attaining a remission. A variety of conventional-dose second-line (salvage) regimens have been used for these patients, but results of treatment with these regimens are disappointing and few patients achieve long-term disease-free survival (DFS).

Hematopoietic cell transplantation

The poor results of conventional-dose salvage therapy for relapsed and refractory HD have led to the use of high-dose therapy (HDT) followed by autologous bone marrow transplantation (BMT) and peripheral blood hematopoietic cell transplantation (PBHCT) for these patients. This approach is based upon the steep dose–response curves exhibited by several drugs, and also radiation therapy, and the fact that dose-limiting toxicities are often related to myelosuppression. The first attempts at using autologous bone marrow infusions to reduce myelosuppression following radiation and chemotherapy were documented in the 1950s. Later, techniques for bone marrow harvesting and cryopreservation were refined and results of autologous BMT for HD began to be reported [6].

Since then, the use of HDT followed by autologous hematopoietic cell transplantation (aHCT) for HD has increased dramatically and this approach has become accepted therapy in a variety of circumstances. In North America, HD is the third most common indication for aHCT after multiple myeloma and NHL. Approximately 1200 aHCTs for HD were reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) in 2009 [7]. It is estimated that this total represents approximately 60% of the transplants being performed for this indication in North and South America. Results from the European Group for Blood and Marrow Transplantation (EBMT) are similar [8]. HD is the third most common indication for aHCT in Europe, and it was reported that 1677 aHCTs for HD were performed there during 2005.

Autologous HCT has become easier, safer, and less expensive over the last 25 years. Mortality within 100 days of transplant is generally under 5% and improved outcomes over time have been demonstrated [7,9]. These improvements are due to increased experience at individual institutions, improved patient selection, the routine use of hematopoietic growth factors, and the increased use of autologous PBHCT, as compared with autologous BMT. aHCT is commonly performed by community oncologists and can be performed on elderly patients, in the outpatient setting [10], and in patients with religious objections to blood transfusion [11].

Results of autologous hematopoietic cell transplantation

The results of some of the larger and most recent series of aHCT for HD are displayed in Table 56.1. It is difficult to compare results because of differences in selection criteria, patient characteristics, HDT regimens, and supportive care. In addition, the length of follow-up varies considerably and some reports include patients transplanted before the routine use of autologous PBHCT and hematopoietic growth factors. Nevertheless, these results demonstrate that a significant proportion of patients achieve long-term progression-free survival (PFS) following transplantation, and that transplantation can be accomplished with low early mortality. It is unknown whether the use of more aggressive and more effective primary chemotherapy regimens will alter the results of transplantation for patients who require salvage therapy [4].

Representative results of HDT followed by aHCT for a large group of patients with relapsed and refractory HD with long follow-up are displayed in Figure 56.1. The results of aHCT for patients with relapsed and refractory HD appear better than those reported with conventional-dose salvage chemotherapy.

Randomized trials

Two randomized trials have compared the results of conventional-dose salvage chemotherapy with aHCT for relapsed and refractory HD. A British National Lymphoma Investigation (BNLI) trial randomized patients with relapsed and refractory HD to receive treatment with mini-BEAM [BCNU = 1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine), etoposide, Ara-C = cytarabine (cytosine arabinoside), melphalan] conventional-dose salvage chemotherapy, or treatment with BEAM (same drugs at higher doses) followed by autologous BMT (aBMT) [12]. This study design tested the role of dose intensity

Table 56.1 Results of autologous hematopoietic cell transplantation for Hodgkin disease

Ref.	*n*	Rescue source	Regimen	Median follow-up	Early mortality (%)	Outcome
[12]	73	BM, PB	VP-16 + Mel	30 mo	4	39% 4-yr DFS
[13]	155	BM	BEAM	NS	10	50% 5-yr DFS
[14]	128	BM, PB	CBV	77 mo	9	25% 4-yr FFS
[15]	85	BM, PB	CY + VP-16 + TBI or CBV	28 mo	8	58% 2-yr EFS
[16]	119	BM, PB	Various	40 mo	5	48% 4-yr EFS
[17]	102	BM, PB	CBV	4.1 yrs	12	42% 3-yr PFS
[18]	280	BM, PB	Various	36 mo	6	60% 4-yr PFS
[19]	70	BM, PB	BEAC	3.6 yrs	13	32% 5-yr EFS
[20]	414	BM, PB	Various	46 mo	7	63% 3-yr OS
[21]	40	PB	BEAM	28 mo	3	69% 3-yr PFS
[22]	56	PB	CY + VP-16 + TBI or CBV	43 mo	4	68% 3-yr EFS
[9]	494	BM, PB	Various	30.5 mo	9	45% 5-yr EFS
[23]	104	BM, PB	Various	5.1 yrs	16	26% 10-yr EFS
[24]	101	BM, PB	BEAM	50 mo		60% 5-yr FF2F
[25]	92	BM, PB	CY + VP-16 + TBI or Bu + Mel + T	6 yrs	15	51% 6-yr EFS
[26]	102	PB	HDS	5 yrs	5	53% 5-yr EFS
[27]	100	BM, PB	CBV ± Plat	11.4 yrs	8	51% 15-yr PFS
[28]	341	BM, PB	Various		8	45% 5-yr EFS
[29]	141	BM, PB	CBV	6.3 yrs	1	48% 5-yr PFS
[30]	127	BM, PB	BU + CY + VP-16	6.7 yrs	6	48% 5-yr PFS

BM, bone marrow; PB, peripheral blood; DFS, disease-free survival; FFS, failure-free survival; EFS, event-free survival; PFS, progression-free survival; OS, overall survival; FF2F, freedom from second failure; NS, not stated; VP-16, etoposide; MEL, melphalan; BEAM, carmustine, etoposide, cytarabine, melphalan; CBV, cyclophosphamide, carmustine, etoposide; BEAC, carmustine, etoposide, cytarabine, cyclophosphamide; CY, cyclophosphamide; BU, busulfan; T, thioTEPA; HDS, high-dose sequential chemotherapy; TBI, total body irradiation; Plat, cisplatin; mo, months; yr, year.

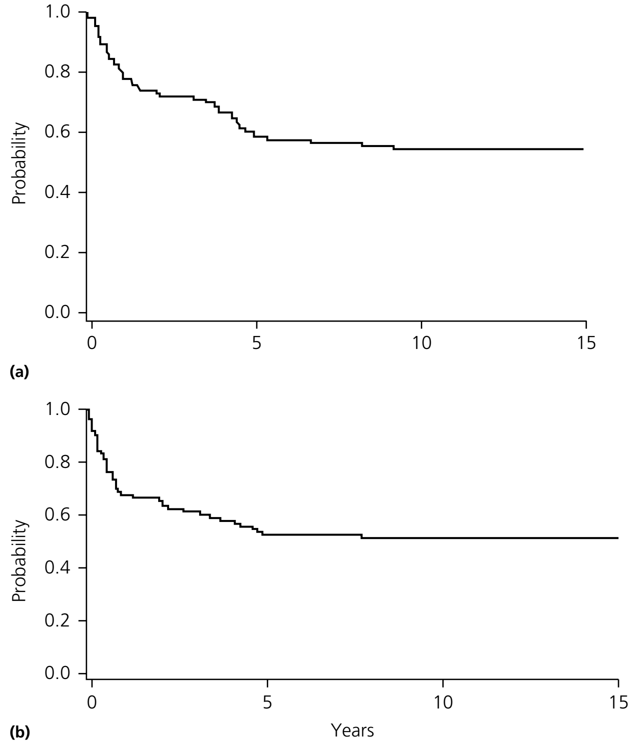


Figure 56.1 (a) Overall survival and (b) progression-free survival following high-dose chemotherapy and autologous hematopoietic cell transplantation for relapsed and refractory Hodgkin lymphoma. Source: Lavoie et al. 2005 [36]. Reproduced with permission of the American Society of Hematology.

in the treatment of HD since the same drugs were used in each treatment arm. The 3-year event-free survival (EFS) was estimated to be 53% in transplanted patients, compared with 10% in the mini-BEAM group (Figure 56.2). The risk of disease progression was also significantly lower in transplanted patients, although no significant differences in overall survival (OS) were observed. Some participants preferred treatment with aBMT and refused randomization, and the study was terminated early.

The German Hodgkin Study Group (GHSG) and the EBMT conducted the other randomized trial, which also closed early because of poor accrual [13]. After randomization, patients received two courses of salvage chemotherapy with Dexa-BEAM (dexamethasone, carmustine, etoposide, cytarabine, melphalan). Responding patients received either two additional courses of Dexa-BEAM, or else high-dose BEAM followed by aHCT. The freedom from treatment failure at 3 years was estimated to be 55% for patients treated with aHCT, compared with 34% for those who received Dexa-BEAM, alone (Figure 56.3). OS rates were estimated to be 71 and 65%, respectively ($p = 0.331$).

Non-randomized trials

Several non-randomized trials have compared the results of conventional salvage chemotherapy with results of aHCT for patients with relapsed and refractory HD. A French registry analysis examined outcomes in patients who failed to respond to treatment or progressed after treatment with a single chemotherapy regimen [14]. The 5-year OS was estimated to be 71% for patients who received aHCT, compared with 32% for others ($p = 0.0001$). Freedom from treatment failure was estimated to be 60 and 20%, respectively ($p = 0.0001$). A multivariate analysis showed that salvage treatment that did not include HDT was associated with significantly worse OS ($p = 0.02$) and freedom from treatment failure ($p = 0.02$). Additional trials discussed in later sections include retrospective registry analyses after first relapse [15], single-institution studies of patients with refractory and relapsed disease [16], and registry analyses of patients with primary refractory disease [14,17,18].

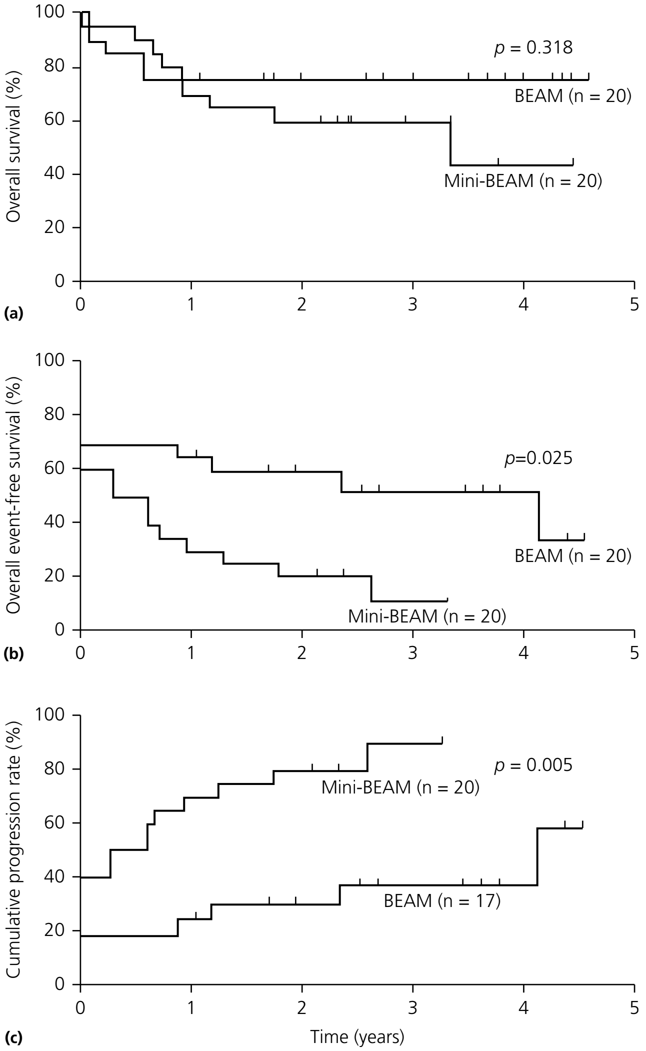
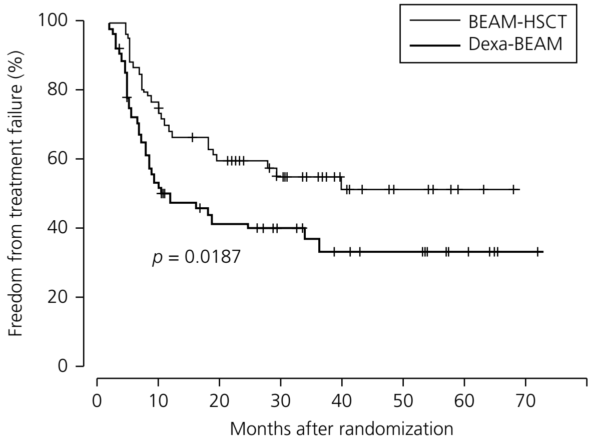


Figure 56.2 (a) Overall survival, (b) event-free survival, and (c) cumulative progression rate in carmustine (BCNU), etoposide, cytarabine, and melphalan plus autologous bone marrow transplant (BEAM) and mini-BEAM groups. Source: Linch et al. 1993 [12]. Reproduced with permission of Elsevier.

Prognostic factors

A large number of factors have been identified that are associated with adverse outcomes for patients who undergo aHCT for HD. Several prognostic models have been developed that use these factors to predict outcome following transplantation. The Vancouver group examined patients undergoing aBMT for HD in first relapse [19]. The presence of systemic symptoms at relapse, extranodal disease at relapse, and initial remission duration less than 12 months were associated with shorter PFS. The 3-year PFS was 100, 81, 40,



Number of patients

BEAM-HSCT	61	43	34	25	13	8	7	0
Dexa-BEAM	56	27	20	15	10	8	5	1

Figure 56.3 Freedom from treatment failure for patients with relapsed chemosensitive Hodgkin disease. Source: Schmitz et al. 2002 [13]. Reproduced with permission of Elsevier.

and 0% for patients with no, one, two, or three risk factors, respectively. Investigators from Memorial Sloan Kettering Cancer Center (MSKCC) found that the same three factors were significant when applied at the time patients received conventional salvage chemotherapy prior to aHCT [20]. The EFS was estimated to be 83% for those with no or one risk factor, 27% for those with two risk factors, and 10% for patients with all three risk factors ($p < 0.001$). The OS rates were 90, 57, and 25%, respectively ($p < 0.001$).

A model for patients transplanted with relapsed or refractory HD was developed at Stanford University [21]. Bone marrow or pulmonary involvement, systemic symptoms at relapse, and more than minimal disease at transplant (>75% reduction in a bulky mass or no nodes >2cm and <10% marrow involvement) were associated with significantly worse OS, EFS, and freedom from progression. Actuarial freedom from progression at 3 years was estimated to be 85, 57, 41, and <20% for patients with no, one, two, and three risk factors, respectively ($p = 0.0001$).

Another model developed in Boston used progressive disease at the time of HCT, more than one extranodal site of relapse, and poor performance status, to construct a prognostic index [22]. The 3-year actuarial survival was 82, 56, and 19% for patients with no, one, or two or more risk factors, respectively ($p = 0.0001$).

A French registry analysis evaluated results of aHCT for relapsed HD [18]. The presence of extranodal disease at relapse and initial remission duration under 12 months were identified as significant risk factors. The 4-year OS was estimated to be 93, 59, and 43%, for patients with no, one, or two risk factors, respectively.

A GHSG study evaluated patients with relapsed HD and determined that initial remission duration less than 12 months, stage III or IV disease at relapse, and anemia at relapse could be used to construct a prognostic model for patients treated with aHCT [18]. An index based upon the presence of no, one, two, or three of these risk factors was able to identify groups with significantly different rates of survival ($p < 0.0001$).

At the University of Nebraska, a prognostic index was developed based upon factors used in the International Prognostic Factors Project Score for advanced HD [5,23]. Low serum albumin (<4g/dL), anemia (<10.5g/dL), age (≥45 years), and lymphocytopenia (<600/mm^3 and/or <8% of total white blood cell count) were associated with inferior outcomes. The estimated 10-year OS rates were 48, 35, 27, and 20%, for patients with no, one, two, or three or more risk factors, respectively.

Another prognostic model was developed at the University of Minnesota [24]. Absence of systemic symptoms at the time of transplant, transplantation in complete remission, and chemotherapy-sensitive disease were independent predictors of improved PFS. The 5-year predicted PFS was 67, 37, and 9% for those with no or one, two, or three risk factors, respectively ($p < 0.001$).

The influence of histologic subtype has not been extensively studied, although one study showed that patients with lymphocyte-predominant histology had significantly longer EFS than other patients [25]. The use of [^{18}F]fluorodeoxyglucose positron emission tomography (FDG-PET) scans has been shown to be useful in the management of HD and NHL. Several studies have evaluated the importance of FDG-PET scans performed prior to aHCT for HD and found that a negative FDG-PET scan is associated with improved PFS [26].

The most important risk factors associated with adverse outcome following aHCT for HD are displayed in Table 56.2. It should be noted that some of these prognostic factors have not been shown to be important in all studies. This is likely related to differences in patient characteristics, selection bias, and sample size. Nevertheless, most studies show that extensive therapy prior to transplant, poor performance status, chemotherapy resistance, and active disease at the time of transplant are predictive of poorer outcome after aHCT for HD.

Pre-transplant "debulking" chemotherapy

Almost all patients with relapsed NHL are treated with conventional-dose salvage chemotherapy to reduce tumor burden prior to treatment with HDT and aHCT. Sensitivity to pre-transplant salvage therapy is one of the most important prognostic factors for PFS and OS following aHCT. The majority of patients with relapsed HD are also treated in this manner and most studies show that chemotherapy sensitivity is an important prognostic factor for these patients, also (Table 56.2). A wide variety of chemotherapy regimens have been used for this purpose, although the ICE (ifosfamide, carboplatin, etoposide) regimen has become popular [20].

The potential impact of administering pre-transplant salvage chemotherapy was evaluated in a study by the Autologous Blood and Marrow Transplant Registry (ABMTR) that compared outcomes of HD patients in first relapse with those in second remission [27]. The actuarial 3-year OS was 58% for patients who received aHCT in first relapse, compared with 75% for patients transplanted in second complete remission ($p < 0.001$).

The Polish Transplant Group showed that actuarial 5-year EFS was 16% for HD transplanted with active disease, compared with 66% for patients in complete remission ($p < 0.001$) [25]. At the University of Rochester, the 5-year EFS was projected to be 46% for patients with minimal disease (all areas ≤2cm) at the time of transplant, compared with 10% for those with bulky disease ($p = 0.0002$) [28]. Autologous HCT results from the Grupo Español de Linfomas/Trasplante Autólogo de Médula Ósea (GEL/TAMO) Spanish cooperative group revealed that the actuarial time to treatment failure was 63.2% at 5 years following HCT for HD patients transplanted in complete remission, compared with 32.2% for those with measurable disease ($p = 0.00001$) [9]. In this cohort, the 5-year time to treatment failure was estimated to be 32.6% for patients transplanted

Table 56.2 Adverse prognostic factors: autologous hematopoietic cell transplantation for Hodgkin disease

Prognostic factor	Ref.
Bulky or "non-minimal" disease at transplant	[12,13,16,19,29,30]
Extensive therapy before transplant	[9,13–15,27–29]
Poor performance status	[14,17,35]
Short initial remission	[13,18,22,26,38]
Extranodal disease at relapse or at transplant	[15–18,27,38]
Systemic symptoms at relapse	[16,26,29,34,38]
Chemotherapy resistance	[9,18–21,23–30]
Female gender	[13]
Male gender	[25]
Age	[40]
Advanced stage at relapse	[21]
Progressive disease at transplant	[17]
Elevated lactate dehydrogenase level at transplant	[20]
Anemia at relapse or transplant	[40]
Hypoalbuminemia at transplant	[40]

with bulky disease, compared with 45.8% for others ($p = 0.045$). At Toronto, the 4-year actuarial DFS was 68% for HD patients who had no evidence of disease at the time of transplant, 26% for patients transplanted with non-bulky disease, and 0% for those with bulky disease ($p = 0.0002$) [29]. At Stanford University, the presence of more than minimal disease (see above) at the time of transplant was associated with significantly worse PFS, EFS, and OS [21]. The Minnesota group showed that relapsed and refractory HD patients who underwent aHCT with residual disease present had a 2.3-fold relative risk of progressive disease compared with those who were transplanted while free of disease ($p = 0.03$) [24]. A similar Cleveland Clinic analysis demonstrated that HD patients transplanted with bulky disease (>10cm) had significantly poorer survival than other patients (hazard ratio 2.9, $p = 0.002$) [30].

These results suggest that treatment with conventional salvage chemotherapy to reduce tumor burden prior to aHCT improves outcomes. However, the absence of bulk may be a surrogate for chemotherapy sensitivity, and some patients might do well without additional therapy prior to transplant. In addition, some patients with relapsed and refractory HD fail to respond to conventional salvage therapy and never proceed to transplantation. For these reasons, a comprehensive two-step transplant regimen was developed at MSKCC so that all patients with relapsed and refractory HD could be evaluated on an intent-to-treat basis [20]. This program consists of two cycles of ICE conventional salvage therapy, followed by accelerated fractionation involved-field radiation, and then HDT and aHCT. The rate of response to ICE was 88%, and the OS and EFS at a median of 43 months were projected to be 73 and 58%, respectively, for all patients. The OS and EFS for the 86% of patients who proceeded to aHCT were 83 and 68%, respectively. This type of intent-to-treat analysis gives a more realistic assessment of the role of transplantation for HD.

The use of pre-transplant chemotherapy may not be necessary in all circumstances. At University College Hospital in London, the actuarial 5-year PFS was 78% in a cohort of patients with HD who underwent aHCT in "untested" relapse [13]. The 5-year PFS was estimated to be 57% for HD patients in the Spanish registry who were transplanted in untested relapse [9]. At the University of Nebraska, the 5-year OS was estimated to be 100% for HD patients who were transplanted without additional therapy after relapse, compared with 45% for patients who received conventional chemotherapy prior to aHCT ($p = 0.048$) [31]. An EBMT retrospective analysis showed no significant differences in OS or PFS when HD patients in untested relapse were compared with those who received conventional salvage therapy prior to aHCT [32].

Patients with HD who are transplanted without conventional salvage chemotherapy may do well because they have minimal tumor burden at the time of relapse. Nevertheless, pre-transplant debulking chemotherapy may not be necessary for all HD patients and it may be possible to eliminate this treatment for patients with minimal tumor burden at the time of relapse, or those without evidence of disease after the site of relapse is resected. Most patients, however, would benefit from pre-transplant chemotherapy, particularly those who have significant tumor burdens.

Pre-transplant radiation therapy

Radiation is an essential tool in the treatment of HD. At some institutions, radiotherapy is used instead of chemotherapy or in addition to chemotherapy to reduce tumor burden prior to aHCT, whereas other institutions administer radiation as consolidation therapy after transplant (see below). There are several unanswered questions regarding the potential advantages and disadvantages of each approach.

The use of radiotherapy prior to transplant can reduce tumor burden and usually does not delay the start of HDT. Multiple institutions have used pre-transplant involved-field radiation for patients undergoing aHCT for HD [21,29,33–37]. Although this may improve local control and decrease the incidence of relapse in irradiated areas, it has been difficult to demonstrate definite survival advantages associated with this therapy [33–35]. At MSKCC, patients with relapsed and refractory HD are treated with an integrated approach utilizing conventional salvage chemotherapy, 1800–3600cGy accelerated fractionation involved-field radiation administered twice daily over a 5–10-day period, followed by aHCT [20].

Pre-transplant involved-field radiation may be associated with increased post-transplant complications such as esophagitis and pulmonary toxicity, especially after mediastinal irradiation. An analysis at the University of California Los Angeles demonstrated that radiation prior to aHCT [using a total body irradiation (TBI)-containing regimen] for HD is associated with significantly higher morbidity and mortality compared with post-transplant radiation [38]. Similar analyses conducted for patients with NHL suggest that pre-transplant radiotherapy may be associated with an increased risk of secondary myelodysplasia or respiratory complications [34]. Hence pre-transplant radiotherapy should be used with caution, especially when high-dose carmustine is given after mediastinal irradiation.

Post-transplant therapy

A number of approaches have been utilized to decrease the risk of relapse following aHCT for HD. At many institutions, involved-field radiation is used following aHCT as consolidation therapy or to treat areas of persistent disease [13,14,24,28,39,40]. Delaying radiation until after aHCT may decrease the incidence of transplant-related complications and may avoid delays in starting HDT due to toxicities of pre-transplant radiation. Post-transplant irradiation can convert a partial remission into a durable complete remission [41].

A retrospective analysis by the University of Rochester demonstrated that actuarial 5-year EFS was 44% for HD patients who received post-transplant consolidative radiotherapy, compared with 26% for patients who did not receive radiation ($p = 0.0056$) [28]. A retrospective analysis by the University of Maryland also demonstrated significantly improved EFS among HD patients who received additional radiotherapy following aHCT [42]. Potential advantages attributed to post-transplant radiation therapy have been seen in other analyses, although randomized trials have not been conducted [35]. Issues relating to selection bias make it difficult to evaluate the advantages of involved-field radiation therapy following aHCT for HD, because patients with early relapse, poor performance status, and delayed engraftment may not be able to be treated. In addition, the use of radiation with primary therapy makes many patients ineligible for additional radiotherapy. Post-transplant radiation may also lead to significant hematologic and non-hematologic toxicity and it is unclear whether it is more advantageous to administer the radiation prior to aHCT. There are also other unresolved issues related to the timing and schedule of treatment, treatment fields, and radiation dose.

In addition to post-transplant involved-field radiation therapy, several groups have conducted phase I/II trials with interferon, interleukin-2 (IL-2), and IL-2 with lymphokine-activated killer cells to decrease relapse rates after aHCT for HD [43,44]. A retrospective analysis by the Polish Lymphoma Research Group suggested that the use of immunotherapy following aHCT for primary refractory HD was associated with significantly better PFS and OS [44]. Although the use of post-transplant immune modulation is feasible and augmentation of various parameters of immune function can be demonstrated, there have been no studies that conclusively show a benefit from this approach.

A number of other approaches have been used to decrease relapse rates following aHCT for HD. Investigators in Baltimore used consolidation chemotherapy administered at 3, 6, 9, and 12 months after transplant [42]. Perhaps the most aggressive form of post-transplant therapy involves the use of a planned second transplant. The group at Valhalla, NY, performed tandem transplants with a regimen containing thioTEPA (*N*-triethylenethiophosphoramide), mitoxantrone, and carboplatin for the first transplant, and ifosfamide, carboplatin, and etoposide for the second [45]. An HD tandem transplant protocol developed at the City of Hope utilized melphalan for the first transplant and TBI or carmustine, combined with etoposide and cyclophosphamide, for the second [46]. Given the efficacy of brentuximab vedotin as salvage therapy for patients with HD relapsing after aHCT [47], a phase III randomized study showed that early consolidation with brentuximab vedotin after aHCT improved PFS in HD patients with risk factors for relapse or progression. Median PFS was 43 months for brentuximab vedotin group compared with 24 months for placebo group [48].

Functional imaging

Positron emission tomography using FDG-PET has been used to assess response to therapy and it is now included in the standard response criteria for lymphoma. FDG-PET has also been shown to provide prognostic information at interim analysis during front-line therapy [49] as well as prior to aHCT in HD patients [49]. A retrospective study from the M.D. Anderson Cancer Center showed that positive FDG-PET before aHCT was predictive of poor outcome with 3-year OS of 58% compared with 87% for FDG-PET negative [50]. Investigators at MSKCC reported that FDG-PET status before aHCT was the only factor significant for EFS and OS in 153 relapsed/refractory HD patients with chemosensitive disease after ICE salvage therapy and before aHCT. The 5-year EFS was 31% for FDG-PET-positive compared with 75% for FDG-PET-negative patients [51] (Figure 56.4). The administration of involved-field radiotherapy with aHCT marginally improved EFS rates. Another Italian study included 24 patients with relapsed HD who had FDG-PET performed after two cycles of salvage chemotherapy IGEV (ifosfamide, gemcitabine, vinorelbine, prednisone) before aHCT. The 2-year PFS was 93% for FDG-PET-negative and 10% for FDG-PET-positive patients [52]. Similar results were reported by investigators at Washington University in 46 patients with relapsed/refractory HD [53]. The pre-aHCT FDG-PET response significantly predicted 3-year EFS: 82% for FDG-PET-negative versus 41% for FDG-PET-positive patients ($p = 0.02$).

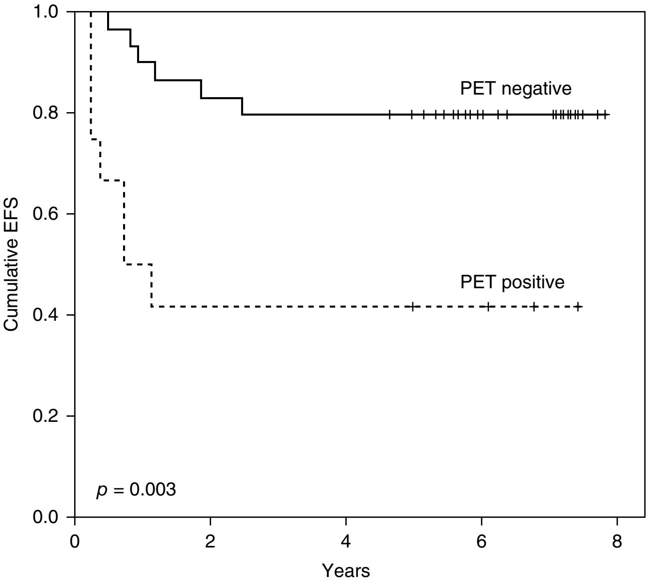


Figure 56.4 Event-free survival according to functioning image (FDG-PET) status before transplantation. Source: Moskowitz et al. 2010 [51]. Reproduced with permission of the American Society of Hematology.

These results suggest that a negative FDG-PET is associated with better outcome after aHCT, and alternative salvage therapy, investigational therapy, or post-transplant therapy may need to be considered for those with positive FDG-PET pre-transplant. This concept was tested at MSKCC in a phase II risk-adapted approach using presalvage risk factors (remission duration <1 year, B symptoms, and extranodal sites of disease)and also FDG-PET response to determine whether additional therapy was warranted before HDT and aHCT [51]. Patients with two risk factors were given two cycles of augmented ICE followed by restaging with FDG-PET. Patients with a negative scan received a transplant whereas those with positive FDG-PET received a non-cross-resistant chemotherapy GVD (gemcitabine, vinorelbine, and liposomal doxorubicin) regimen administered every 2 weeks for four doses. Patients without evidence of disease progression proceeded to HDT and aHCT; those with progressive disease were taken off the study. At a median follow-up of 51 months, patients with negative FDG-PET transplanted after one or two salvage programs had an EFS of >80% compared ith 28.6% for patients with a positive scan ($p < 0.001$).

The prognostic value of FDG-PET has also been reported in patients undergoing allogeneic HCT. In a study in Italy, 46 patients with relapsed HD underwent reduced-intensity allogeneic HCT [54]. Patients with negative FDG-PET had significantly better outcome with a 3-year OS rate of 82% versus 28% ($p = 0.006$) for FDG-PET-positive patients and a 3-year PFS rate of 72% versus 30% ($p = 0.027$). The risk of relapse was significantly higher for FDG-PET-positive patients, 59% versus 27% ($p = 0.066$) for FDG-PET-negative patients. These results emphasize the importance of FDG-PET response in predicting the outcome of both aHCT and allogeneic HCT. Patients with persistent positive FDG-PET after salvage therapy before HCT have a poor outcome, and a trial of new investigational agents such as brentuximab vedotin and bendamustine should be considered. These agents can be used as a potential bridge to transplant.

Timing of transplantation

An important issue concerning aHCT for HD relates to the appropriate time to perform this procedure during the course of disease. Some patients with relapsed HD may be cured with conventional salvage chemotherapy or radiotherapy, and it may be appropriate to delay transplantation until after these approaches fail. Conversely, transplantation should not be delayed to a point when resistance to chemotherapy or poor performance status makes cure unlikely with any approach. Potential times when aHCT for HD can be performed are displayed in Table 56.3. Although data from randomized trials are lacking, results in each situation can be compared with results of conventional salvage therapy.

Failure to attain initial complete remission

HD patients who do not achieve complete remission with initial chemotherapy have a poor prognosis [4] and aHCT may be the only curative treatment for them. Several cooperative groups and institutions have reported results of aHCT for these patients.

The EBMT identified 175 HD patients who failed to achieve an initial complete or partial remission after one or more regimens [55]. The 5-year actuarial OS and PFS were 36 and 32%, respectively (Figure 56.5). OS was significantly better for patients who were transplanted within 18 months of diagnosis. The ABMTR analyzed aHCT results for 122 HD patients who never achieved remission after completing at least one combination chemotherapy regimen [56]. The 3-year OS and PFS were estimated to be 50 and 38%, respectively. OS was significantly worse in patients with systemic symptoms at diagnosis and in patients with a poor performance status at the time of transplant. Other reports of aHCT for primary refractory HD are displayed in Table 56.4.

No randomized trials have compared the results of aHCT with conventional salvage chemotherapy for patients with primary refractory HD, although other comparisons have been performed. At Stanford University, induction failure was defined as progression during initial therapy or relapse within 2 months [16]. Results of aHCT for these patients were compared with those for historical controls who received conventional salvage therapy. The EFS at 4 years was projected to be 52 and 19%, respectively ($p = 0.01$). However, there were no significant differences in OS (44% vs. 38%, respectively, $p = 0.32$). A case–control study from the French database examined the outcome of HD patients who progressed during first-line treatment or within 3 months of completing treatment [17]. OS 6 years from the time of diagnosis was 38% for transplanted patients compared with 29% for patients who received conventional salvage therapy ($p = 0.058$). A retrospective analysis in Cologne, Germany, examined outcomes in 67 patients with primary progressive HD, defined as progression during first-line therapy or within 3 months after initial therapy [57]. The mean survival was 56.2 months for patients treated with aHCT compared with 11.2 months for those who received conventional salvage therapy. The actuarial 5-year OS rates were 53 and 0%, respectively. The use of aHCT was identified as a significant factor associated with improved freedom from treatment failure ($p = 0.031$) and OS ($p = 0.039$). A retrospective analysis by the Gruppo Italiano per lo Studio dei Linfomi (GISL) reviewed the results of therapy in 72 patients with HD who progressed or had minimal response to initial chemotherapy [58]. HDT followed by aHCT was attempted in 27 of these patients. The actuarial 4-year OS was 81% for this group, compared with 38% in patients who received chemotherapy alone ($p = 0.019$).

Interpretation of results of aHCT in these patients is difficult because of different definitions of "primary refractory" and "induction failure." Definitions include patients with disease progression during initial therapy, stable disease after initial therapy, progression within 90 days of completing initial therapy, and partial remission with initial therapy. Patients who progress on primary therapy, and those who have no response, are likely to have a different

Table 56.3 Potential timing of autologous hematopoietic cell transplantation for Hodgkin disease

- Failure to attain initial complete remission
  - Primary refractory
  - First partial remission
- First relapse
  - Short vs. long initial remission
- Second or subsequent relapse
- First remission

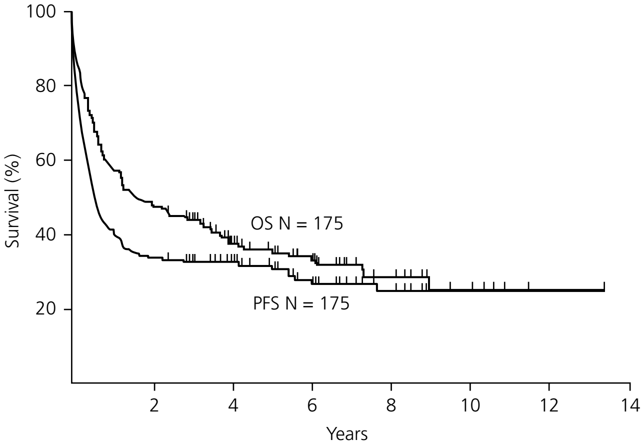


Figure 56.5 Overall survival (OS) and progression-free survival (PFS) following autologous hematopoietic cell transplantation for primary refractory Hodgkin disease. Source: Sweetenham et al. 1999 [55]. Reproduced with permission of the American Society of Clinical Oncology.

Table 56.4 Results of Autologous hematopoietic cell transplantation for primary refractory Hodgkin disease

Ref.	*n*	Outcome	
[54]	175	32% 5-yr PFS	36% 5-yr OS
[55]	122	38% 3-yr PFS	50% 5-yr OS
[35]	86	25% 5-yr EFS	35% 5-yr OS
[56]	75	45% 10-yr EFS	48% 10-yr OS
[37]	70	31% 5-yr PFS	43% 5-yr OS
[57]	62	15% 5-yr TTF	26% 5-yr OS
[49]	65	36% 3-yr PFS	55% 3-yr OS
[9]	49	13% 5-yr PFS	
[13]	46	33% 5-yr PFS	
[58]	30	42% 5-yr PFS	60% 5-yr OS
[59]	28	26% 3-yr EFS	34% 5-yr OS
[36]	27		81% 4-yr OS
[29]	21	45% 10-yr PFS	48% 10-yr OS
[34]	13	52% 4-yr PFS	44% 4-yr OS

PFS, progression-free survival; OS, overall survival; EFS, event-free survival; TTF, time to treatment failure; DFS, disease-free survival; yr, year.

prognosis than patients who have a partial remission or near-complete remission. This is supported by French registry data demonstrating significantly worse OS and PFS when aHCT results for primary refractory HD patients were compared with results from patients in first partial remission [59]. Another French study identified 27 HD patients with stage IV disease and/or large mediastinal masses who were in an incomplete response (≥50%) following three cycles of ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) chemotherapy [60]. This group was treated with aHCT and the 5-year EFS was estimated to be 87%, compared with 61% for control patients treated with conventional therapy ($p = 0.02$). The actuarial 5-year OS rates were 92 and 77%, respectively ($p = 0.2$). Other investigators have also reported good outcomes following aHCT for HD patients in first partial remission [61–63]. It would seem that patients who attain a good partial remission following primary therapy can do well following aHCT, although reports may classify these patients in the category of primary refractory disease.

Interpretation of results is also hampered by the fact that patients may not have biopsy-proven residual disease before transplantation, and that some merely had residual radiographic abnormalities without active disease. It is hoped that the use of modalities such as FDG-PET scans will help with this difficulty. Finally, it is important to recognize that a significant proportion of patients with primary refractory disease will not be able to proceed to transplant and that patients reported in these series may be selected [14,18]. Nevertheless, the results in Table 56.4 appear superior to those attainable with conventional salvage chemotherapy, and aHCT may be the best option for patients who fail to respond to initial chemotherapy. Additional chemotherapy or radiation may be considered prior to HDT for patients with significant tumor burden, whereas other patients with minimal disease remaining after primary therapy should proceed directly to transplantation [55].

First relapse

HDT followed by aHCT for HD is most often considered for patients who relapse following their initial chemotherapy-induced complete remission. Several reports of aHCT in this situation have been published and allow comparison with results of conventional salvage therapy. Selected results of aHCT for HD after first relapse are displayed in Table 56.5, which includes series that specifically address this population of patients [13,15,19,27,31,32,64].

Some of these trials compared the results of aHCT with those of conventional salvage chemotherapy. The duration of initial remission is an important prognostic factor for outcome with conventional salvage chemotherapy for HD [4,18]. In the randomized GHSG trial, HD patients in first relapse were divided into those with early (<12 months) or late (>12 months) relapses [13]. The time to treatment failure at 3 years was estimated to be 41% for early relapse patients treated with aHCT compared with 12% for those who received conventional salvage chemotherapy ($p = 0.008$). OS was estimated to be 43 and 40%, respectively ($p = 0.623$). The actuarial 3-year time to treatment failure for patients with long first remissions was 75% following aHCT, compared with 44% for patients who received conventional salvage chemotherapy ($p = 0.025$). OS survival rates were 93 and 75%, respectively ($p = 0.088$). An analysis by Stanford University compared the outcome of aHCT for patients after first relapse of HD with the results of conventional salvage chemotherapy in historical controls [16]. The 4-year EFS for patients with initial remissions of 12 months or less was estimated to be 56% following aHCT compared with 19% for those who received conventional salvage chemotherapy ($p < 0.01$). OS rates were 58 and 38%, respectively ($p = 0.15$). No significant differences in OS or EFS were seen in patients with longer initial remissions. A retrospective French–Belgian analysis of HD treatment after first relapse found that OS was 69% following aHCT, compared with 55% for patients treated with conventional chemotherapy (p = NS) [15]. Among patients with initial remissions less than 12 months in duration or with stage III–IV disease at relapse, the freedom from treatment failure was estimated to be 64% following aHCT and 40% for those who received conventional chemotherapy. The OS rates were estimated to be 55 and 30%, respectively. A subsequent analysis demonstrated that the actuarial 4-year OS following aHCT was 81% for patients with late relapses as compared with 65% for patients with shorter initial remissions ($p < 0.05$) [65].

Table 56.5 Results of autologous hematopoietic cell transplantation for Hodgkin disease after first relapse

Ref.	*n*	Outcome	
[65]	357	49% 5-yr TTF	57% 5-yr OS
[20]	295	46% 3-yr DFS	58% 3-yr OS
[33]	187	36% 5-yr FF2F	54% 5-yr OS
[43]	139	45% 5-yr PFS	49% 5-yr OS
[29]	120	45% 10-yr PFS	47% 10-yr OS
[42]	85	40% 5-yr FFS	51% 5-yr OS
[27]	60	62% 15-yr DFS	67% 15-yr OS
[26]	59	63% 5-yr EFS	77% 5-yr OS
[38]	58	64% 3-yr PFS*	72% 3-yr OS[a]

TTF, time to treatment failure; OS, overall survival; DFS, disease-free survival; FF2F, freedom from second failure; PFS, progression-free survival; FFS, failure-free survival; EFS, event-free survival; yr, year.

[a] Median follow-up 2.3 years.

Other groups have also analyzed transplant outcome in relation to the duration of the first HD remission. A report from the Spanish registry demonstrated that 5-year time to treatment failure was estimated at 57% following aHCT after a first remission of at least 12 months, compared with 43% for those with shorter remissions ($p = 0.0014$) [64]. The OS rates were 67 and 50%, respectively ($p = 0.001$). The Minnesota group showed that 5-year OS following aHCT was estimated to be 60% for those with long initial remission (>12 months) compared with 44% for those with shorter remissions ($p = 0.04$) [24]. At Vancouver the PFS was estimated to be 85% for HD patients treated with aHCT after an initial remission of at least 1 year, compared with 48% for those with shorter remissions ($p = 0.016$) [19]. In contrast, other reports have failed to note statistically significant differences in outcome related to the duration of initial remission [22,31,32,40,66].

Prognostic factors for aHCT after first HD relapse are similar to those reported in Table 56.2. Results from the Spanish registry revealed that the actuarial 5-year OS was 75% for patients who achieved a second remission with conventional salvage therapy prior to aHCT, 43% for patients who were sensitive to salvage therapy, but did not attain a second remission, and 19% for resistant patients [64]. Results from the EBMT and ABMTR also demonstrated prolonged OS in patients who were transplanted in second complete remission, compared with patients who had sensitive relapse, but did not achieve a second remission [27,55].

Although OS advantages have not been demonstrated, most investigators would recommend aHCT for HD patients who relapse within 1 year of attaining remission with initial chemotherapy. There is more controversy regarding the management of patients with longer remissions since a significant proportion may achieve prolonged second remissions with radiation or conventional salvage chemotherapy. It may be reasonable to delay transplantation until second relapse in some patients, although transplantation regardless of length of initial remission is usually advocated [4,13,32,67].

Second or subsequent relapse

Prolonged DFS is unusual following treatment with third-line chemotherapy regimens for HD. No significant differences in time to treatment failure or OS were identified when aHCT and conventional salvage chemotherapy were compared in patients with multiple relapses in the randomized GHSG trial [13]. However, only 24 patients were included in the comparison and most investigators would recommend transplantation for HD patients following a second relapse from chemotherapy.

First remission

Some trials have demonstrated that NHL patients with adverse prognostic characteristics benefit from strategies that employ aHCT in first remission or as part of primary therapy. There is less experience with early aHCT for HD, although a number of variables have been identified that might be used to identify potential candidates [5].

Italian investigators reported results of high-dose chemotherapy followed by aHCT in 22 first remission HD patients [68]. All patients had adverse characteristics at diagnosis and had been treated with mechlorethamine, vincristine (Oncovin), procarbazine, prednisone (MOPP)/ABVD. Their outcome was compared with those of concurrently treated patients with similar characteristics who also achieved complete remission, but did not desire transplantation. Eighteen patients were alive at the time of publication and only one had relapsed. The actuarial survival of transplanted patients was 80% with a median follow-up of 83 months. The actuarial PFS was estimated to be 77%. Only eight of 24 patients who were not consolidated with aHCT were alive and free of disease. A prospective European intergroup trial also evaluated the role of early aHCT for HD [69]. Patients with adverse prognostic characteristics were treated with ABVD or similar regimens. Those in complete or partial remission after four cycles of therapy were randomized to treatment with four additional cycles of therapy or to treatment with HDT followed by aHCT. The 5-year failure-free survival was estimated to be 75% for patients randomized to transplant compared with 82% for patients who continued on chemotherapy ($p = 0.4$). The 5-year OS rates were estimated at 88% in each arm ($p = 0.99$). Results of other phase II trials and retrospective analyses examining results of aHCT for patients with HD in first remission are displayed in Table 56.6. The results suggest that PFS may be prolonged by early transplantation for poor-prognosis HD. However, there is little evidence that OS is prolonged, since transplantation or other salvage therapies can be used at relapse. In addition, most series only report outcomes for the selected poor-prognosis patients who are able to be transplanted. It is likely that many patients with adverse prognostic features do not attain remission or may not be transplanted for other reasons, and this “denominator” is rarely reported [62]. Until prospective trials demonstrate significant advantages for patients undergoing aHCT for HD in first remission, this approach should not be considered standard therapy.

Table 56.6 Results of autologous hematopoietic cell transplantation for Hodgkin disease in first remission

Ref.	*n*	Outcome
9	57	70% 5-yr TTF
59	45	70% 5-yr EFS
66	22	77% PFS (median follow-up 86 mo)
62	20	100% CCR (13.2–149.2 mo)[a]
64	16	87% 5-yr DFS
63	13	77% 5-yr DFS

[a] Some patients in first partial remission.

TTF, time to treatment failure; EFS, event-free survival; PFS, progression-free survival; CCR, continuous complete remission; DFS, disease-free survival; mo, months; yr, year.

Autologous hematopoietic cell transplantation for children

The prognosis for children and adolescents with HD is better than for adults and most are cured with primary therapy. Nevertheless, approximately 10–15% of these patients are not cured and aHCT has been extended to the pediatric population.

A case-matching study from the EBMT compared the results of aBMT in 81 HD patients who were under the age of 16 at diagnosis with results from 81 adults [70]. The median age at the time of transplant was 16.9 years. The PFS (median follow-up 36 months) for pediatric patients was estimated at 39% compared with 48% in adults (median follow-up 34 months) ($p = 0.64$). The patterns of relapse and the incidence of transplant-related toxicities were also similar.

A retrospective study by the University of Nebraska analyzed the results of aHCT in 53 children and adolescents (age ≤21 years) with relapsed and refractory HD [71]. The 5-year failure-free survival and OS were estimated to be 43 and 31%, respectively. No significant differences in outcome were observed when younger pediatric patients were compared with adolescents, and no significant differences in outcome were observed when these patients were compared with a historical control group of patients over the age of 21 years who were transplanted during the same period. A similar analysis by Stanford University examined the results of aHCT in 41 pediatric patients with relapsed and refractory HD [72]. The median age was 18 years. The actuarial 5-year PFS and OS were 63 and 68%, respectively.

Results of salvage therapy for pediatric patients with relapsed and refractory HD following treatment in German–Austrian trials were analyzed [73]. The median age at the time of relapse or progression was 14.7 years. The actuarial 10-year OS was 51% for patients treated with aHCT following first recurrence. These results were similar to those for children who received conventional salvage chemotherapy. However, the 6-year OS of children who were transplanted after a second recurrence was estimated to be 52%, compared with 29% for those receiving conventional treatment ($p = 0.04$). A retrospective study by the United Kingdom Children’s Cancer Study Group also examined results of aHCT for childhood HD [74]. The median age of transplanted patients was 12 years. When results for patients treated with aHCT were compared with those for controls who received conventional salvage chemotherapy after first relapse, there were no significant differences in survival. However, patients with primary refractory disease had significantly longer survival following aHCT.

Prolonged PFS can be seen after aHCT for children and adolescents with relapsed and refractory HD and the results are comparable to those for adults. The use of aHCT appears to be justified, particularly in patients with refractory disease and those with multiple relapses. Long-term growth and development are normal, although delayed onset of ovarian function has been observed [71,74]. Of greater concern are reports of respiratory complications [72] and myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) following aHCT for HD in children [71,74]. Recently, the long-term outcome of 144 children with HD who underwent HDT and aHCT at Stanford University was reported [75]. At a median follow-up of 10.2 years, the 10-year cumulative incidence of death from HD and other causes was 21.7 and 12.7%, respectively. The risk ratio for second malignancies was 8.0 compared with the general population and 1.5 compared with HD patients not undergoing aHCT. Hence children who are survivors of aHCT may be at increased risk of death from HD and other causes compared with the general population, but not compared with the HD population as a whole.

Transplantation for elderly patients

Data from the CIBMTR indicate that 26% of patients treated with aHCT between 2001 and 2004 were older than 60 years of age [76]. Age was not a significant prognostic factor in the majority of trials evaluating results of aHCT for HD, although relatively few patients over the age of 60 years were included, and they may be highly selected. In one report, patients who were ≥45 years of age had significantly worse EFS and OS, however [23].

Most reports of aHCT in the elderly have included patients with a variety of hematologic malignancies and have not reported separate results for HD patients. Results from Stanford showed that regimen-related toxicity and EFS were worse in patients over the age of 50 years who underwent aHCT [77]. Similar results have been reported for older NHL patients [78].

Patients over the age of 60 years can have prolonged failure-free survival following aHCT for relapsed and refractory HD. Although toxicity may be greater, patients should not be excluded from treatment based upon age alone. It may be appropriate to perform more extensive pre-transplant tests of cardiac, pulmonary, and renal function in these patients [78].

Transplantation for HIV-associated Hodgkin disease

Although HD is not an AIDS-defining illness, the incidence of HD is increased 8–10-fold in patients infected with the human immunodeficiency virus (HIV). Highly active antiretroviral therapy (HAART) has changed the natural history of HIV infection and it now is possible for patients with HIV-related HD to receive the same primary chemotherapy treatment that HIV-negative individuals receive.

Earlier results for HDT and aHCT in HIV-associated HD were limited to only a few patients and included patients with HIV-associated NHL. Investigators in Paris reported the results of aHCT in four patients with relapsed HIV-associated HD [79]. All patients received HAART before transplantation and were continued on HAART during hematopoietic cell collection, during and after transplantation. Three patients were alive in complete remission at 4, 13 and 15 months after transplant. An Italian group described results of aHCT in four patients with relapsed and refractory HIV-associated HD [80]. All patients received HAART during treatment. Two patients with relapsed HIV-associated HD were treated with aHCT at the City of Hope [81]. Patients received HAART and had to be free of opportunistic infections for 1 year. The patients were reported to be alive and in remission at 56 and 61 months following HCT.

Recent studies have shown that the outcome of HDT and aHCT for patients with HIV-associated lymphoma are similar to those for HIV-negative patients with lymphoma. Long-term results from the Italian Cooperative Group included 50 HIV-positive HAART-responding patients with refractory/relapsed lymphoma, 38% of whom had HD [82]. At a median follow-up of 44 months, the DFS and OS were 74 and 75%, respectively. Low CD4 count, marrow involvement, and poor performance status independently affected survival. Another study in Spain reported the results of HDT and aHCT in 68 patients with HIV-related lymphoma including 18 patients with HD [83]. Non-relapse mortality (NRM) was 7.5% at 12 months after aHCT, mainly due to bacterial infections. At a median follow-up of 32 months, DFS and OS were 56 and 61%, respectively. These findings were supported by a retrospective analysis by EBMT comparing survival between HIV-positive and -negative lymphoma patients undergoing HDT and aHCT that included 53 patients (66% NHL and 34% HD) within each cohort [84]. Both groups were comparable except for a higher proportion of males and mixed cellularity HD. Incidence of relapse, OS, and PFS were similar in both cohorts. A higher NRM within the first year after aHCT was observed in the HIV-positive group (8% vs. 2%), predominantly because of early bacterial infections, although this was not statistically significant and did not influence survival.

These results demonstrate that peripheral blood hematopoietic cells can be collected in HIV-infected individuals on HAART, and that aHCT can be performed in selected individuals. HIV-related lymphoma patients should be considered for aHCT using the same criteria as for HIV-negative patients. Potential candidates for aHCT should be entered in clinical trials or referred to centers with experience in this patient population.

Late events

Increasing numbers of people are long-term survivors after aHCT for HD and other hematologic malignancies. There are several reports on quality of life after HCT for hematologic malignancies, although no reports have dealt exclusively with outcomes following aHCT for HD alone.

Long-term follow-up of HD patients treated with aHCT in British Columbia demonstrated that 89% had a performance level of at least 90% [36]. Nevertheless, some patients remained on disability and others described significant levels of anxiety and depression. A survey conducted by the International Bone Marrow Transplant Registry and the ABMTR showed that transplant survivors were more likely to report poorer health than controls and reported more problems with fatigue, sleep, and sexual function [85]. Survivors were also more likely to report problems with anxiety, depression, and social functioning. Patients with HD who had been treated with aHCT formed a relatively small percentage of patients in this cohort, however. A similar survey of long-term function following aHCT was conducted in Germany [86]. Patients with HD represented only a small percentage of patients in this survey also. Most patients (67%) rated their global quality of life as good to excellent, and scores improved over time. The majority of patients had returned to their former employment, although difficulty with holding a job has been reported in other studies [87]. Sexual dysfunction was commonly observed.

Patients who undergo aHCT for HD are at risk for late relapse. Although some series have not reported late relapses, others have demonstrated that patients continue to be at risk of relapse for long periods. Relapse more than 7 years following aHCT has been reported [9,36]. These results indicate that life-long follow-up is necessary after aHCT for HD.

The Bone Marrow Transplant Survivor Study (BMTSS) examined long-term outcomes in patients who had survived at least 2 years after aHCT [87]. Patients transplanted for HD had a 28.2-fold risk of late death in comparison with the general population. This risk included relapse and non-relapse causes of death. A nation-wide study of patients undergoing aHCT in Finland noted that patients who had received aHCT for HD had an 8.1% incidence of NRM [88].

A large number of reports have described the occurrence of secondary MDS and AML following aHCT for both HD and NHL [89]. The 5-year risk of developing AML or MDS following aHCT for HD ranged between 4 and 15%. This complication has been observed within months of the transplant. Risk factors that have been reported to be associated with the development of post-transplant MDS/AML include age, extent of prior therapy with alkylating agents, use of total-body irradiation conditioning, or prior use of radiotherapy, history of splenectomy, and use of autologous PBHCT compared with autologous BMT.

There is evidence that the risk of secondary MDS/AML following aHCT for HD is largely related to treatment administered prior to transplant rather than the high-dose conditioning regimen itself. An analysis in Vancouver did not find an increased risk of secondary AML or other malignancies when HD patients who had received aHCT were compared with others who only received conventional chemotherapy [90]. Investigators have also used fluorescence in situ hybridization (FISH) analysis to demonstrate that the cytogenetic abnormalities present in post-transplant MDS/AML can be detected in stored pre-transplant blood or marrow samples from patients with HD [91]. This observation led to the recommendation that cytogenetic analysis should be performed on bone marrow from all patients prior to hematopoietic stem cell collection. In addition, most studies of post-transplant MDS/AML have identified abnormalities of chromosomes 5 and 7 that are associated with typical alkylating agent-based therapy administered prior to aHCT. The short latency period between aHCT and development of MDS/AML in many cases also suggests that malignancy results from prior therapy and not the high-dose chemotherapy regimen.

The occurrence of solid tumors has also been described following aHCT for HD. The 15-year cumulative risk of developing a solid tumor was estimated to be 3.2% following aHCT for HD in the analysis in British Columbia [90].

Other late complications of aHCT for HD include infections [9,27,32,66], cardiac complications [37,66], and pulmonary complications [9,27,37]. The BMTSS found that the risk of late mortality from cardiac dysfunction was increased 4.3-fold and the risk from pulmonary compromise was increased 29.1-fold in survivors of aHCT for HD [87]. Virtually all women will develop amenorrhea following aHCT for HD, although return of ovarian function is not unusual. Successful pregnancies, both assisted and unassisted, have been described following aHCT for HD [41,66], and patients should be counseled about the need for contraception.

Management of relapse

A significant proportion of patients will relapse following aHCT for HD. The median survival following disease progression after aHCT has been reported to be less than 12 months in some series, although some reports show a better prognosis [92,93]. Options are frequently limited because of prior therapy, low blood counts, and poor performance status. Radiation therapy should be considered for patients with localized relapse. High response rates and occasional long-term remissions have been noted with single-agent vinblastine [66,94], and patients will often respond to other conventional salvage regimens for HD [95].

More recently, new agents have been shown to provide effective salvage therapy for relapsed HD post-aHCT. Brentuximab vedotin is an antibody–drug conjugate that selectively delivers monomethyl auristatin E, an antimicrotubule agent, into CD30 express cells. Since CD30 is expressed on malignant Hodgkin Reed–Sternberg cells and has limited expression on normal cells, CD30 is an attractive target for anti-HD therapy. In a phase I dose-escalation trial that enrolled 45 patients with relapsed or refractory CD30$^+$ hematologic malignancies, objective responses were observed in 17 patients and tumor regression was observed in 86% of evaluable patients. A total of 73% of the patients had undergone aHCT [96]. A pivotal phase II study of 102 HD patients with relapsed and refractory disease after aHCT showed an overall response of 75%, with complete remission in 34% of the patients [47] The estimated 12-month survival was 89% and the median PFS for all patients was 5.6 months. The median duration of response for those who achieved complete response was 20.5 months. The treatment was well tolerated and the most common treatment-related adverse events were peripheral sensory neuropathy, nausea, fatigue, neutropenia, and diarrhea.

Panobinostat, a potent pan-deacetylase inhibitor, is another new agent for the treatment of relapsed HD post-aHCT. In a phase II study, 129 heavily pretreated patients with a median of four prior systemic regimens and 41% refractory to chemotherapy were enrolled [97]. Panobinostat 40mg was administered orally three times per week. Tumor reduction was observed in 74% with an objective response of 27%. The median duration of response was 6.9 months and median PFS was 6.1 months. The estimated 1-year OS was 78%. Non-hematologic toxicities include nausea, diarrhea, and fatigue. Grade III–IV thrombocytopenia, anemia, and neutropenia were manageable.

Bendamustine, a bifunctional alkylating agent, was evaluated in a phase II trial in 36 patients with relapsed/refractory HD who were ineligible for aHCT or had relapsed after aHCT [98]. The median number of prior regimens was four and 75% had relapsed after aHCT. Patients received bendamustine at 120mg/m^2 for two consecutive days every 28 days for six cycles. Thirty-four were evaluable for response and the overall response rate was 53%, including 33% complete response and 19% partial response. Responses were seen in patients with refractory disease and also in patients with prior aHCT and allogeneic HCT; however, no response was seen in patients who relapsed within 3 months of aHCT. The median duration of response was 5 months. Similar results were reported by the Italian group for 41 patients, including 85% relapsed after aHCT [99]. The overall response rate was 58% with 31% complete response. Eight responding patients subsequently underwent allogeneic HCT. Median PFS and OS exceeded 11 and 21 months respectively. Therefore, bendamustine may be used in palliative therapy in addition to salvage therapy before allogeneic HCT.

Some patients may be candidates for a second transplant. The EBMT reported that 50% of patients were alive and in remission 2 years after a second aHCT for HD [100]. Others have reported similar results [95]. A report from the IBMTR analyzed results of allogeneic HCT in 114 patients, including 35 with HD, who relapsed after aHCT for lymphoma [101]. All patients received high-dose myeloablative conditioning, and some received unrelated or mismatched transplants. The 100-day mortality rate was 21% and the 5-year cumulative incidence of disease progression was 70%. The 5-year actuarial survival was 24% and no evidence of a survival plateau was noted. RIC followed by allogeneic HCT has also been used to decrease the transplant-related mortality of allogeneic HCT for HD patients with a prior aHCT (see below). At the M.D. Anderson Cancer Center, the 18-month OS and PFS were estimated to be 61 and 32%, respectively, in a cohort of 30 HD patients (75% with a prior aHCT) who received reduced-intensity allogeneic HCT [102]. The 3-year probability of PFS was estimated to be 8% for patients with HD in a series of patients from several institutions who were treated with a reduced-intensity allogeneic HCT after relapsing from a prior transplant [103]. A prospective trial in Spain examined the use of reduced-intensity allogeneic HCT in 29 HD patients who had progressed after aHCT [104]. The actuarial 2-year OS and EFS were 52 and 23%, respectively. These values were 75 and 50%, respectively, for patients whose remission duration following aHCT was at least 12 months.

Source of hematopoietic cells

Most autologous HCT procedures in North America and Europe are now performed with mobilized peripheral blood hematopoietic cells, and autologous BMT is rarely performed [8,76]. Hematopoietic recovery is more rapid following autologous PBHCT and this approach allows cells to be collected without general anesthesia. Most importantly, this approach allows the use of HDT in patients whose marrow cannot be harvested due to tumor contamination or prior radiation.

Results from the series of aHCT for HD displayed in Table 56.1 have not shown differences in survival when results of autologous PBHCT and autologous BMT were compared. A Spanish registry analysis showed that the 5-year time to treatment failure was estimated to be 41% for HD patients treated with autologous BMT, compared with 55.3% for patients who were treated with autologous PBHCT (p = 0.007) [9]. These differences were not significant in the multivariate analysis, however. In contrast, the analysis in Vancouver showed that first-relapse HD patients treated with autologous PBHCT had inferior PFS to recipients of autologous BMT (p = 0.04) [36]. Again, these differences were not significant in the multivariate analysis.

An EBMT matched-pair analysis compared outcomes of autologous PBHCT and autologous BMT for HD patients [105]. The actuarial 4-year PFS was 52% following BMT compared with 38% following PBHCT (p = 0.0082). The actuarial OS rates were 65 and 53%, respectively (p = 0.0198). A single-center matched-pair analysis at University College Hospital in London also compared outcomes of autologous BMT and PBHCT for HD [106]. The 3-year PFS was estimated to be 59% following autologous BMT and 58% for those who received autologous PBHCT (p = 0.255). The 3-year OS rates were 69 and 78%, respectively (p = 0.078).

Prospective trials in cohorts containing patients with both HD and NHL have also compared outcomes in relation to hematopoietic cell source [107–109]. No significant differences in survival were noted, although improvements in secondary outcomes such as engraftment rate, duration of hospital stay, costs, and quality of life have been described in patients treated with autologous PBHCT. Therefore, the use of autologous PBHCT is now preferred owing to secondary advantages associated with ease of collection and engraftment rate.

Allogeneic hematopoietic cell transplantation

The use of allogeneic HCT may provide additional antitumor activity compared with aHCT, but is associated with a higher NRM. It has also been suggested that allogeneic HCT might correct the lifelong immunologic defects seen in HD patients. Allogeneic HCT may also result in a graft-versus-Hodgkin effect similar to the graft-versus-leukemia effect observed after allogeneic HCT for leukemia. Although it is unknown whether infused Reed–Sternberg cells are clonogenic, the use of allogeneic donors also eliminates the risk of reinfusing malignant cells. Allogeneic HCT may be considered for patients with inadequate hematopoietic cell collections or for patients with dysplastic bone marrow or cytogenetic bone marrow abnormalities.

The role of allogeneic HCT for HD is controversial. Results of myeloablative allogeneic HCT are poor, although most series have been composed of patients who were extensively pretreated and those with advanced chemoresistant disease [110,111]. The actuarial 3-year DFS was 15% in 100 patients with advanced HD reported to the IBMTR who underwent myeloablative allogeneic BMT from matched siblings [111]. The 3-year risk of treatment-related mortality was 61%. Factors associated with treatment failure include older age, poor performance status, and recent infection. The 4-year actuarial PFS and NRM were 15 and 48%, respectively, following myeloablative allogeneic HCT in a series of 45 patients reported to the EBMT [112]. The results of allogeneic and autologous HCT for HD have been compared retrospectively. A case-matching analysis from the EBMT identified no significant differences in PFS, OS, relapse, or transplant-related mortality when autologous and allogeneic HCT for HD were compared [112].

At the Johns Hopkins Oncology Center, patients with relapsed and refractory HD were preferentially assigned to receive allogeneic HCT if they had a matched sibling donor and were under the age of 56 years [113]. Patients were otherwise treated with aHCT. A retrospective comparison revealed no significant differences in relapse, EFS, or OS between groups. Investigators in Seattle compared the outcome of allogeneic HCT and aHCT for HD [110]. The relapse rate following allogeneic HCT was lower (45% vs. 76%, p = 0.05), although OS differences were not observed because of higher transplant-related mortality in allogeneic HCT. A more recent EBMT analysis evaluated the outcome of 167 patients with HD who were treated with allogeneic HCT [114]. The donor was a human leukocyte antigen (HLA)-identical sibling in 81.5% of cases. The 4-year PFS and OS were estimated to be 15.5 and 24.7%, respectively. The procedure-related mortality was estimated to be 51.7% at 4 years. A matched-pair analysis demonstrated that aHCT was associated with superior PFS compared with allogeneic HCT.

Graft-versus-Hodgkin disease (GVHL)

Some comparisons have demonstrated that the rate of relapse is lower after allogeneic HCT for HD compared with aHCT [110,112,113]. The data supporting the existence of a GVHL effect derived from the response observed either after donor lymphocyte infusions (DLIs) or after the development of graft-versus-host disease (GVHD).The existence of a GVHL effect is supported by reports of response rates of 3055% after donor leukocyte infusions [115–117]. In a large EBMT retrospective study, DLIs were administered to 64 patients at a median of 6 months (range 1–38 months) for persistent or progressive disease following RIC allogeneic HCT. The response rate was 32% and the median OS following DLI was 20 months. In a subset of 18 patients receiving DLI without additional chemotherapy, the response rate was 44% [118]. Similar results were reported from the M.D. Anderson Cancer Center in 27 patients who developed progressive disease after T-repleted allogeneic HCT. Ten of 27 patients (37%) had a complete or partial response and the median duration of response was 7.5 months (range 0.5–20 months) [119]. Not only has DLI been shown to induce remission, it also has been shown to reduce relapse risk following T-depleted allogeneic HCT. In another study in the United Kingdom, 76 patients with multiple relapsed or refractory HD underwent allogeneic HCT that incorporated in vivo T-cell depletion [120]. DLIs were given in a dose-escalating fashion to 22 patients for mixed chimerism and to 24 patients for relapse. Nineteen of 22 patients (86%) receiving DLI for mixed chimerism converted to full donor status. The 4-year relapse incidence was 5% in these patients compared with 43% in the group with full donor chimerism (p = 0.00071). For those 24 patients receiving DLI for relapse, the response rate was 79% with a 4-year OS of 59%. These results suggest that preemptive DLI based on mixed chimerism may reduce relapse risk following T-depleted allogeneic HCT.

There is also evidence that relapse rates may be lower in patients who develop GVHD [112,121]. An EBMT analysis of 168 HD patients treated with allogeneic HCT found that the development of chronic GVHD significantly decreased the incidence of relapse, which translated into a trend for a better PFS with no impact on NRM [121]. Similar results were reported by the Gruppo Italiano per il Trapianto di Midollo Osseo (GITMO) on 122 patients who underwent allogeneic HCT following relapse post-aHCT. They found that patients with chronic GVHD experienced a significantly better OS ($p < 0.001$) and PFS ($p = 0.024$) compared with those without it or with acute GVHD [122].

Reduced-intensity conditioning followed by allogeneic transplantation

Several groups of investigators have explored the use of RIC allogeneic HCT for HD to exploit potential GVHL effects and to reduce transplant-related mortality [102,104,116,123,124]. These studies have shown the feasibility of this approach in patients who have relapsed after aHCT and in patients who are not candidates for allogeneic HCT using conventional myeloablative regimens. A variety of conditioning regimens have been used from non-myeloablative (fludarabine 90mg/m^2 and TBI 200Gy) to RIC regimens such as fludarabine and melphalan with or without alemtuzumab.

The largest series of RIC and allogeneic HCTs for HD was initially reported by the EBMT [125]. Fifty-two patients were conditioned with a variety of regimens, although the majority received fludarabine-based treatments. The 2-year PFS and OS were estimated to be 42 and 56.3%, respectively. The 1-year transplant-related mortality was 17.3%. Updated results in 285 patients were subsequently published [118]. Most had chemosensitive disease and 80% had prior aHCT. The 1-year TRM was low at 11%, but the 5-year progression rate was 59% without a plateau on the progression curve. The 3-year OS and PFS were 29 and 25%, respectively. The only factor that was significantly associated with DFS and OS was chemosensitivity prior to transplant (Figure 56.6).

Encouraging results of RIC and allogeneic HCT for HD have recently been reported from registry data and also single-center studies. The GEL/TAMO and the Lymphoma Working Party of EBMT reported a phase II trial of allogeneic HCT in 78 patients with relapsed HD (unrelated donors = 23) [126]. Fifty were in complete or partial remission and 28 had stable disease. Fludarabine 150mg/m^2 and melphalan 140mg/m^2 were used as conditioning regimen. Antithymocyte globulin was added for GVHD prophylaxis in recipients of unrelated donor transplants. The NRM was 15% at 1 year. Relapse was the major cause of treatment failure. The PFS rate was 48% at 1 year and 24% at 4 years. Chronic GVHD was associated with a lower incidence of relapse. Patients who were transplanted in complete remission had a significantly better outcome with an OS rate of 71% at 1 year and 43% at 4 years .Refractory disease and poor performance status were associated with significantly worse survival. There was no difference in survival between recipients of match-related and unrelated donors. CIBMTR analyzed the outcome of 143 patients undergoing unrelated donor RIC/non-myeloablative stem cell transplantation (NST) for relapsed/refractory HD [127]. Various conditioning regimens were used. Patients were heavily pretreated with ≥3 prior regimens and 89% had prior aHCT. With a median follow-up of 25 months, the probability of TRM at day 100 and 2 years was 15 and 33%, respectively. The 2-year PFS and OS were 20 and 37%, respectively. Extranodal disease and poor Karnofsky Performance Score (KPS) were significant risk factors for TRM and survival. Relapse and infection were the most common causes of death.

Results from single-center experiences showed a lower incidence of TRM. At the M.D. Anderson Cancer Center, 40 patients with relapsed and refractory HD were treated with RIC allogeneic HCT (matched sibling $n = 20$; matched unrelated donor $n = 20$) [102]. The conditioning regimens consisted of fludarabine plus cyclophosphamide (± antithymocyte globulin), and fludarabine plus melphalan. The actuarial PFS and OS at 18 months were 32 and 61%, respectively. The cumulative incidence of progressive disease was 55%. The OS at 18 months was estimated to be 73% for patients who received fludarabine–melphalan compared with 39% for patients treated with fludarabine–cyclophosphamide ($p = 0.03$). An update in 58 patients receiving fludarabine and melphalan showed a low incidence of TRM of 7% at day 100 and 15% at 2 years [128]. At a median follow-up of 2 years, projected 2-year overall and PFS were 64% and 32%, respectively, with 2-year disease progression/relapse rate of 55%. There was no statistical difference in survival between matched related and unrelated donors transplants. Investigators in the United Kingdom reported results of RIC allogeneic HCT (matched sibling $n = 31$; matched unrelated donor $n = 18$) in 49 patients with multiply relapsed HD [116]. The majority (90%) had progressed after a previous aHCT. The conditioning regimen consisted of fludarabine, melphalan, and alemtuzumab. The 4-year PFS and OS were estimated to be 39 and 55.7%, respectively. Patients in complete remission at the time of transplant had significantly better PFS ($p = 0.0389$) and OS ($p = 0.0398$) compared with patients in partial remission or with refractory disease. PFS was also poorer in patients with a history of aHCT ($p = 0.0267$). Their updated results showed that alemtuzumab significantly reduced the risk of GVHD without impact on survival outcome and durable response to DLI was seen following the inclusion of alemtuzumab in the conditioning regimen [129]. Baron et al. (see earlier) reported results or RIC allogeneic HCT in 35 HD patients who had failed a previous transplant [103]. The conditioning regimen consisted of fludarabine and 2Gy TBI in the majority of patients. The actuarial 3-year PFS and OS were 8 and 35%, respectively.

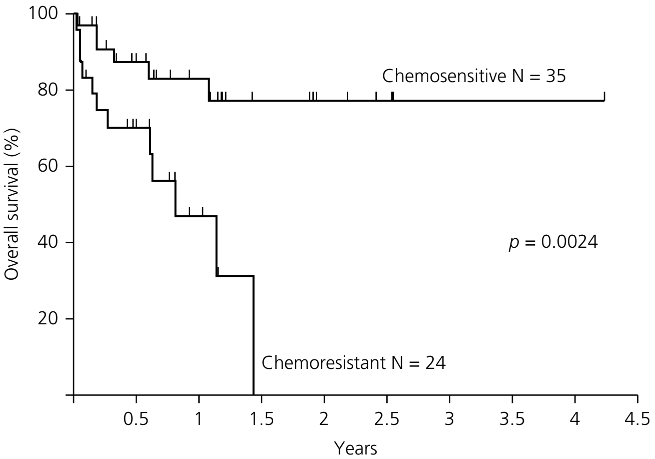


Figure 56.6 Overall survival of patients with chemosensitive or chemoresistant Hodgkin disease following non-myeloablative conditioning. Source: Schmitz et al. 2004 [123]. Reproduced with permission of Elsevier.

Investigators in Spain have also reported on the results of RIC allogeneic HCT (matched sibling $n = 37$) in 40 patients with relapsed or refractory HD [104]. The majority ($n = 29$) had been treated previously with aHCT. Conditioning consisted of fludarabine and melphalan. The 2-year PFS and OS were estimated to be 32 and 48%, respectively. Resistant disease at the time of transplant was associated with significantly worse PFS and OS. Similar outcomes, including the adverse influence of chemotherapy resistance, have been reported by other groups using RIC allogeneic HCT for HD [124].

A comparative analysis of allogeneic HCT with conventional salvage chemotherapy for relapsed HD after aHCT was performed by the retrospective analysis of 185 patients by the Italian investigators [122]. Of 185 patients, 122 found donors (related $n = 55\%$, unrelated $n = 32\%$ and haploidentical $n = 13\%$) and 63 did not. There were no differences in clinical characteristics between the two groups. At a median follow-up of 48 months, the 2-year PFS and OS were better in the donor group (39.3% vs. 14.2% and 66% vs. 42%, respectively, $p < 0.001$). In multivariate analysis, having a donor was significant for better PFS and OS ($p < 0.001$). Patients who were transplanted in complete remission showed a better PFS and OS. This study supports the role of allogeneic HCT in patients with HD who had relapsed after aHCT.

The comparative analysis of allogeneic HCT after RIC or conventional high-dose conditioning in patients with relapsed or refractory HD was reported by the EBMT [121]. In this study, the clinical outcomes of 89 patients who underwent RIC allogeneic HCT were compared with the outcomes of 79 patients who underwent conventional high-dose allogeneic HCT. The two groups were comparable except that more patients in the high-dose group were transplanted in earlier years and had received bone marrow. RIC patients were more heavily pretreated, and more patients had relapsed after a prior aHCT (61.8% vs. 40.5%, $p = 0.005$). There was no difference in hematopoietic recovery or the incidence of GVHD between the two groups. However, the NRM was significantly decreased in the RIC group (48% vs. 24%, hazard ratio 2.85, $p < 0.001$). The 5-year OS was also better in the RIC group and there was a trend for better 5-year PFS in the RIC group. Patients who developed chronic GVHD had a significantly decreased risk of relapse, which translated into a trend for a better PFS (Figure 56.7). In another study reported from the United Kingdom, the outcome

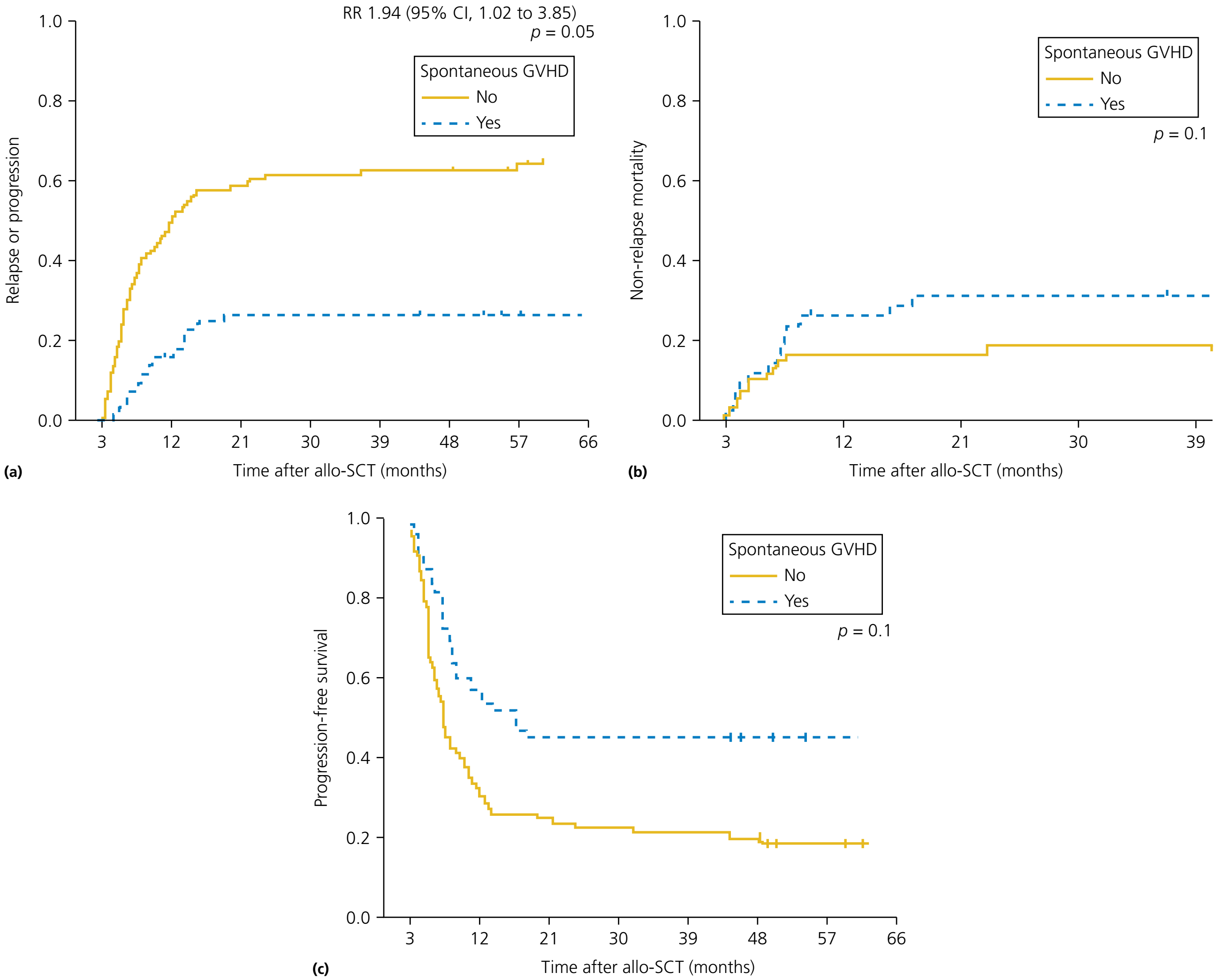


Figure 56.7 Influence of chronic graft-versus-host disease on the outcome after allogeneic hematopoietic cell transplantation. Cumulative incidence of (a) relapse or progression, (b) non-relapse mortality, and (c) Kaplan–Meier estimation of progression-free survival. Source: Sureda et al. 2008 [121]. Reproduced with permission of the American Society of Clinical Oncology.

of RIC allogeneic HCT was compared with the outcome of conventional non-transplant salvage therapy in 72 patients with HD relapsing following autograft [130]. Thirty-eight underwent alemtuzumab-containing RIC and 34 did not. The OS from both diagnosis and autograft was significantly better in the RIC allogeneic HCT group, with actuarial survival at 10 years from diagnosis of 48% compared with 15% ($p = 0.0001$) and survival at 5 years from autograft of 65% compared with 15% ($p \leq 0.0001$) These results support the use of RIC followed by allogeneic HCT in patients with relapsed HD following aHCT. Moreover, these studies confirm the existence of a GVHL effect associated with the development of chronic GVHD.

Results of allogeneic HCT for HD are displayed in Table 56.7. The results of allogeneic HCT for HD using RIC appear to be better than those reported with full-intensity myeloablative conditioning, especially in patients who have relapsed after a prior aHCT. This approach should be considered for patients who relapse after aHCT. As emerged from the above studies, chemosensitive disease and complete remission status at transplant are the most important factors to improve survival after allogeneic HCT. Alternative treatment strategies should be explored in patients with chemoresistant disease. Recent studies have shown that brentuximab vedotin [131] and bendamustine [98,99] can induce remission in patients with relapsed HD that enabled them to undergo allogeneic HCT. Additional studies are required to compare results with those of other approaches, determine the appropriate timing of transplantation, develop optimal RIC regimens, and design strategies to decrease toxicity and the risk of GVHD.

Other investigators have studied the strategy of aHCT followed by RIC allogeneic HCT for HD (see above). This approach is designed to exploit a GVHL effect in patients who have achieved a minimal disease state following an HDT regimen. Carella et al. demonstrated the feasibility of this approach [47]. Ten patients with relapsed and refractory HD received HDT followed by aHCT. At a median of 84 days after aHCT, patients underwent allogeneic HCT after conditioning with fludarabine and cyclophosphamide. Five patients were alive between 210 and 430 days after the second transplant. Additional studies are required to assess the curative potential of this approach.

Haploidentical related transplantation

To permit HLA-haploidentical HCT after non-myeloablative conditioning, investigators at Johns Hopkins University and the Fred Hutchinson Cancer Research Center incorporated high-dose post-transplantation cyclophosphamide to achieve the selective depletion of alloreactive T cells. Subsequently, immunosuppressive therapy with tacrolimus and mycophenolate mofetil was begun to control both rejection and GVHD. The outcomes of haploidentical related HCT ($n = 28$) were compared with those for HLA-matched related donor ($n = 38$) and unrelated donor ($n = 24$) HCT patients with relapsed or refractory HD who underwent non-myeloablative conditioning regimens [132]. Patients were heavily pretreated with a median of five regimens and 92% had failed aHCT and local radiation therapy (83%). There was no significant differences in OS between the three groups; however, there were significantly decreased risks of relapse for haploidentical related recipients compared with HLA-matched related ($p = 0.01$) and unrelated ($P = 0.03$) recipients. NRM was also significantly lower for haploidentical recipients than HLA-matched related recipients ($p = 0.02$). There were no differences in the incidences of severe grade III–IV acute or chronic GVHD between the groups. These results suggest that alternative donor sources are a viable option, particularly for those patients who may have a limited window of opportunity to proceed to allogeneic HCT.

Umbilical cord transplantation

The majority of candidates of allogeneic HCT will not have an HLA-matched sibling donor. Matched unrelated donors may be used, although many patients will not have suitable donors, and this option may be associated with a higher risk of GVHD and transplant-related mortality. The use of unrelated umbilical cord blood (UCB) has the advantages of rapid availability and lower risk of GVHD despite donor-recipient HLA disparity.

Investigators from Minnesota compared the results of RIC allogeneic HCT with UCB in 9 patients, with the results of transplantation in 12 patients with HLA-matched siblings [133]. The cumulative incidences of acute and chronic GVHD, as well as treatment-related mortality, were similar in both groups. The 2-year PFS

Table 56.7 Results of allogeneic hematopoietic cell transplantation for Hodgkin disease

Ref.	*n*	Regimen	Mortality	Outcome
[113]	100	Myeloablative	61% 3-yr TRM	15% 3-yr DFS
[113]	53	Myeloablative	43%	27% 10-yr EFS
[112]	45	Myeloablative	65% 4-yr TRM	15% 4-yr PFS
[110]	53	Myeloablative	53% 5-yr TRM	22% 5-yr EFS
[114]	167	Myeloablative	51.7% 4-yr TRM	15.5% 4-yr PFS
[123]	94	RIC (55% prior aHCT, 10 URD)	20%	39% 2-yr PFS
[102]	40	RIC (75% prior aHCT, 20 URD)	22% 18-mo TRM	32% 18-mo PFS
[116]	49	RIC (90% prior aHCT, 18 URD)	16% 730-day NRM	39% 4-yr PFS
[104]	40	RIC (73% prior aHCT, 2 URD)	25% 1-yr TRM	32% 2-yr PFS
[103]	35	RIC	32% 3-yr NRM	8% 3-yr PFS
[102]	58	RIC (83% prior aHCT, 33 URD)	15% 2-yr TRM	32% 2-yr PFS
[133]	21	Myeloablative, RIC (66% prior aHCT, 9 UCB)	11% UCB, 17% MSD 100-day TRM	25% 2-yr PFS UCB, 20% 2-yr PFS MSD
[124]	14	RIC (50% prior aHCT)	None	36% 1-yr PFS
[121]	168	RIC = 89, 61.8% prior aHCT	23% at 1 yr	28% 5-yr OS
		Myeloablative = 79, 41% prior auto	46% at 1 yr	22% 5-yr OS
[118]	285	RIC (85% prior aHCT, 105 URD)	19% at 1 yr	52% 2-yr OS
[122]	122	RIC (100% prior aHCT, 32 URD)	12% at 1 yr	31% 2-yr PFS
[126]	92	RIC (86% prior aHCT, 23 URD)	8% at 1 yr	48% 1-yr PFS
[127]	143	RIC (89% prior aHCT, 100 %URD)	15% at 2 yrs	30% 1-yr PFS

TRM, transplant-related mortality; DFS, disease-free survival; EFS, event-free survival; PFS, progression-free survival; RIC, reduced-intensity conditioning regimen; aHCT, autologous hematopoietic cell transplant; URD, unrelated donor; UCB, umbilical cord blood; MSD, matched sibling donor; OS, overall survival; mo, month; yr, year.

was estimated to be 25% following UCB allogeneic HCT, as compared with 20% for patients with matched sibling donors ($p = 0.67$). The actuarial 2-year PFS was 0% for patients with primary refractory disease, and those who relapsed within one year of aHCT. These results demonstrate that allogeneic HCT using UCB may be an alternative for patients without matched sibling donors or fully-matched unrelated donors.

High-dose therapy regimens

A wide variety of HDT regimens have been used with aHCT for HD (Table 56.8). No prospective trials have been performed, although regimens have been compared retrospectively. HDT regimens are frequently divided into those that use TBI and those that contain only drugs. Use of radiation with prior treatment often eliminates the possibility of using TBI.

Investigators in Seattle examined the influence of the preparative regimen in a cohort of HD patients who were treated with both autologous and allogeneic HCT [110]. No significant differences in EFS were observed when TBI-containing and drug-only regimens were compared, although 5-year OS rates were 28 and 14%, respectively ($p = 0.081$). The actuarial 5-year survival was 25% for patients who received cyclophosphamide and TBI compared with 11% for patients who received busulfan and cyclophosphamide ($p = 0.0610$). Patients treated with cyclophosphamide and TBI had similar outcomes to patients treated with cyclophosphamide, carmustine, and etoposide. A retrospective analysis of HD patients undergoing autologous or allogeneic HCT at Johns Hopkins University also failed to note differences in outcome when TBI-containing regimens were compared with regimens using chemotherapy alone [113]. Patients at City of Hope received a regimen of TBI, etoposide, and cyclophosphamide, or else carmustine, etoposide, and cyclophosphamide if they had received prior radiation [37]. No significant differences in response rate, DFS, OS, or toxicity were observed. At Stanford University, patients received TBI, etoposide, and cyclophosphamide, or regimens where carmustine or lomustine were substituted for TBI [21]. No significant differences in OS, freedom from progression, or EFS were noted. Similarly, there were no differences in outcome when TBI-containing regimens were compared with drug-only regimens in an ABMTR analysis [27]. An EBMT analysis also failed to show significant differences in PFS or OS associated with the use of TBI [55]. At MSKCC, there were no significant differences in EFS and OS when HD patients were treated with a regimen using accelerated fraction total lymphoid irradiation combined with cyclophosphamide and etoposide, compared with a high-dose chemotherapy regimen [20].

Table 56.8 Commonly used high-dose therapy regimens for Hodgkin disease

Regimen	Total dose administered
CBV	Cyclophosphamide, 4.5–7.2g/m^2 or 100mg/kg Carmustine, 300–600mg/m^2 or 15mg/kg Etoposide, 750–2400mg/m^2 or 60mg/kg
BEAC	Carmustine, 200–400/mg/m^2 Etoposide, 600–1200mg/m^2 Cytarabine, 800–1200mg/m^2 Cyclophosphamide, 140–180mg/kg or 6–7.5g/m^2
BEAM	Carmustine, 300–400mg/m^2 Etoposide, 400–1600mg/m^2 Cytarabine, 400–1600mg/m^2 Melphalan, 140mg/m^2
CY, TBI	Cyclophosphamide, 120–200mg/kg Total body irradiation, 800–1320cGy
VP-16, CY, TBI	Etoposide, 30–60mg/kg Cyclophosphamide, 60–120mg/kg Total body irradiation, 1200–1375cGy
BU, CY	Busulfan 16mg/kg Cyclophosphamide 120–200mg/kg

The use of TBI may be associated with increased pulmonary toxicity, particularly in patients who have received prior mediastinal irradiation. Spanish registry results showed that the use of TBI-containing regimens prior to aHCT for HD was associated with a significantly higher risk of early transplant-related mortality (relative risk 2.3, $p = 0.02$) [9]. A multivariate analysis of another Spanish registry study of aHCT for HD identified the use of TBI-containing regimens to be associated with a 6.09-fold risk of NRM ($p = 0.0001$) [64]. The actuarial 5-year OS was 33% for patients transplanted with TBI-containing regimens compared with 60% for those transplanted with chemotherapy regimens ($p = 0.01$), although this difference was not significant on multivariate analysis.

One of the most common high-dose chemotherapy regimens used prior to aHCT for HD is the CBV [cyclophosphamide, BCNU (carmustine), etoposide] regimen developed at the M.D. Anderson Cancer Center. The original CBV regimen has been modified and individual institutions have used widely differing schedules (Table 56.8). No prospective trials have examined whether variations in the CBV regimen lead to different outcomes, although higher doses of carmustine have been associated with an increased risk of pulmonary toxicity, particularly in patients who have received prior chest irradiation [110,134]. The addition of cisplatin to the CBV regimen did not result in significant differences in outcome, although this modification allowed the dose of carmustine to be reduced [36,135]. Another high-dose chemotherapy regimen for HD aHCT is the BEAM regimen, which was developed by investigators in London and has also been widely modified (Table 56.8) [66]. Retrospective analyses have failed to show significant differences in survival when CBV-type regimens and BEAM-type regimens were compared [25,65]. In one retrospective comparison, CBV was associated with significantly higher rates of transplant-related morbidity and mortality, although an unusually high dose of carmustine (800mg/m^2) was used [136]. In one study, escalation of the total dose of etoposide in the BEAM regimen to 1600mg/m^2 did not significantly improve survival [137].

A variety of other HDT regimens have also been used prior to aHCT for HD. In Seattle, a retrospective analysis compared results of a regimen containing busulfan, melphalan, and thioTEPA with results from the combination of etoposide, cyclophosphamide, and TBI for patients undergoing aHCT for HD [39]. No significant differences in toxicity, EFS, or OS were observed. Other HDT regimens used prior to aHCT for HD include busulfan, etoposide, and cyclophosphamide [30], carmustine, etoposide, and cisplatin [138], thioTEPA, mitoxantrone, and carboplatin [139], and high-dose sequential chemotherapy regimens [40].

It is unclear from existing data whether any HDT regimen is superior for patients with HD. Prospective trials will be required to prove the superiority of any regimen and it is difficult to recommend any single regimen, although TBI and higher doses of carmustine should be avoided in patients with pulmonary disease or a history of chest irradiation.

Several institutions have tested the use of double (tandem) aHCT for HD (see earlier). This approach is feasible and good outcomes are possible, although a significant proportion of patients are not able to receive the second planned transplants [45,46].

Summary and future directions

Over the last 25 years, the use of HDT followed by autologous HCT has become the treatment of choice for most patients with relapsed and refractory HD. The outcomes are better in chemotherapy-sensitive patients and those with good prognostic factors at relapse. New approaches are needed for patients with primary refractory HD, since the chance of long-term remission remains poor even with autologous HCT. Novel approaches using brentuximab vedotin as salvage therapy before transplant are being explored. Long-term survivors of autologous HCT for HD are at higher risk of NRM due to prior exposure to multiple chemotherapies and radiotherapy. New strategies to reduce long-term complications are needed. Incorporation of early interim FDG-PET and at completion of therapy may help identify patients who may not be curable with conventional therapy and thus benefit from early transplant. Despite the lower incidence of NRM, the role of reduced-intensity allogeneic HCT for relapsed and refractory HD requires further studies. At present, patients with chemosensitivity benefit from allogeneic HCT and the existence of a GVHL effect has been clearly demonstrated. The optimal choices of RIC regimens, the best GVHD prophylaxis, the type of hematopoietic stem cell, and the role of DLI require further studies. Nevertheless, allogeneic HCT should be considered in selected patients with HD who relapsed after autologous HCT.

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CHAPTER 57

Hematopoietic Cell Transplantation for Non-Hodgkin Lymphoma (B Cell)

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Introduction

Non-Hodgkin lymphomas (NHLs) are a diverse group of malignant neoplasms derived from either B-cell progenitors, T-cell progenitors, mature B cells, or mature T cells. It was estimated that approximately 72,000 men and women would be diagnosed with NHL and 20,000 men and women would die of lymphoma in 2015 [1]. Since hematopoietic cell transplantation (HCT) is recognized as a potentially curative treatment for NHL, the use of HCT in the treatment of patients with NHL has risen steadily. In 2009, over 3500 HCTs were performed in the United States for treatment of NHL, of which over 2500 were autologous HCT (autoHCT) [2]. This chapter reviews the use of autoHCT and allogeneic HCT (alloHCT) for B-cell NHL. HCT for T-cell NHL is discussed in Chapter 58. Readers are referred to other chapters for complete and related discussions of preparative regimens (Chapters 20 and 21), radioimmunotherapy (Chapter 23), minimal residual disease (Chapter 25), hematopoietic cell sources (Chapter 4), complications after HCT (chapters in Section 7) and adoptive immunotherapy (Chapter 69).

Diffuse large B-cell lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of NHL and accounts for approximately 30% of NHLs diagnosed each year with the median age at diagnosis being 64 years [3]. The addition of the anti-CD20 monoclonal antibody rituximab (RTX) to CHOP [cyclophosphamide, hydoxydaunomycin (doxorubicin), vincristine (Oncovin), prednisone] has significantly improved outcomes for DLBCL patients. The International Prognostic Index (IPI), developed in 1993, is used for risk stratification for patients with DLBCL, and the age-adjusted IPI (aaIPI) is also widely applied particularly to tailor more intensive therapy such as autoHCT [4]. The risk factors included in the IPI include age, performance status, lactate dehydrogenase level, multiple extranodal sites of involvement, and stage of disease. The number of risk factors present at diagnosis segregates patients into low-, intermediate-, and high-risk groups. Patients treated with CHOP or CHOP-like regimens have predicted 5-year survival rates ranging from 73% in the low-risk group to 26% in the high-risk group, with an increased risk of death due to both a lower complete remission (CR) rate and a higher rate of relapse from CR. However, over 50% of patients who achieve a CR will relapse within 2–3 years and autoHCT is the standard of care for patients who can demonstrate chemosensitive disease with salvage therapies. AutoHCT can also salvage a subset of patients who are induction failures to front-line chemoimmunotherapy. AlloHCT is typically reserved for patients who are not candidates for autoHCT, such as those with marrow involvement, patients with very high-risk features such as the "double hit" lymphomas, and those who have relapsed after autoHCT.

Autologous HCT

Several phase II trials demonstrated survival advantages for autoHCT as upfront therapy in high-risk patients, which led to randomized trials of autoHCT as consolidation therapy for aggressive lymphomas [5]. A meta-analysis with 11 trials with over 2000 patients who received autoHCT as consolidation demonstrated comparable mortality in both HCT and control arms; however, significant heterogeneity was noted among the trials with regard to trial design. No overall survival (OS) benefit was noted [6]. Many of the original studies examining the efficacy of autoHCT for relapsed or refractory DLBCL were conducted prior to the addition of RTX to CHOP. In the "post-RTX age," response rates and both OS and progression-free survival (PFS) were improved after conventional chemotherapy and remain superior at 10 years of follow-up. Hence justification of upfront autoHCT for high-risk presentation DLBCL requires a more stringent risk-to-benefit ratio [7]. A position statement from the American Society for Blood and Marrow Transplantation in 2011 concluded that autoHCT as first-line therapy is not recommended for any risk group of the IPI at that time; however, none of the published studies included RTX in their treatment protocols, and ongoing studies that include RTX may change this recommendation [5].

In the RTX era, two randomized studies have been conducted testing the role of autoHCT in the front-line therapy of unfavorable (high- and high–intermediate-risk patients) DLBCL. A Southwest Oncology Group study used either CHOP or R-CHOP (CHOP with rituximab added) induction therapy followed by either additional cycles of R-CHOP or autoHCT [8]. There was a significant difference in 2-year PFS between the autoHCT arm and the standard arm, 69% versus 56%, respectively ($p = 0.005$), but for OS the differences were not significant, 74% versus 71% ($p = 0.32$). By exploratory analyses, when the high–intermediate IPI and high IPI groups were analyzed separately, the high IPI patients who received HCT had a stronger improvement in 2-year PFS and OS compared with

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the standard arms (PFS 75% vs. 41%; OS 82% vs. 64%). The trial was not powered to detect differences between these subsets. No difference was seen between treatment effects according to induction therapy or between B-cell and T-cell histology.

The Italian Lymphoma Foundation conducted an upfront HCT trial as a multicenter randomized trial with a 2 × 2 factorial design for high-risk (aaIPI 2 or 3) patients [9]. Patients were first randomized to either of one of two RTX dose-dense treatments (RCHOEP14 vs. RmegaCHOP) and responding patients then underwent a second randomization to either HCT or observation. The primary end-point was event-free survival (EFS), and all analyses were performed with an intent-to-treat analysis. The 2-year PFS was 72% in the HCT arm versus 59% in the no-HCT arm ($p = 0.008$), with no difference seen in OS.

For DLBCL patients receiving front-line chemotherapy, the use of interim [^{18}F]fluorodeoxyglucose positive emission tomography (FDG-PET) scanning after 2–4 cycles of chemotherapy has demonstrated prognostic significance. Patients with a positive interim FDG-PET scan after two cycles of CHOP or R-CHOP were correlated with a poorer outcome in a study by Haioun et al. [10]. Additional cycles of the same chemotherapy were not sufficient to cure these patients. Upfront autoHCT has been offered to DLBCL patients in the context of interim FDG-PET scanning during induction chemotherapy. Investigators at the Memorial Sloan Kettering Cancer Center prospectively studied interim FDG-positive disease using a risk-adapted strategy in 98 patients with DLBCL treated with four cycles of R-CHOP14 [11]. After four cycles, a PET scan was performed and patients with FDG-negative scans received ICE (ifosfamide, carboplatin, etoposide) for three cycles followed by observation. Patients with a positive interim PET scan were biopsied and, if positive for residual NHL on biopsy, received ICE followed by autoHCT. Of the 38 patients with a positive interim scan, only five had a positive biopsy and received autoHCT. Three of these five patients remained alive and free of disease, and all 33 patients with positive interim PET scans and negative biopsies received ICE without autoHCT. Twenty-six of these 33 patients remained alive and without subsequent relapse. This is a cautionary note for investigators considering the use of an interim PET scan as an indication for autoHCT.

Autologous HCT for DLBCL – relapsed disease

Patients who relapse after primary therapy, or who fail to achieve CR with primary treatment, should be considered for autoHCT. The PARMA trial was a randomized, multicenter trial that compared autoHCT with consolidation therapy in 215 patients with chemotherapy-sensitive NHL [12]. All patients had achieved a first remission with an anthracycline-containing chemotherapy regimen. All patients received DHAP (dexamethasone, high-dose cytarabine, cisplatin) with harvesting of autologous bone marrow, followed by a second cycle of DHAP. Patients were then randomized to four more cycles of DHAP versus autoHCT. There were no differences in prognostic factors between the groups. After a median follow-up of 63 months, the response rate was 84% after HCT versus 44% after conventional therapy. At five years, EFS was 46% in the HCT group and 12% in the group receiving chemotherapy only ($p = 0.001$) The OS was 53 and 32%, respectively ($p = 0.038$). Subsequent results from the PARMA study indicated that patients with a longer initial remission (relapse after >1 year vs. <1 year) had a higher response to DHAP (69% vs. 40%) and superior OS at 8 years (29% vs. 13%). Survival advantages were only seen in patients with an IPI of >0 at the time of relapse [13].

The addition of rituximab to front-line chemotherapy has allowed more patients to achieve and maintain remission. However, increasing evidence has shown that patients who had received RTX prior to salvage therapy and autoHCT fared significantly worse than patients who were RTX-naïve prior to relapse and subsequent autoHCT. In a retrospective analysis, Spanish investigators from the Grupo Español de Linfomas/Trasplante Autólogo de Médula Ósea (GEL/TAMO) evaluated the influence of prior RTX use in 163 patients with relapsed or refractory DLBCL who received R-ESHAP (rituximab plus etoposide, cytarabine, cisplatin, methylprednisolone) salvage chemotherapy followed by autoHCT. Sixty-nine patients had not received RTX prior to relapse. The response to salvage therapy and also PFS and OS were significantly worse in the patients who had received RTX as part of front-line therapy. With a median follow-up of 29 months, the PFS for the RTX-exposed group versus the RTX-naïve group was 17% versus 57% ($p < 0.0001$) and the OS was 38% versus 67% ($p = 0.0005$) [14].

This observation was confirmed in the Collaborative Trial in Relapsed Aggressive Lymphoma (CORAL), which was a European intergroup prospective phase III study that enrolled 396 patients with DLBCL in first relapse or with primary refractory disease. Patients were randomized to salvage chemotherapy with R-ICE (rituximab, Ifosfamide, carboplatin, etoposide) or R-DHAP (rituximab, dexamethasone, high-dose cytarabine, cisplatin) [15]. Responding patients proceeded to autoHCT and underwent a second randomization after HCT to either observation or RTX maintenance for 1 year. A total of 244 patients (63%) had received RTX as part of first-line therapy. On comparing the R-ICE and R-DHAP arms, there was no difference in response rates, 63.5% versus 62.8%, respectively or in EFS, 26% versus 35%, respectively. The factors that significantly affected response to salvage and EFS were refractory/relapse <12 months from diagnosis, secondary aaIPI >1 and prior exposure to RTX. The group of patients who relapsed <12 months from diagnosis and had prior RTX exposure had an inferior PFS compared with early relapsed patients without prior RTX exposure (Figure 57.1a). For patients who relapsed >12 months from diagnosis, prior RTX exposure did not affect PFS.

Regarding the impact of maintenance RTX after HCT, no differences in EFS, PFS, or OS were seen in the maintenance versus observation arm. The 4-year post-HCT EFS was 52% in the maintenance arm versus 53% in the observation group ($p = 0.7$). More adverse events after day 100 were reported in the maintenance arm, the majority of which were infections. The secondary aaIPI was highly significant ($p < 0.001$) for EFS, PFS, and OS (Figure 57.1b). A striking observation was the significant difference in PFS between males and females at the time of second random assignment and after maintenance therapy. The females had a superior EFS at both time points, attributed to the higher RTX clearance in males resulting in lower RTX exposure. The EFSs for males and females were 43% and 69%, respectively. In the Cox model for maintenance therapy, the secondary aaIPI ($p < 0.001$) and male gender ($p = 0.01$) were significant prognostic factors [16].

The CORAL investigators retrospectively evaluated the prognostic value of the molecular subtype or cell of origin in the relapsed/refractory DLBCL patients [17]. Based on the availability of histologic material, tumor biopsies of 232 patients were analyzed by immunohistochemistry for CD10, *bcl-6*, MUM1, and FOXP1 and by fluorescence in situ hybridization (FISH) for *bcl-2*, *bcl-6*, and *c-MYC* breakpoints. On the basis of the Hans algorithm, which utilizes immunohistochemistry to segregate patients into germinal center B-cell-like (GCB) and activated B-cell-like (ABC)

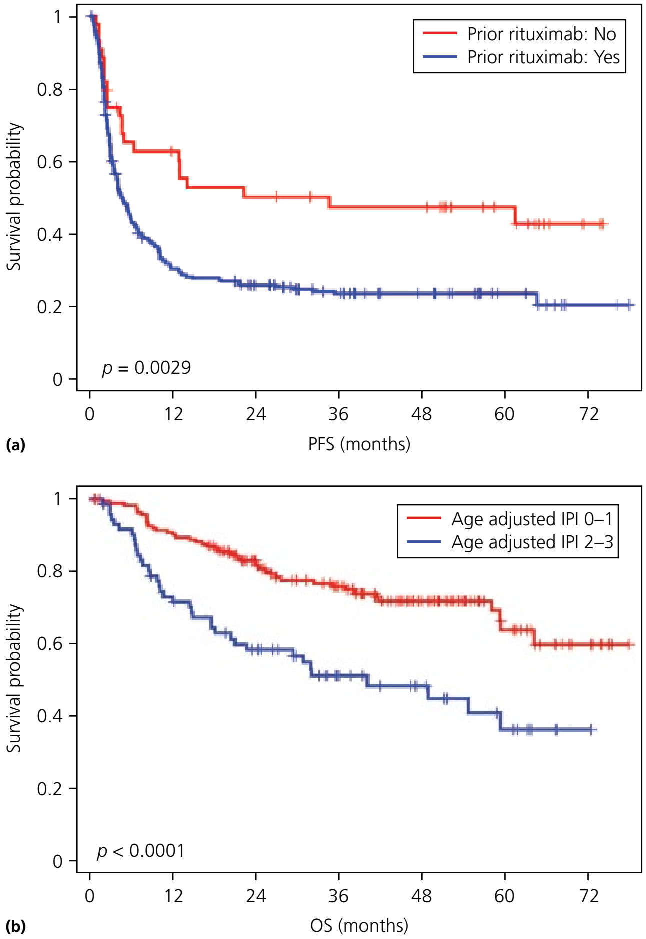

Figure 57.1 (a) Progression-free survival from diagnosis by prior rituximab therapy in diffuse large B-cell lymphoma. Failure from diagnosis was <12 months. CORAL study. (b) Overall survival according to the age-adjusted International Prognostic Index at the time of relapse in diffuse large B-cell lymphoma. CORAL Study. Source: Gisselbracht et al 2010 [15]. Reproduced with permission of the American Society of Clinical Oncology.

subtypes, 46% of the patients were classified as "GCB-like" and 49% as "ABC-like" [18]. The GCB-like patients had a significantly better PFS and OS compared with the ABC-like patients, with PFS of 70% versus 28% and OS of 74% versus 40%, respectively. When response to salvage was analyzed by cell of origin and by salvage regimen, the GCB-like patients treated with R-DHAP had a 100% 3-year PFS whereas the 3-year PFS for the GCB-like patients treated with R-ICE was only 27%. The ABC-like DLBCL patients had an unfavorable outcome irrespective of the salvage regimen. Hence prospective randomized trials with cell of origin stratification are needed to evaluate survival benefit with R-DHAP over R-ICE in patients with GCB-like DLBCL.

The role of radioimmunotherapy (RIT) in HCT is covered comprehensively in a separate chapter (Chapter 23) but selected trials are worth mentioning here. Radioisotope-labeled monoclonal antibodies deliver targeted radiation to malignant cells expressing a specific antigen with the added benefit of irradiating neighboring malignant cells with lower or absent antigen expression. RIT is an ideal agent to incorporate into HCT preparative regimens for B-cell NHL. Myelosuppression, the major adverse effect of RIT, can be overcome by infusion of hematopoietic stem cells. Several phase I and II trials have demonstrated the feasibility and safety of adding standard dose or escalating doses of RIT to the HCT preparative regimen using anti-CD20 RIT with [^{90}Y]ibritumomab tiuxetan or [^{131}I]tositumomab [19–21].

A multicenter phase III trial, conducted by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) randomized chemosensitive persistent or relapsed DLBCL patients to autoHCT with either the rituximab plus BEAM [BCNU (carmustine), etoposide, cytosine arabinoside, melphalan] preparative regimen (n = 113) or BEAM plus [^{131}I]tositumomab (n = 111). There was no significant difference in 2-year PFS (48.6% vs. 47.9%, p = 0.97) and 2-year OS (66% vs. 61%, p = 0.38). The treatment-related mortality (TRM) was also comparable (4.1% vs. 4.9%, p = 0.97), although the RIT arm had a significantly higher mucositis score [22]. There is an ongoing multicenter phase III trial with a similar trial design to the previously mentioned BMT CTN trial that is testing the efficacy of [^{90}Y]ibritumomab tiuxetan with BEAM. Patients with relapsed or persistent DLBCL are randomized to autoHCT with a conditioning regimen of BEAM versus BEAM + [^{90}Y]ibritumomab tiuxetan. It also should be noted that the most promising outcomes in the phase I and II studies incorporating RIT preparative regimens used higher doses of RIT and further study is perhaps necessary with such doses.

Allogeneic HCT for DLBCL

AlloHCT for relapsed DLBCL has lower rates of relapse than autoHCT but at the expense of higher treatment-related morbidity and mortality. The earliest retrospective studies of alloHCT in NHL lent support to the existence of a graft-versus-lymphoma (GVL) effect [23]. This is demonstrated by lymphoma regression after withdrawal of immunosuppression or after infusion of donor lymphocytes.

This finding of lower relapse with alloHCT was also observed in a large European Group for Blood and Marrow Transplantation (EBMT) Registry study, which analyzed 1185 lymphoma patients transplanted between 1982 and 1998, and compared the results with those of approximately 14,000 autologous procedures during the same period [24]. In a matched analysis, for patients with intermediate-grade NHL, the relapse rates for allogeneic and autologous recipients were approximately 35 and 60%, respectively. However, there was no difference in OS due to the significantly higher TRM seen in the allogeneic group. A registry study by the Center for International Blood and Marrow Transplant Research (CIBMTR) compared outcomes of 916 DLBCL patients undergoing first autoHCT (n = 837) or myeloablative alloHCT (n = 79) [25]. Allogeneic recipients were more likely to have advanced disease, more prior chemotherapy regimens, and resistant disease. With a median follow-up of 60 months for the autologous patients versus 81 months for the allogeneic patients, alloHCT was associated with a higher 1-year TRM [relative risk (RR) 4.88], treatment failure (RR 2.06), and mortality (RR 2.75). The risk of disease progression was similar in both groups. The disparity in risk features may explain the absence of an observed GVL effect.

For most DLBCL patients, alloHCT is more often utilized after failure of prior autoHCT. Three retrospective trials analyses reported a PFS ranging from 35 to 49% and OS ranging from 45 to 59% in DLBCL patients who received fludarabine-based reduced-intensity conditioning (RIC) regimens (Table 57.1). Over 70% of patients in all three studies had failed a prior autoHCT. Two large registry studies in Europe reported the outcomes of DLBCL patients

Table 57.1 Reduced intensity alloHCT for relapsed diffuse large B-cell lymphoma

Ref.	*n*	Prior autoHCT (%)	Conditioning regimen	PFS (%)	OS (%)	NRM (%)	Relapse/ progression (%)	Follow-up
SFGM [124] 2010	68	79	Flu-based	49	49	23	41	49 months
UK [125] 2009	48	70	Flu, Mel, alemtuzumab	48	47	33	32	4 years
FHCRC [108] 2008	31	75	TBI ± Flu	35	45	25	41	45 months

SFGM, Société Française de Greffe de Moelle et de Thérapie Cellulaire; FHCRC, Fred Hutchinson Cancer Research Center; OS, overall survival; PFS, progression-free survival; Flu, fludarabine; Mel, melphalan; TBI, total body irradiation.

after RIC or ablative alloHCT and included only patients who had relapsed after autoHCT. The long-term survival approached 30–40% with a non-relapse mortality (NRM) of 28% in both studies [26,27]. The most important prognostic factor was disease status at the time of alloHCT and not the intensity of the conditioning regimen. An analysis was conducted of 147 patients with hematologic malignancies who relapsed after autologous (n = 135), allogeneic (n = 10), or syngeneic (n = 2) HCT and then were subsequently treated with human lymphocyte antigen (HLA)-matched related or unrelated grafts after non-myeloablative conditioning with 2Gy of total body irradiation (TBI) with or without fludarabine [28]. The best outcomes were observed in patients with NHL (24 aggressive NHL, 12 indolent, and 14 mantle cell lymphoma), as compared with myeloma or classical Hodgkin lymphoma patients. Being in a partial remission (PR) or CR at the time of alloHCT and developing chronic graft-versus-host disease (GVHD) were associated with a lower risk or progression or relapse. Factors associated with a better OS were remission status, lack of comorbidities, and absence of acute GVHD. Other groups have identified increased NRM in patients who were older, or whose initial remission after autoHCT was less than 12 months [27].

Double hit lymphoma

It is now recognized that DLBCL with concurrent abnormalities including the *MYC* gene rearrangement and the t(14;18) (*c-myc*$^+$/*bcl-2*$^+$) constitute a subtype commonly referred to as “double hit (DH) lymphoma” with particularly poor clinical outcomes, even with intensive upfront therapy [29]. The double hit subtype has been observed in 2–11% of newly diagnosed DLBCL patients. Evaluation with FISH for *MYC* should be performed for NHL patients with a mature B-cell phenotype who are CD10 positive and either *bcl-6* or *bcl-2* positive. This is especially relevant if a diffuse pattern with or without a “starry sky pattern” is seen, and in the entity B-cell lymphoma, “unclassifiable, intermediate between DLBCL and BL (U-DLBCL/BL)”.

The standard of care for newly diagnosed double hit DLBCL patients has not been established [30]. Most DH NHL patients present with high IPI scores and with bone marrow and central nervous system (CNS) frequently involved. A review of published clinical studies in which DH lymphomas were separately analyzed demonstrated a variety of approaches, including doxorubicin-based chemotherapy regimens and autoHCT with a median OS of only 0.2–1.5 years [31–35]. In a recent study with 303 patients with DLBCL treated with R-CHOP, 35 patients had a MYC rearrangement, of which 27 were DH cases [36]. All *MYC* cases had an inferior outcome. All four patients with DLBCL with a DH and who received ifosfamide-based high-intensity regimens died within 5 months after the start of treatment. The poor prognosis of these patients led some centers to advocate the use of autoHCT or alloHCT in the upfront setting for patients. However, given the lack of published literature for this approach, its merits are unclear. In one retrospective study of 16 patients, the DH patients treated with autoHCT or alloHCT as part of front-line therapy still had a poor median survival of less than 6 months [31].

HCT for double hit lymphoma – relapsed disease

Although standard of care for patients with relapsed DLBCL includes salvage chemotherapy followed by autoHCT, recent studies by Cuccuini et al. indicated that *MYC*-positive DLBCL is not salvaged by classical R-ICE or R-DHAP followed by autoHCT [37]. Of the 477 patients in the previously mentioned CORAL study, 28 patients (17%) were confirmed to have a *MYC* rearrangement. Twenty-one of these patients had one or more concurrent translocations, including either *bcl-2* and, in some cases, also *bcl-6*. Patients with the DH markers had a 4 year PFS and OS much lower than those of patients in the *MYC*-negative category (18% vs. 42% and 29% vs. 62%, respectively). Type of salvage (R-ICE vs. R-DHAP) had no impact on survival. These findings suggest that novel therapies and possibly alloHCT should be considered in such patients. However, it is not clear if even these approaches will improve survival.

Mantle cell lymphoma

Mantle cell lymphoma (MCL) is a B-cell neoplasm comprising 3–10% of all NHLs. It occurs in middle-aged to older individuals with a median age of 50 years and a male predominance. In addition to lymph nodes, other sites commonly involved include spleen, bone marrow, peripheral blood, gastrointestinal tract, and Waldeyer’s ring. Most patients present with stage III or IV disease. Although some MCL patients can be followed without aggressive treatment at the time of diagnosis, most require multi-agent chemotherapy. There is currently no standard therapy for newly diagnosed or relapsed disease, but autoHCT is often offered as part of front-line therapy. Early treatment with CHOP chemotherapy resulted in low CR rates of 25% or less with patients relapsing after a median of only 1–2 years [38–41]. The use of RTX in the upfront setting improved outcomes, with R-CHOP yielding a CR rate of 35%. A recent study added bortezomib to R-CHOP and increased the CR rate to 64% [42]. The R-hyperCVAD (RTX, vincristine, doxorubicin, dexamethasone) regimen has gained widespread use following demonstration of CR rates as high as 90% in the front-line setting with a prolonged 5-year failure-free survival (FFS) of 60% in younger patients [43]. A risk-adapted approach at the M.D. Anderson Cancer Center demonstrated that as treatments changed over the years, the best improvements were seen with the additional RTX and the combination of R-hyperCVAD [44].

The IPI developed for DLBCL has been applied to MCL but is less predictive of survival compared with the Mantle Cell Prognostic Index (MIPI) [4,45]. The outcome of patients with MCL varies from a median OS of 60% at 5 years for the low-risk group to only 29 months for the high-risk group. The MIPI appears to assist with prediction of survival both at the time of initial diagnosis and after autologous transplantation. Budde et al. reported that the MIPI was independently associated with survival after HCT in patients who underwent autoHCT as initial consolidation [46]. For patients with low-, intermediate-, and high-risk MIPI, the OS at 2.5 years after HCT was 93, 60, and 32%, respectively. After adjustment for the MIPI, an intensive induction regimen was not associated with improved survival after HCT in all patients.

Front-line application of Autologous HCT

AutoHCT has yielded disappointing results in relapsed MCL patients, which led to consideration of autoHCT during CR1. A key trial was a prospective study conducted by the European Mantle Cell Consortium of 122 patients with advanced-stage MCL. Patients were randomly assigned to either high-dose cyclophosphamide and autoHCT or maintenance therapy with interferon-α after CHOP-like therapy. The results showed a significantly longer PFS in the autoHCT arm, 39 months versus 17 months ($p = 0.01$) for the interferon patients. No significant difference in OS was observed, 83% versus 77% ($p = 0.18$) for the autologous and interferon arms, respectively. However, the crossover design of the trial may have interfered with obtaining a meaningful OS end-point [41].

The Nordic group has extensively studied the role of autoHCT in CR1. The MCL-2 Nordic trial incorporated R-CHOP with higher doses of cyclophosphamide and doxorubicin alternating with high-dose cytarabine and consolidation with autoHCT using RTX in vivo-purged autoHCT [47]. The overall response and CR rate achieved were 96 and 54%, respectively. No relapses were reported after 5 years with the longest follow-up at 7.5 years, suggesting that some long-term remissions could be obtained. In the intent-to-treat analysis, the 10-year OS and EFS for all 160 patients was 58 and 43%, respectively [48]. The median OS was >10 years and the EFS was 7.4 years. The Ki-67 proliferation rate was the sole independent predictor of EFS. Compared with the Nordic MCL-1 trial, which had a similar trial design but did not use RTX or high-dose cytarabine, the MCL-2 trial demonstrated a higher molecular remission rate and improved EFS and OS compared with the historical controls from the MCL-1 trial [47,49] (Figure 57.2).

The ability of high-dose cytarabine and rituximab to increase CR rates and improve outcomes has been studied in several other prospective trials [50–52] (Table 57.2). A notable trial was reported by the GELA group in which cisplatin was incorporated in the induction regimen for 60 newly diagnosed MCL patients. Patients received R-CHOP followed by R-DHAP and responding patients proceeded to autoHCT. Either BEAM or a TBI-based regimen with cytarabine was given as a preparative regimen. The median EFS was 84 months and the OS was not reached with a follow-up of 67 months. No toxic deaths were reported [51].

At least two clinical trials have demonstrated rationale for administering maintenance RTX after autoHCT for MCL. Brugger et al. performed a prospective, multicenter phase II study of 31 patients, including 10 MCL cases, using a single course of RTX weekly for 4 weeks, beginning approximately 8 weeks after autoHCT [53]. Patients were monitored with polymerase chain reaction (PCR) for minimal residual disease. RTX consolidation post-HCT was tolerable, with the most common toxicity being non-life-threatening infection. At a median follow-up of 42 months (range 13–96 months) after HCT, the actuarial EFS was 81% at 4 and 5 years. PCR negativity was observed in 22% of the patients before HCT, 53% post-HCT ($p = 0.0547$), 72% after RTX maintenance ($p = 0.0018$), and 100% at 6 months post-HCT.

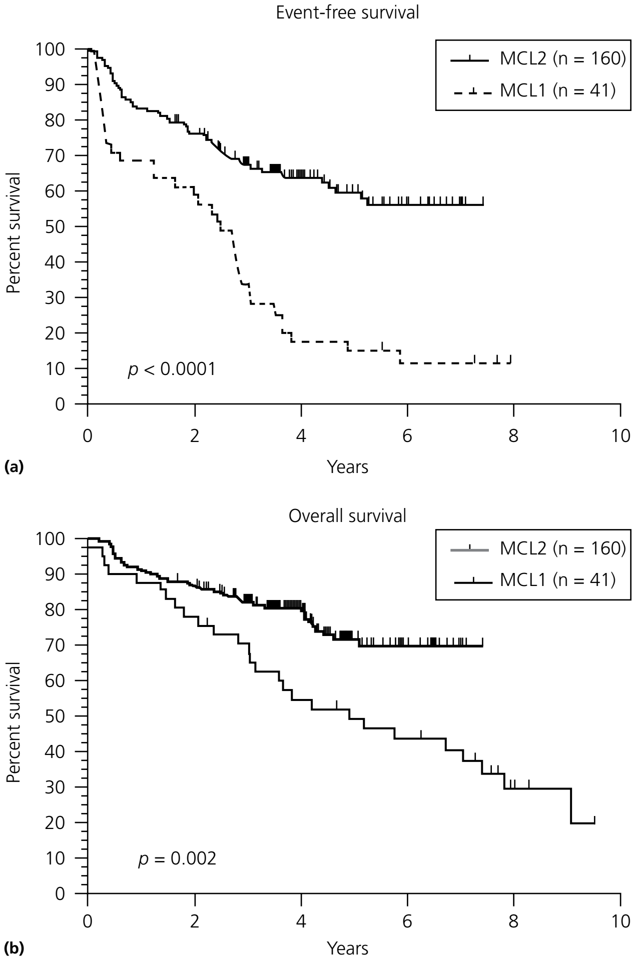

Figure 57.2 (a) Event-free survival and (b) overall survival in the Nordic MCL-1 and Nordic MCL-2 trials.

Table 57.2 Phase II trials containing rituximab and cytarabine with autoHCT for newly diagnosed patients with mantle cell lymphoma

Ref.	*n*	Median age (years)	CR rate	Overall RR (%)	OS (%)	EFS/PFS (%)	Follow-up
GELA [51] 2013	60	57	75	82	75	64	67 months
SWOG [126] 2013	59	57	55	86	63	49	4.8 years
CALGB [52] 2009	79	57	69	88	64	56	4.7 years
HOVON [127] 2009	87	55	64	70	79	46	41 months

GELA, Groupe d'Etude des Lymphomes de l'Adulte; SWOG, Southwest Oncology Group; CALGB, Cancer and Leukemia Group B, HOVON, Dutch Haematology–Oncology Cooperative Group; CR, complete remission; RR, response rate; OS, overall survival; EFS/PFS, event-free survival/progression-free survival.

In the previously mentioned Nordic MCL-2 trial, the use of preemptive treatment with RTX after autoHCT was also studied

[54]. Twenty-six patients with evidence of molecular relapse after successful autoHCT (as evidenced by rising PCR levels of bcl-1/IgH (immunoglobulin H) and clonal IgH rearrangements in blood and marrow following HCT), were offered preemptive RTX 375mg/m^2 weekly for 4 weeks. A total of 92% of these patients were able to achieve molecular remission after "preemptive treatment." Median molecular and clinical relapse-free survival after preemptive treatment were 1.5 and 3.7 years, respectively.

The 2013 European Society for Medical Oncology consensus guidelines for MCL recommend that autoHCT should be incorporated into first-line therapy. No conditioning regimen has shown a clear superiority; however, TBI may improve PFS in patients in PR after induction [55].

Autologous transplant for relapsed disease

AutoHCT for MCL has been offered to patients beyond CR1 but high relapse rates remain problematic. The CIBMTR reported the outcomes of 151 MCL patients with relapsed disease who underwent autoHCT [56]. The 5-year OS was 40%, with 50% of patients eventually showing progressive disease. owing to these disappointing results, intensified conditioning regimens have been explored. A compilation of both EBMT and CIBMTR data on MCL patients receiving autoHCT between 1988 and 1998 resulted in a cohort of 195 patients with a median follow-up of 4 years. The 2- and 5-year OS were 76 and 50% and the PFS were 55 and 33%, respectively. Disease status at transplant was the most significant factor affecting survival: patients with chemosensitive disease but not in first CR were 2.99 times [95% confidence interval (CI) 1·66–5·38, $p < 0{\cdot}001$] more likely to die than patients transplanted in CR1.

Allogeneic HCT

Long-term DFS and cures have been reported in MCL patients after alloHCT, including patients who had failed a prior autoHCT. A retrospective analysis of 70 consecutive patients with refractory or relapsed MCL from 12 centers treated with RIC alloHCT was reported [57]. Forty-seven of the patients had previously been treated with autoHCT with the median number of prior therapies being two and median time from diagnosis to RIC alloHCT of 44 months. The 2-year EFS, OS, and TRM rates were 50, 53, and 32%, respectively. No difference in outcome was seen based on number of prior lines of therapy, but disease status at the time of RIC alloHCT reached statistical significance with regard to EFS. The EFS for CR patients was 62.5%, for PR patients 53%, and for patients with stable disease or progressive disease (PD) only 11%. The 3-year EFS for patients who had received a prior autoHCT was 49%.

A risk-adapted strategy for transplant for MCL was described by the group at the M.D. Anderson Cancer Center [44]. When RIC alloHCT was compared retrospectively with autoHCT, either in CR1 (AUTO1) or in the relapsed setting (AUTO2), the 6-year actuarial PFS for the allogeneic patients was 46% versus 39% for AUTO1 and 10% for AUTO2 patients. The respective actuarial 6-year OS were 53, 61, and 35%, respectively. These results showed that long-term DFS can be obtained in patients undergoing autoHCT in CR1 with an RTX-containing regimen or in patients with relapsed or refractory disease with RIC alloHCT.

A recent retrospective report from the CIBMTR demonstrated that alloHCT can salvage some patients with chemorefractory disease [58]. A total of 202 patients with refractory MCL were treated with either myeloablative (MA) or RIC conditioning; 33% of the RIC group and 13% of the MA group had had a prior autoHCT. At 3 years post-HCT, no significant differences were seen between the groups in NRM (47% MA vs. 43% RIC), relapse/progression (33% vs. 32%), PFS (20% vs. 25%), or OS (25% vs. 30%). Receipt of a bone marrow or T-cell-depleted allograft was associated with an increased risk of NRM and inferior PFS and OS.

Follicular lymphoma

Follicular lymphoma (FL) comprises 20% of all NHLs and is the second most common type of NHL diagnosed in the United States. The incidence increases with age with a median age at diagnosis of 60 years. FL cells are characterized by the cell surface markers CD19, CD20, and CD10 and express surface immunoglobulin. The t(14;18) translocation, which involves the *IgH*/*bcl-2* genes, occurs in up to 90% of follicular lymphomas and is also found in 20–30% of DLBCL patients [59]. The median survival of patients with FL ranges from 8 to 12 years but there is wide variability as some patients do not require treatment for many years whereas 15% of patients die within 2 years of diagnosis due to progressive or transformed disease [60]. FL remains incurable with standard therapy but the "watch and wait" approach remains acceptable in patients without bulky disease and who are asymptomatic. For patients who require treatment, treatment options include radiotherapy, chemotherapy, monoclonal antibody therapy, radioimmunoconjugates, and HCT. There is no consensus as to the optimal first-line or salvage therapeutic regimen but HCT should be considered for patients beyond first remission.

Autologous HCT for follicular lymphoma

First remission

The role of autoHCT as consolidation therapy for front-line treatment of FL patients has been explored in three large randomized trials in Europe [61–63]. In all three trials, patients with newly diagnosed FL received upfront combination chemotherapy and were then randomized to interferon maintenance therapy versus autoHCT. There was no difference in OS between the conventional chemotherapy arms and the autoHCT arms in all three trials, although a superior PFS was seen in the patients who underwent autoHCT in two of the trials. It should be noted that these trials were completed before RTX was routinely incorporated into front-line therapy.

A disturbing finding was the higher incidence of therapy-related neoplasms in the patients who received HCT. In the French GELA trial and the GOELAMS trial, this incidence was 14% in the HCT arms and ranged from 1 to 7% in the conventional chemotherapy arms [61,63]. A prospective, multicenter trial by the Italian GITMO group represents the only randomized trial that incorporated RTX in the front-line HCT setting [64]. Newly diagnosed FL patients with high-risk disease received R-CHOP induction therapy followed by either RTX maintenance or intensified chemotherapy with RTX during mobilization and after autoHCT. The 4-year EFS was significantly higher in the HCT arm (61% vs. 28%, $p < 0.001$), with no difference in OS. Molecular remission was significantly higher in the HCT arms (80% vs. 44%, $p < 0.001$) and was the strongest independent predictor of outcome. Patients who relapsed in the R-CHOP arm were crossed over to autoHCT, which resulted in a 3-year EFS of 68% in those patients. Thus, based on the above results, there is no role for autoHCT as consolidation of FL patients in first remission.

Autologous HCT for follicular lymphoma – relapsed disease

For patients with relapsed disease, autoHCT has consistently been shown to improve DFS, with one prospective study showing improved OS. Both prospective and retrospective studies reported

5-year PFS ranging from 40 to 50% and one study showed a 10-year freedom from progression of 48% [61–71]. Optimal patients for autoHCT are those who are not heavily pretreated (i.e., fewer than three prior regimens), patients with chemosensitive disease, and patients who have a lower risk Follicular Lymphoma International Prognostic Index (FLIPI) score at the time of autoHCT (Figure 57.3).

The largest retrospective series came from the EBMT and reported the outcomes of 693 FL patients who underwent autoHCT [69]. The 10-year PFS and OS were 31 and 52%, respectively, with an NRM of 9%. The relapse incidence was 54%, with relapse occurring at a median of 1.5 years (range 1 month–13.5 years) after autoHCT. By multivariate analysis, older age, chemoresistant disease, and use of a TBI-based conditioning regimen correlated with inferior survival. The incidence of a second malignancy was 9% and occurred at a median of 7 years after HCT. Another large retrospective series of 241 FL patents who underwent autoHCT reported a 10-year OS and PFS of 75 and 49%, respectively [70]. The relapse incidence was 47%, with a median time to relapse of 20 months (range 2–128 months). Five patients developed a therapy-related malignancy. There has been only one randomized study, known as the CUP (chemotherapy versus unpurged versus purged arm), that prospectively addressed the role of autoHCT versus standard therapy in relapsed FL patients [67]. A total of 140 FL patients with chemosensitive disease after salvage chemotherapy were randomized to receive further conventional therapy versus autoHCT with a purged graft-versus-autoHCT with an unpurged graft. It should be noted that this trial was conducted in the pre-RTX era. The 4-year OS was 46% for the chemotherapy arm, 71% for the unpurged arm and 77% for the purged HCT arm, with a similar advantage seen for the HCT groups with regards to PFS. The impact of ex vivo purging could not be assessed owing to the small sample size in both HCT arms. Unfortunately, this trial closed prematurely owing to poor accrual. There was one large retrospective analysis in the RTX era that included FL patients who received an autoHCT. The GELA/GOELAMS groups evaluated the role of autoHCT versus conventional salvage in 175 FL patients at first relapse who received conventional chemotherapy only or autoHCT [71]. Forty patients (25%) underwent autoHCT. Nearly half of the patients had prior RTX exposure. With a median follow-up of 31 months, the 3-year OS was significantly higher in patients who proceeded to HCT than in patients who did not undergo HCT (92% vs. 63%, $p = 0.0003$). Prior RTX exposure did not affect outcome. It should be noted that the favorable impact of HCT may have been affected by selection bias as only patients responding to salvage therapy proceeded to HCT.

Based on the current literature, the best candidates for autoHCT are relapsed FL patients who are chemosensitive, had fewer than three prior regimens, and who are less than 70 years old. However, for patients with limited stage at relapse, involved field radiotherapy and/or conventional treatment should be considered.

Allogeneic HCT for follicular lymphoma

AlloHCT is the only known curative therapy for patients with FL. FL patients who underwent myeloablative alloHCT demonstrated lower relapse rates than autoHCT recipients [72,73]. However, the high NRM related to ablative regimens was prohibitive and offset the benefit of a lower relapse risk. Two large registry studies by the CIBMTR and the EBMT compared the outcomes of relapsed FL patients who underwent either autoHCT or myeloablative alloHCT [24,72]. In both studies, the relapse risk was significantly lower in the alloHCT group than in the autoHCT patients, but this did not translate into improved OS in the alloHCT patients owing to the higher NRM. The OS for both the autologous and allogeneic groups ranged from 50 to 62% in both studies. Three single-institution retrospective analyses with myeloablative regimens have reported durable remissions in FL patients, with 5-year EFS ranging from 45 to 75% and with outcomes consistently dependent on chemosensitivity at the time of alloHCT [74–76].

Reduced-intensity allogeneic HCT

RIC has been increasingly offered to FL patients and the results have shown durable remissions and less NRM compared with myeloablative regimens. The use of RIC regimens has broadened patient eligibility and usually includes patients older than 70 years of age, patients who had failed a prior autoHCT, and patients with comorbid conditions who otherwise would not be eligible to receive an ablative regimen.

Table 57.3 provides outcomes in four selected prospective trials of FL patients undergoing RIC alloHCT. All trials included patients who had failed a prior autoHCT and utilized a fludarabine-based preparative regimen [77–80]. With a median follow-up ranging from 2 to 9 years, the DFS/EFS ranged from 57 to 85% and the OS ranged from 54 to 88%. Chemosensitivity at the time of HCT was a consistent predictor of outcome. The trial by the M.D. Anderson Cancer Center group had the longest follow-up of 107 months and incorporated the FCR (fludarabine, cyclophosphamide, RTX)

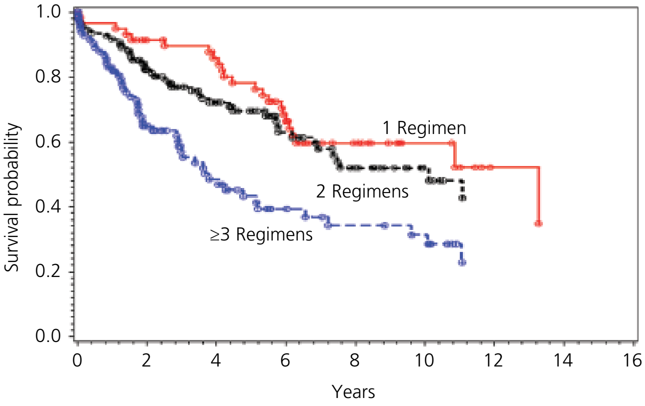

Figure 57.3 Overall survival based on the number of prior chemotherapy regimens for relapsed follicular lymphoma patients. Source: Vose 2008. Reproduced with permission of Elsevier.

Table 57.3 Prospective phase II trials for reduced intensity alloHCT for relapsed follicular lymphoma

Ref.	*n*	Median age (years)	Regimen	PFS (%)	OS (%)	TRM (%)	Follow-up (months)
MDACC [80] 2011	47	53	Flu/Cy/Rit	72	78	13	107
MDACC [80] 2011	26	55	Flu/Cy/^{90}Y	85	88	8	23
CALGB [78] 2011	44	53	Flu/Cy	75	81	9	55
GEL/TAMO [79] 2010	37	50	Flu/Mel	57	54	24	52
UK [77] 2010	82	45	Flu/Mel/Alem	76	76	15	43

MDARC, M.D. Anderson Cancer Center; CALGB, Cancer and Leukemia Group B; GEL/TAMO, Grupo Español de Linfomas/Trasplante Autólogo de Médula Ósea; PFS, progression-free survival; OS, overall survival; TRM, treatment-related mortality; Flu, fludarabine; Cy; cyclophosphamide; ^{90}Y, [^{90}Y]ibritumomab tiuxetan; Mel, melphalan.

regimen [80]. This regimen included high doses of RTX in which three or the four planned doses were given at a dose of 1000mg/m^2. The 11-year EFS and OS were 72 and 78%, respectively, with only three relapses observed. Based on these impressive results, the BMT CTN conducted a phase II multicenter trial using the same FCR regimen in relapsed FL patients with chemosensitive disease and who had either a matched related or matched unrelated donor identified. Accrual completed in late 2012 and results are eagerly awaited.

The EBMT retrospectively analyzed the outcomes of 164 patients who had matched sibling donors with the specific goal of assessing the impact of in vivo T-cell depletion (TCD) [81]. In this report, patients were divided into three groups: patients who received antithymocyte globulin (ATG) ($n = 46$) or alemtuzumab ($n = 42$) as part of the preparative regimen and a third group ($n = 76$) who received neither agent. Although the patients in the TCD group experienced significantly lower incidences of acute and chronic GVHD compared with the non-TCD recipients, no observed difference in NRM was seen between the TCD and non-TCD groups. However, a significantly higher relapse rate was see in the TCD group (28% vs. 14%, $p = 0.05$). Disease status at HCT was the strongest predictor of all outcomes, including PFS, OS, NRM, and relapse. A retrospective report from the CIBMTR of FL patients who underwent alloHCT with either an ablative or an RIC regimen also further established that chemosensitivity rather than the intensity of the conditioning regimen was a stronger determinant of outcome [82].

The CIBMTR and EBMT directly compared the outcomes of FL patients who underwent either myeloablative or a RIC alloHCT in separate registry analyses. In both reports, the RIC recipients were significantly older and a higher proportion had failed a prior autoHCT compared with the ablative recipients. The CIBMTR analyses included only matched related donors and reported no difference in PFS and OS based on intensity of the conditioning regimen [83]. However, the RIC group had a significantly higher risk of progression. The EBMT report was limited to patients with unrelated donor grafts [84]. In contrast to the CIBMTR report, recipients of the RIC regimens had a lower NRM and experienced a significantly improved PFS and OS by multivariate analysis, although relapse was comparable in the two groups. These findings suggested that an unrelated donor graft may confer a more robust GVL effect. In both the CIBMTR and EBMT reports, chemoresistance and a lower performance status adversely affected outcomes.

In summary, both retrospective and prospective trials reveal that chemosensitivity rather than conditioning intensity is the most reliable predictor prior to alloHCT. Therefore, there are few indications for an ablative regimen outside of a clinical trial.

Novel conditioning regimens

For patients with chemorefractory disease, options are more limited. Therefore, novel regimens have been explored to augment cytoreduction while limiting toxicity, most prominently the use of radioimmunotherapy. Radioimmunotherapy has proven efficacy in the treatment of B-cell lymphomas, including in patients who are refractory to RTX and combination chemotherapy [85]. The M.D. Anderson group tested the novel YFC ([^{90}Y]ibritumomab tiuxetan, fludarabine, cyclophosphamide) regimen in 26 patients with advanced FL including 10 who were chemorefractory [80]. With a median follow-up of 33 months, the 3-year PFS for the chemorefractory and chemosensitive patients were 80 and 87%, respectively, with a 1-year TRM of 8%. A German group reported the results of a prospective trial that used an RIC regimen comprised of [^{90}Y]ibritumomab tiuxetan, fludarabine and low-dose TBI in 40 patients with advanced NHL, including 17 with FL [86]. All patients either had refractory disease or had relapsed after prior autoHCT. The 2-year OS and EFS for the FL patients were 67 and 57%, respectively. However, the 2-year NRM was 45% for all patients, with infection being the leading cause of death.

Another strategy for FL patients with advanced disease is tandem autoHCT followed by RIC alloHCT. A Canadian group prospectively enrolled 27 FL patients with chemorefractory and transformed disease [87]. The 3-year PFS and OS were both 96%, with one death attributed to NRM. A retrospective Italian series reported the results for 34 high-risk NHL patients, including 14 with FL who also underwent tandem autoHCT followed by RIC alloHCT [88]. With a median follow-up of 4 years, the 5-year OS and PFS were 77 and 68%, respectively. The 2-year TRM was 6%. These encouraging results suggest that perhaps the intense cytoreduction conferred by the high-dose chemotherapy with autoHCT followed by eradication of minimal residual disease via the GVL effect may overcome chemoresistance. The low TRM is also encouraging.

Maintenance therapy after HCT

Strategies to eradicate minimal residual disease after HCT leading to extension of remission duration are paramount, as relapse remains the primary cause of treatment failure after autoHCT. Rituximab (RTX) represents an ideal agent for this purpose owing to its tolerability and its immune-mediated mechanism of action. The safety and feasibility of maintenance therapy with RTX after autoHCT have been reported in several phase II trials with patients with MCL, FL, and DLBCL [89–91]. In these early trials, the most common adverse effects of maintenance RTX included prolonged neutropenia and prolonged hypogammaglobulinemia. Severe infections were seen in some but not all of the trials, despite the impaired immune reconstitution.

There have been two large multicenter randomized trials that studied the impact of RTX maintenance therapy after autoHCT for patients with DLBCL. The GELA group reported the results for 476 patients with newly diagnosed high-risk DLBCL who were randomized to induction therapy with either ACE [Adriamycin (doxorubicin), cyclophosphamide, etoposide] or ACVBP [Adriamycin (doxorubicin), cyclophosphamide, vindesine, bleomycin, prednisone] [92]. A total of 330 patients proceeded to autoHCT. After HCT, 269 patients underwent a second randomization to maintenance RTX or observation. After a median of 4 years of follow-up from the second randomization, there was no significant difference in the primary end-point of EFS between the RTX arm and the observation arm.

As mentioned earlier, the CORAL study was a large European intergroup trial eligible to DLBCL patients with relapsed or primary refractory disease. After a first randomization that determined the administered salvage regimen prior to autoHCT, a second randomization assigned patients to maintenance RTX or observation after HCT [16]. No differences were observed in EFS, PFS, or OS between the maintenance and observation arms. The 4-year post-HCT EFS was 52% in the maintenance arm versus 53% in the observation group ($p = 0.7$). Adverse events, most of which were infection, were significantly higher in the RTX maintenance arm.

The EBMT conducted the first prospective randomized trial exploring the efficacy of RTX when administered as an in vivo purging agent prior to mobilization of hematopoietic stem cells and as post-HCT maintenance to consolidate remission [93]. A total of 280 patients with chemosensitive relapsed FL were enrolled. In the first randomization that occurred after salvage therapy, patients received RTX weekly with four doses or observation prior to mobilization. After HCT, patients underwent a second randomization to receive RTX every 2 months for 4 infusions or observation. There was no significant difference in PFS between the purging and no purging groups, but maintenance therapy had a significant effect on 10-year PFS (54% vs. 37%, $p = 0.012$) in favor of the maintenance arm. A higher incidence of neutropenia was seen in the RTX maintenance arms.

Hence definitive conclusions cannot be drawn based on the emerging data on RTX maintenance therapy after autoHCT. Immunotherapy options with interleukin-2 (IL-2) and interferon-α as post-HCT maintenance versus observation have also been explored in randomized trials [94,95]. Treatment with IL-2 alone showed no difference in OS or PFS in a randomized Southwest Oncology Group (SWOG) trial, but the combination of IL-2 and interferon-α improved OS in another trial reported from Israel. Lenalidomide, an oral immunomodulator, has potential as a maintenance agent owing to its reported activity as a salvage agent for NHL patients relapsed after autoHCT [96]. Ibrutinib, a selective and covalent inhibitor of Bruton's tyrosine kinase enzyme, has impressive activity against B-cell malignancies and represents another oral agent that may eventually be explored in the maintenance setting [97].

HCT for transformed lymphoma

For patients with FL, the risk of histologic transformation (HT) to a more aggressive histology is estimated to be 20% at 5 years and 30% at 10 years, and is often associated with a poor prognosis. The median survival after transformation has been reported to be 1–2 years, although outcomes are thought to be improving in the RTX era [98,99]. There is no standard treatment for HT patients and the choice of treatment depends on therapies previously delivered. Most patients are treated with an RTX plus an anthracycline-containing regimen as front-line therapy. Retrospective data suggest that survival after HT is significantly longer in patients who received RTX than in transformed patients who did not receive RTX [98].

High-dose chemotherapy with autoHCT has resulted in prolonged PFS in patients with HT, although most of the studies were in the pre-RTX era and all were retrospective. The 5-year PFS ranged from 25 to 47% with OS ranging from 37 to 58% in these series [100–103]. The only prospective series came from Norway, where 47 patients with HT underwent salvage chemotherapy with responding patients proceeding to autoHCT [104]. For the 30 patients (63%) who proceeded to autoHCT, the 5-year PFS and OS were 32 and 47%, respectively, with a median OS of 47 months. For the 17 patients who did not proceed to HCT, the median OS was only 10 months. Inferior survival outcomes were seen in patients who had received two or more courses of chemotherapy prior to HCT compared with patients who had received only one prior regimen. The first report describing the impact of RTX in the setting of autoHCT for transformed patients came from the University of Rochester, in which 18 patients received RTX-containing therapy before or after HT and then proceeded to autoHCT [105]. The 2-year PFS and OS were 59 and 82%, respectively. For the six patients who did not receive RTX pre-transformation, a significantly better 2-year PFS was seen post-HCT compared with the 12 patients who were not exposed to RTX before transformation. This observation is similar to the results seen in the CORAL study, where PFS after autoHCT was inferior in patients exposed to RTX prior to relapse of DLBCL [15]. The Canadian Blood and Marrow Transplant Group published the largest study to date, in which the outcomes of transformed FL patients were compared after alloHCT ($n = 22$), autoHCT ($n = 97$), or conventional chemotherapy with RTX ($n = 53$) [106]. Over 95% of the alloHCT patients received a myeloablative regimen. The outcomes were similar among all three groups, with the 5-year PFS for the alloHCT, autoHCT, and conventional chemotherapy groups being 46, 55, and 40%, respectively, and the corresponding 5-year OS were 46, 65, and 61%. AlloHCT was an independent prognostic factor for improved PFS but the higher TRM offset any OS benefit. The 5-year TRM for the alloHCT group was 23% versus 5% for the autoHCT group. Based on the above results, patients with transformed lymphoma may modestly benefit from autoHCT as it appears to improve OS compared with conventional chemotherapy with RTX. Hence patients with a good performance status and chemosensitive disease should be offered autoHCT early in their course.

There have been only a few retrospective series describing the outcomes of transformed patients after alloHCT and the results overall are only fair at best. The largest series comprised 40 patients with relapsed composite and transformed lymphoma patients (n = 25 transformed) who underwent myeloablative alloHCT [107]. The 5-year EFS and OS were 23%. The 3-year TRM was 36%, with a 5 year relapse incidence of 42%. In another retrospective study, 16 patients with transformed disease underwent RIC alloHCT with disappointing results. The 3-year PFS and OS were 18 and 21%, respectively, with an NRM of 42% [108]. Tandem autoHCT followed by RIC alloHCT has also been studied in 10 patients with transformed disease. The 4-year PFS and OS were both an encouraging 70% [109]. Based on these limited results and poor outcomes, alloHCT should not be routinely offered to transformed patients except with within the context of a clinical trial.

Burkitt lymphoma

Burkitt lymphoma (BL) is a highly aggressive but curable mature B-cell NHL that accounts for less than 5% of all NHLs in adults. The median age at diagnosis is 45 years and patients often present with bulky extranodal disease, CNS involvement, and a rapidly progressive clinical course. BL cells display a high proliferation index with a Ki-67 proliferation index of nearly 100%. The hallmark of BL is the overexpression of MYC due to a balanced translocation between the MYC/8q24 locus and an immunoglobulin gene, most commonly IgH t(8;14)(q24;q32) [110]. Front-line therapy of BL is comprised of brief-duration, high-intensity chemotherapy that includes RTX and yields CR rates close to 100%, with cure rates approaching 65–70% [111,112]. AutoHCT has not improved outcome compared with front-line therapy.

There have been few reports and no randomized studies evaluating the role of autoHCT in patients with BL. With the exception of a recent CIBMTR analysis [113], none of the reports published to date included patients who had previously received RTX, which limits the applicability of such results. The feasibility of autoHCT as front-line therapy for BL has been established, but none of the reports have demonstrated superior outcomes compared with the current results seen with modern front-line chemoimmunotherapy alone. The largest and most recent retrospective series came from the CIBMTR, which reported the results for 113 BL patients who underwent autoHCT in various states of remission [113]. Only 5% of the 113 BL patients had received RTX prior to HCT. The 5-year OS was 83% for patients in CR1, 53% for patients in subsequent CR, and 22% for patients not in CR. Relapse or progression was the primary cause of death for all groups, with a significantly higher relapse incidence at 5 years in the CR1 versus non-CR1 group (61% vs. 18%, respectively, $p < .0001$). This retrospective analysis included the period from 1985 to 2007, in which only 19% of patients underwent HCT after 2001. Hence the use of autoHCT has significantly declined over time. The EBMT conducted a retrospective analysis of 117 adult BL patients who received autoHCT in which 70 patients (60%) were in CR1 [114]. The 3-year OS and PFS were 72 and 73%, respectively. For patients in chemosensitive relapse, the 3-year OS was 34%. Disease bulk was predictive of outcome, as patients with bulky disease had inferior outcomes. However, none of the patients in the EBMT study had received RTX. These results indicated that autoHCT in CR1 yielded comparable survivals before the use of modern brief, high-intensity regimens that incorporated RTX. Hence autoHCT in CR1 does not offer an additional advantage over current dose-intensive regimens.

Results for BL patients with alloHCT are not noticeably superior to autoHCT outcomes, although in most series the alloHCT recipients had either more advanced disease or chemoresistant disease or were more heavily pretreated compared with autoHCT patients. The above-mentioned CIBMTR analyses included 128 BL patients who underwent alloHCT [113]. The 5-year OS for patients in CR1, subsequent CR, and not in CR were 53, 28, and 12%, respectively. As observed with the autoHCT recipients in this CIBMTR report, progression/relapse was significantly lower in the remission patients than the non-remission patients, 27% versus 51%, respectively. Hence the alloHCT cohort was not directly comparable to the autoHCT group. The EBMT analyzed outcomes of 1185 NHL patients after alloHCT, including 71 patients (6%) who had BL [24]. The 4-year OS and PFS were 37 and 35%, respectively, with a TRM of 31% for the BL patients. Disease status at transplant was the most significant prognostic factor by multivariate analysis.

In summary, based on current results, there is no role for autoHCT or alloHCT as consolidative therapy for the treatment of BL. For patients with relapsed disease, achieving a subsequent CR prior to HCT is the most important determinant of outcome prior to either autoHCT or alloHCT. As mentioned above, the results obtained from retrospective analyses cannot accurately gauge whether autoHCT or alloHCT is more suitable, since the alloHCT patients had more high-risk features. Patients who relapse after modern intensive RTX-containing regimens are a subset of patients with particularly aggressive disease and should be offered alloHCT if a suitable donor is available. For patients with a matched sibling or matched unrelated donor, alloHCT with myeloablative conditioning is preferred. However, autoHCT is a suitable option if an appropriate donor is not available and a second CR can be achieved prior to autoHCT.

Lymphoblastic lymphoma

Lymphoblastic lymphoma (LBL) is a rare, aggressive form of NHL that is morphologically indistinct from acute lymphoblastic leukemia (ALL), as both entities express lineage-specific markers and terminal deoxynucleotidyl transferase (TdT) [3]. The clinical features used to distinguish LBL from ALL include <25% marrow involvement, predominantly nodal disease involvement, and a large mediastinal mass. LBL is treated very similarly to ALL with chemotherapy phases consisting of induction, intensification, consolidation, CNS prophylaxis, and maintenance therapy [115]. These multidrug regimens yield CR rates >90% and OS of 60–70% at 3 years [116,117]. There is no definitive evidence that autoHCT or alloHCT improves survival if offered in CR1. The Vancouver group conducted a retrospective analysis of 34 newly diagnosed LBL patients who proceeded to autoHCT or alloHCT soon after induction therapy [118]. For the 29 patients who actually proceeded to HCT (25 autologous recipients, four allogeneic recipients), the 4-year OS and EFS were 79 and 73%, respectively, with a median follow-up of 51 months. All four alloHCT patients were alive and disease free whereas the autoHCT group had a 4-year EFS of 69%. The GOELAMS group identified high-risk patients who underwent autoHCT in first remission and compared their outcomes with those of standard risk patients randomized to either conventional chemotherapy or autoHCT. There were no differences in outcomes among all three groups, although the definitions of risk factors among the groups were not consistent.

The CIBMTR published results for one of the largest retrospective series of LBL patients in various states of remission or relapse, which included 128 autoHCT patients and 76 patients who received alloHCT with a matched sibling donor [119]. The 5-year relapse incidence was significantly lower in the alloHCT than the autoHCT group (34% vs. 56%, $p = 0.004$), but the OS was comparable (39% vs. 44%, $p = 0.47$) due to the significantly higher TRM in the alloHCT recipients (25% vs. 5%, $p = 0.0003$). No prospective trial with alloHCT has been reported to date. Therefore, for LBL patients, HCT should be reserved for patients who are induction failures or beyond CR1. For young patients with a suitable donor, alloHCT would generally be recommended to take advantage of the GVL effect. However, if a donor is not available or if a patient is unable to proceed to alloHCT, autoHCT is also acceptable.

Involved field radiotherapy after HCT

Retrospective analyses show that ~50–75% of failures after autoHCT occur at prior sites of disease. Thus, involved field radiation therapy (IFRT) is a logical strategy to improve local control after autoHCT. No studies have prospectively evaluated the impact of IFRT in NHL patients after HCT, but several retrospective series have yielded mixed results. Some series have unequivocally demonstrated improved local control and PFS with the use of IFRT but without definitive impact on OS, while other studies have shown a positive impact on OS [120–123].

A recent study of a large, retrospective series of 164 patients examined the effect of IFRT in DLBCL patients after autoHCT and showed a significantly improved survival outcome. A total of 79 patients (48%) underwent IFRT after autoHCT. IFRT reduced local failure by only 10% ($p = 0.17$), but on multivariate analysis IFRT was associated with a significantly improved OS and disease-specific survival [120]. Additionally, a CIBMTR report on 184 patients with primary refractory diffuse aggressive NHL who received IFRT after autoHCT showed that the omission of IFRT before or after HCT was an adverse prognostic factor [123]. The timing of IFRT in the HCT setting is also a matter of debate, as pre-transplant IFRT reduces disease volume prior to HCT but the incidence of pneumonitis and venocclusive disease may be increased. Post-transplant IFRT will have less impact on the timing of HCT and allows for refinement of radiation dose and field according to response after HCT. Despite the lack of randomized trials, IFRT is routinely recommended owing to its benefit in achieving local control and its ability to convert patients with persistent disease to CR after HCT. It is often administered 1–3 months post-HCT when blood counts have fully recovered.

Conclusion

NHL patients are a heterogeneous group, hence the recommendation of autoHCT or alloHCT in NHL patients must be tailored to the specific histology, disease status, age, and performance status of the patient. The addition of RTX to front-line therapy in patients with B-cell NHL has improved response rates and survival outcomes. However, an increasing amount of data has shown that prior RTX exposure is an adverse risk factor for relapsed DLBCL patients and for transformed NHL patients who subsequently proceed to autoHCT. It is still a matter of debate as to the optimal therapy for this specific patient group and investigation therapies should also be considered in this population. However, autoHCT remains the standard of care for relapsed DLBCL patients and confers long-term DFS in a significant number of patients with chemosensitive disease. AutoHCT also can extend DFS and OS in MCL patients when offered as consolidation therapy during CR1 and should be considered for all patients <65 years old and without significant comorbidities.

AlloHCT is not commonly offered as front-line therapy for B-cell NHL patients owing to the high NRM compared with autoHCT. RIC alloHCT can salvage a subset of patients who relapse after autoHCT, provided that chemosensitive disease can be demonstrated prior to alloHCT. The most challenging subtype are the "double hit" aggressive lymphomas. Unfortunately, it is not clear that autoHCT or alloHCT can alter the poor prognosis associated with this group of patients. In summary, the outcomes of B-cell NHL patients continue to improve and the number of eligible patients for HCT continues to grow owing to improved supportive care, more precise donor matching, and an increasing availability of donors.

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CHAPTER 58

Hematopoietic Cell Transplantation for Non-Hodgkin Lymphoma (T Cell)

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Introduction

Mature T-cell and natural killer (NK)-cell non-Hodgkin lymphomas (T-cell NHLs) comprise about 12% of all NHLs and 15–20% of aggressive lymphomas worldwide [1,2]. T-cell NHL exhibits great morphologic diversity and histological variation even within individual disease entities; the current 2008 World Health Organization (WHO) *Classification of Tumours of Haematopoietic and Lymphoid Tissues* recognizes 20 types of mature T- and NK-cell neoplasms and three provisional entities [3]. The systemic disease histologies are often collectively referred to as peripheral T-cell lymphomas (PTCLs) and the most common nodal subtypes typically include peripheral T-cell lymphoma not otherwise specified (PTCL-nos), anaplastic large-cell lymphoma (ALCL), and angioimmunoblastic T-cell lymphoma (AITL); however, there are also geographic pockets of high-prevalence endemic disease, especially for human T-cell lymphotropic virus 1 (HTLV-1)-associated T-cell lymphoma/leukemia, Epstein–Barr virus (EBV)-associated NK/T-cell nasal type, and enteropathy-associated T-cell lymphoma (EATL) [4]. Cutaneous T-cell lymphomas (CTCLs) represent a clinically distinct subgroup of T-cell NHLs [5], generally having more indolent behavior, with the exception of a few subtypes, including Sézary syndrome and transformed mycosis fungoides.

For aggressive lymphomas, T-cell phenotype tends to confer poorer clinical outcomes than does B-cell phenotype, with the exception of ALCL [6,7]. The most aggressive T-cell NHL histologies include hepatosplenic T-cell lymphoma (HSTCL), γ/δ T-cell malignancies and NK/T-cell lymphomas [8], with long-term survival at 5 years only 10–30% for most histologies [4]. Advanced disease stage, high prognostic index at presentation [9], and inherent chemomoresistance [10] contribute to the unsatisfactory outcomes for this disease group. Recently identified biomarkers with potential diagnostic and prognostic implications for PTCL include cell lineage markers such as γ/δ T-cell receptor (TCR) rearrangement; NK-cell receptors and killer immunoglobulin-like receptors (KIRs); cytotoxic markers such as TIA-1, granzyme and perforin; and follicular helper T-cell markers such as CXCL13 and PD-1 [11]. Table 58.1 provides the frequency distribution and 5-year overall survivals for the major PTCL histologies as determined by the International T-Cell Lymphoma Project [4].

Patients with aggressive CTCL histologies, Sézary syndrome, and advanced mycosis fungoides typically have prolonged disease courses with multiple failed treatment regimens, ultimately resulting in chemoresistance. Table 58.1 also provides frequency distribution and disease-specific survival data for the cutaneous T-cell NHLs as determined by the WHO and European Organization for Research and Treatment of Cancer (EORTC) classification [5].Current therapeutic strategies for T-cell NHL remain poorly defined and tend to be extrapolated from treatment paradigms for B-cell NHL. As a result, relapsed and chemorefractory disease remain significant clinical dilemmas in the care of these patients.

Diagnosis and treatment of T-cell NHL

Current treatment paradigms for T-cell NHL are grossly inadequate, as indicated by the poor overall outcomes (Table 58.1). Despite a lack of randomized prospective trials for these rare diseases, the National Comprehensive Cancer Network (NCCN) has created treatment guidelines using available clinical data and the principles of evidence-based medicine [12]. These guidelines are acceptable as current standard of care and are modified in real time as new data emerges.

As per the NCCN guidelines, most histologic subtypes are treated similarly with the exception of a few clinically distinct subtypes: anaplastic lymphoma kinase (ALK)-positive ALCL, which has excellent prognosis compared with other subtypes; extra nodal NK/T-cell lymphoma nasal type, which is radiosensitive; and adult T-cell leukemia/lymphoma (ATLL), which is considered uniquely both by the NCCN and in this book (Chapter 69). At the time of presentation, patients undergo induction therapy that may be consolidated with high-dose therapy and autologous hematopoietic cell transplantation (autoHCT) if remission is achieved. At the time of relapse, a treatment plan is formulated based on whether or not the patient is a transplant candidate.

Disease characteristics and recommended treatments for the three major treatment groups follow.

PTCL-nos excluding CTCL and NK/T-cell lymphomas

PTCL-nos is a heterogeneous group of diseases that includes both nodal and extranodal presentations. The median age at presentation is less than 35 years for ALK^+ ALCL and over 60 years for most other subtypes, with a slight male predominance. T-cell lymphomas tend to present with a higher International Prognostic Index (IPI) and are more likely to have systemic involvement including bone marrow,

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Table 58.1 PTCL and CTCL frequency and survival

PTCL histology[a]	Frequency (%)	5-year OS (%)
Peripheral T-cell lymphoma – nos	25.9	32
Angioimmunoblastic	18.5	32
Extranodal NK/T-cell lymphoma	10.4	32
Nasal type NK/T		42
Extra-nasal type NK/T		9
Adult T-cell leukemia/lymphoma	9.6	14
Anaplastic large cell lymphoma, ALK^+	6.6	70
Anaplastic large cell lymphoma, ALK^-	5.5	49
Enteropathy-type T-cell lymphoma[c]	4.7	20
Primary cutaneous ALCL	1.7	90
Hepatosplenic T-cell lymphoma	1.4	7
Subcutaneous panniculitis-like lymphoma	0.9	64
CTCL histology[b]	**Frequency (%)**	**5-year DSS (%)**
Mycosis fungoides	61.2	88
Lymphomatoid papulosis	16.0	100
Primary cutaneous ALCL	9.9	95
Sézary syndrome	3.5	24
Primary cutaneous PTCL, unspecified	3.2	16
Primary cutaneous CD4^+ small/medium pleomorphic[d]	2.6	75
Subcutaneous panniculitis-like lymphoma	1.2	82
Primary cutaneous aggressive CD8^+ T-cell lymphoma[d]	0.9	18
Primary cutaneous γ/δ T-cell lymphoma[d]	0.9	Not reached
Primary cutaneous NK/T-cell lymphoma, nasal type	0.5	Not reached

OS, overall survival; DSS, disease-specific survival.

[a] All PTCL data are from 1314 patients from 22 worldwide centers on the International Peripheral T-cell and Natural Killer/T-cell Lymphoma Study [94].

[b] All CTCL data are from 1905 patients in the Dutch and Austrian Cutaneous Lymphoma Group Registry (of whom 1476 were T or NK cell) described in the WHO–EORTC classification for cutaneous lymphomas [5].

[c] The classification Enteropathy-type T-cell lymphoma used in this study is not the same as the WHO 2008 enteropathy-associated T-cell lymphoma (EATL), but was a generic category that included most T-cell lymphomas involving the intestines, including EATL and γ/δ T-cell lymphoma.

[d] Provisional classification.

skin, and central nervous system (CNS) compared with B-cell lymphomas. The broad range of disease subtypes and clinical behavior poses a challenge to studying treatment for these diseases [13].

Initial treatment with anthracycline-containing regimens shows favorable outcomes for patients with ALK^+ ALCL, but the data for other histologies are not convincing, and treatment on clinical trial is recommended if available. A retrospective analysis from a large multicenter study demonstrated no difference in outcomes using anthracycline- or non-anthracycline-containing regimens, with 5-year survival for most histologies remaining below 30% [4]. In contrast, a more recent retrospective report of 442 PTCL patients from North America shows that up front anthracycline-containing regimens are associated with an improved PFS (35% vs. 2%) and OS (60% vs. 2%), even in high risk patients [14]. A German retrospective analysis s did show an event-free survival (EFS) benefit for patients younger than 60 years of age who received etoposide-containing regimens for patients with ALCL [15]. Presently, CHOP [cyclophosphamide, hydroxydaunomycin (doxorubicin), vincristine (Oncovin), prednisone] or CHOP-like regimens remain the backbone of upfront therapy. Attempts to improve outcomes include the use of targeted agents in addition to CHOP-like regimens, such as denileukin diftitox, pralatrexate, alemtuzumab, and brentuximab vedotin. While some trials are ongoing, the findings so far have not been convincing enough to change treatment paradigms. Attempts to use non-CHOP-based regimens in the upfront setting have not been successful; for example, the PEGS (platinum, etoposide, gemcitabine, methylprednisolone) induction regimen results in a 2-year progression-free survival (PFS) of 12% and an overall survival (OS) of 30% [16]. It is possible that as new targeted agents are developed for T-cell lymphomas, novel combinations of these agents with or without cytotoxic chemotherapy will prove to be more effective than chemotherapy alone.

The NCCN guidelines recommend consideration of high-dose therapy and autoHCT as consolidation of first remission for eligible patients with most histologies of PTCL. The only exceptions are ALK^+ ALCL where upfront CHOP-like therapy results in a 5-year OS of over 80%.

NK/T-cell lymphoma nasal and extranasal type

NK/T-cell lymphomas are rare tumors primarily seen in Asia and South and Central America. They are categorized as extranodal NK/T-cell nasal type leukemia (ENKL) and aggressive NK-cell leukemia (ANKL). The median age at presentation is 43 years with a male predominance. These tumors exhibit angiodestructive behaviors, with a predilection for the nasal cavity and paranasal sinuses, but can present at other sites, including skin, gastrointestinal tract, testis, kidney, and upper respiratory tract. NK/T-cell lymphomas are associated with EBV, express P-glycoprotein and have an aggressive clinical course [17].

NK/T-cell tumors are extremely radiosensitive and where possible radiation should be part of the managment plan. For localized disease of the nasal type without any local tumor invasion or nodal involvement, radiation therapy given at doses of >50Gy provides improvement in survival [18]. Similarly, there is an improvement in EFS and OS when radiation therapy is given early or in the upfront setting, either concurrently or sequentially, with combination chemotherapy. For advanced-stage disease or extranasal variants, it is difficult to deliver localized radiation therapy, hence treatment relies primarily on chemotherapy; Recommended regimens incorporate agents like asparaginase, methotrexate, etoposide and ifosphamide [19]. Except for stage I disease, it is recommended that once a patient reaches a state of remission, HCT should be considered [17].

CTCL and Sézary syndrome

Cutaneous T-cell lymphomas have a more indolent clinical course characterized by multiple relapses and occasional spontaneous remissions, with the exception of primary cutaneous γ/δ T-cell lymphoma. The unique biologic features of this group of diseases include primarily skin manifestations, waxing and waning of lesions, relative chemoresistance of tumors, and an immunosuppressive disease state. The combined immunosuppression and breach of the skin barrier are associated with high infection rates, particularly with staphylococcal and streptococcal species, and successful treatment of these infections results in improvement of disease flares. The clinical course varies widely, but only 5–10% of patients develop more advanced aggressive diseases, such as transformed mycosis fungoides and Sézary syndrome.

Most patients experience prolonged survival with the use of only skin-directed therapies and may not require any systemic therapy for extended periods. Treatments can be combined to optimize therapeutic effects and minimize side effects and can be reinitiated at a later time if the disease progresses after an initial response. Supportive treatments play an important role in the overall management and include the use of emollients and ointments, antipruritic agents, and infection control/prevention. Owing to the indolent nature of the disease, maintenance treatment or tapering of the regimen plays an important role and should be considered in patients achieving remission. For most modalities, efficacy data are derived from small studies with a heterogeneous population of patients and an evolving staging system. Despite this, treatment recommendations have been developed by the NCCN [12], the United States Cutaneous Lymphoma Consortium [20], and the EORTC [21].

Skin-directed therapies alone are recommended for stages IA, IB, and IIA, but may also be used in addition to systemic therapies for relapsed or advanced disease. Topical therapies include steroids, nitrogen mustard (mechlorethamine), carmustine, imiquimod, and bexarotene. Light and radiation therapies include phototherapy [UVB, narrow-band UVB, PUVA (psoralen UVA irradiation)] and total skin electron beam therapy (TSEB) for generalized skin involvement. Local radiation can be used to treat ulcers, tumors, or transformed disease.

Systemic therapies are indicated for stages IIB and higher, folliculotropic subtype or transformed disease, as well as after failure of skin-directed therapies in earlier stage disease. These include the US Food and Drug Administration (FDA)-approved treatments bexarotene, denileukin diftitox, vorinostat, romidepsin, and extracorporeal photopheresis (ECP). Other therapies include interferon, bortezomib, alemtuzumab and single-agent chemotherapies including pralatrexate, methotrexate, liposomal doxorubicin and gemcitabine. Sézary Syndrome patients respond to ECP either alone or in combination, alemtuzumab, and romidepsin. Combination chemotherapies used for the treatment of aggressive lymphomas can be applied in advanced-stage CTCL or transformed disease. Brentuximab vedotin has shown promising activity in CD30$^+$ cutaneous lymphoproliferative disorders such as mycosis fungoides [22]. Allogeneic transplant is reserved for more advanced disease and is discussed below.

HCT for systemic or peripheral NK/T-cell lymphoma (PTCL)

Autologous HCT

High-dose therapy and autoHCT are used extensively to treat patients with PTCL, both in the relapsed setting and as first-line consolidation. AutoHCT in first remission was first explored due to low response rates to primary therapy and frequent early relapses. The data to support this is presented below.

Two randomized trials by the French Groupe d'Etude des Lymphomes de l'Adulte (GELA) in the late 1990s examined the role of autoHCT for aggressive NHLs including T-cell lymphomas. LNH-87-02 included 452 patients with high-risk lymphoma, 17% with T-cell phenotype, who achieved CR and were randomized to either a combination chemotherapy consolidation regimen or high-dose chemotherapy followed by autoHCT [23]. The autoHCT arm was shown to have a better outcome than the chemotherapy arm with an 8-year OS of 64% versus 49%. In LNH-93, 370 patients were enrolled and 23% had T-cell phenotype [24]. The authors compared standard induction consolidation at that time with a shortened induction and autoHCT consolidation. In this approach, the transplant arm yielded an inferior 5-year OS of 60% versus 46%. Notably, the T-cell phenotype was clearly associated with poor prognosis in this trial ($p = 0.009$). In 2004, the data from both GELA trials was pooled for all subtypes of NHL to determine whether there was a benefit to autoHCT in CR1 [25]. There were 51 (16%) patients with T-cell lymphomas including precursor T-cell lymphomas and ALK$^+$ ALCL, but no benefit was evident for autoHCT. It is difficult to apply these data to current practice, as these trials were not designed to look specifically at PTCL and included aggressive histologies such as lymphoblastic lymphoma. Also, the LNH-87 and LNH-93 trials used regimens no longer in standard practice, which may compromise their applicability to modern regimens.

Retrospective studies

Over the past 20 years, there have been over 24 retrospective studies with more than 1400 PTCL patients, assessing the role of autoHCT for the treatment of PTCL at various disease stages. These studies have an inherent selection bias in terms of favoring younger, healthier patients who are able to undergo transplant. In addition, most studies are heterogeneous in terms of patient populations, upfront treatment regimens, and conditioning regimens. Almost all studies exclude patients with leukemic variants of PTCL and are limited to otherwise healthy patients with median ages under 60 years. There are several excellent reviews that describe these studies in detail [26,27]. Data from the larger studies in recent years are summarized in Table 58.2.

Table 58.2 Recent retrospective PTCL trials for autoHCT

Ref.	*n*	Histologies	Disease status at transplant	OS	PFS
Beitinjaneh 2011 [32]	126	42 PTCL-nos, 47 ALCL, 7 ALK$^+$, 15 AITL, 6 NK/T, 6 HSTCL	CR/PR 84% 33% 1st line	39%(4 yr) For CR1 87% (4 yr)	30% (4 yr) For CR1 67% (4 yr)
Nademanee 2011 [34]	67	30 ALCL 16% ALK+, 30 PTCL-nos, 7 AITL	CR/PR 70% 18% 1st line	54% (5 yr)	40% (5 yr)
Numata 2010 [95]	39	23% ALCL ALK$^+$	CR/PR 82% 59% 1st line	62% (5 yr) CR1/PR1 72% (5 yr)	61% (5 yr) CR1/PR1 73% (5 yr)
Kyriakou 2008 [96]	146	AITL	CR/PR 84%	67% (2 yr)	53% (2 yr)
Yang 2009 [97]	64	PTCL-nos	CR/PR 91% 44% 1st line	53% (3 yr)	44% (3 yr)
Nickelsen 2008 [33]	424	9 ALCL ALK$^-$, ALK$^+$ 19, 176 PTCL-nos, 120 AITL, 30 aggressive variants	CR/PR 87%	50% (3 yr)	62% (3 yr)
Chen 2008 [35]	53	34% ALK$^+$ ALCL	CR/PR 81% 28% 1st line	48% (5 yr) CR1/PR1 87%	25% (5 yr) CR1/PR1 51%
Feyler 2007 [98]	64	30 PTCL-nos, 20 ALCL, 14 other/unknown	CR/PR 77%	53% (3 yr) CR1 59% (2 yr)	50% (3 yr) CR1 62% (2 yr)
Smith 2007 [99]	32	66% ALK$^+$ ALCL	CR/PR 74%, 81% in 2nd line	34%	18%

n, Number of patients; PTCL-nos, peripheral T-cell lymphoma not otherwise specified; ALCL, anaplastic large-cell lymphoma; ALK, anaplastic lymphoma kinase; AITL, angioimmunoblastic T-cell lymphoma; NK/T, NK/T-cell lymphoma; HSTCL, hepatosplenic T-cell lymphoma; CR, complete response; PR, partial response; OS, overall survival; PFS, progression-free survival; yr, year(s).

The advent of the Revised European–American Lymphoma (REAL) and later the WHO classifications provided the impetus to look at the autoHCT experience in NHLs and tease out distinguishing characteristics for the T-cell immunophenotype. Vose et al. [28] first described outcomes for autoHCT performed in the relapsed setting for T-cell phenotype and compared them with aggressive B-cell lymphoma outcomes. In this study, there were 17 T-cell and 24 B-cell patients based on immunophenotype, and the authors found no difference in outcomes in terms of OS and EFS; the 2-year OS was 30–35% for both groups, thus establishing autoHCT as an acceptable treatment option for T-cell NHL. Fanin et al. [29] examined outcomes in 64 patients with CD30$^+$ T- and null-cell ALCL based on the REAL classification, and concluded that the 15 patients transplanted in CR1 had >90% long-term survival. This was the first suggestion that autoHCT might provide cure if performed in CR1 for certain PTCL histologies. In both studies, patients were young, with median ages of 25 and 33 years, in keeping with the practice patterns of that time.

In 2003, Rodriguez et al. [30] analyzed data from 115 PTCL patients (by REAL classification) in the GEL/TAMO (Grupo Español de Linfomas/Trasplante Autólogo de Médula Ósea) database who underwent autoHCT. At 5 years, the median OS was 56% and time-to-treatment failure was 51%. However, the 5-year OS was 80% for those transplanted in CR1 versus 50% for non-CR1, and 0% for patients with refractory disease. Longer survival was associated with age <41 years, transplantation in CR, or chemosensitive disease. Within the same group, Rodriguez et al. [31] reported separately on a cohort of 35 patients who did not achieve remission after anthracycline-based induction therapy. Thirty-one of these patients underwent transplantation and 23 (66%) achieved CR. The 5-year OS was 37% and disease-free survival (DFS) was 55%, indicating that one-third of patients with PTCL who failed to respond to initial chemotherapy could be salvaged with autoHCT. This study also emphasized the superior outcomes obtained from consolidation in first remission.

The largest single-center study of 126 PTCL autoHCT patients was reported by the M.D. Anderson Research Center [32] at the 2011 meeting of the American Society of Clinical Oncology. At the time of transplant, 33% of patients were in CR1, 51% had chemosensitive relapse, and 16% were refractory to therapy. The median follow-up was 39 months, with 4-year OS of 39% and PFS of 30%. The best outcomes were seen in patients transplanted in CR1, with a 4-year OS of 87% and a PFS of 67%. In comparison, the 4-year OS and PFS for patients with chemosensitive relapse was 39 and 36% and for refractory disease 24 and 15%, respectively. This study indicates that although it is possible to achieve long-term remission for chemosensitive relapsed disease with autoHCT in PTCL, the best outcomes are seen for consolidation of first state of remission. The largest registry study to date from the European Group for Blood and Marrow Transplantation (EBMT) was presented as an abstract at the American Society of Hematology meeting in 2008. Nickelsen et al. [33] reported on 424 patients undergoing autoHCT for PTCL with an OS of 62% and a DFS of 50% at a median of 3 years. This study also supported the assertion that the best outcomes following autoHCT are in CR1.

The most common conditioning regimens used for autoHCT in PTCL patients are: BEAM (BCNU, etoposide, Ara-C, melphalan), BEAC (BCNU, etoposide, Ara-C, cyclophosphamide), CBV (cyclophosphamide, BCNU, etoposide), and busulfan (BU)/cyclophosphamide (CY). A fair number of patients have also received total body irradiation (TBI)-based regimens, usually in combination with cyclophosphamide or etoposide or both. The City of Hope group compared the outcomes of 41 patients treated with TBI-based regimens with those of 26 patients with non-radiation-based regimens and reported no differences [34]. The Stanford group questioned the effect of T-cell depletion in a cohort of 53 patients, of whom 86% received a T-cell-depleted graft, but found no differences in outcome [35]. Currently there are no specific recommendations for the choice of a conditioning regimen for this group of patients. The highest transplant-related mortality (TRM) at 11% was reported by Jantunen et al. in a small study of 37 patients [36] that included the uncommon and aggressive EATL. In other sudies, TRM rates range from 3.7% [32] to 7.5% [37]. No information is available on long-term complications as the maximum follow-up is 5 years.

Multiple studies have focused on specific histologies of T-cell NHL. ALCL has been reported by several groups to have superior outcomes compared with non-ALCL histologies [29,33,36–38]. An EBMT Registry study [39] focused on the outcomes of patients with AITL, another common nodal PTCL. In this cohort of 146 patients, about 70% were transplanted in CR1. The 2-year OS and PFS were 67 and 53%, respectively. Patients who were transplanted in CR1 demonstrated a 4-year PFS of 56%, versus 30% for non-CR1 and 23% for relapsed disease. Hence this study supports the use of autoHCT for patients with AITL in CR1. NK/T-cell lymphoma is more common in Asia, and Hwang et al. reported on the use of autoHCT in 10 patients with NK/ T-cell lymphoma [40]. For patients undergoing stem-cell transplant in CR/PR (partial remission), the 5-year OS was 53% compared with only 20% for those not in CR/PR.

Other than transplantation in CR1, there is no real consensus on prognostic factors that affect outcomes after autoHCT in PTCL. Retrospective studies suggest that autoHCT is feasible in patients with PTCL and the 5-year median OS varies from 34 to 70% and EFS from 30 to 61%. However, if transplants are performed in first remission reports show up to 80% 5-year OS and 67% EFS. In most studies, the outcome for refractory or chemoresistant disease remains dismal, with fewer than 15% long-term survivors. The use of autoHCT can salvage about one-third of patients with relapsed chemosensitive disease, again with best outcomes for ALCL, particularly ALK$^+$.

Prospective trials

There is now a substantial body of literature supporting autoHCT in CR1 for PTCL, albeit lacking confirmation by randomized trials. The NCCN guidelines for NHL recommend consideration of autoHCT for patients with PTCL who achieve a CR or PR after primary therapy and are eligible based on age and comorbidity [12]. Exceptions to this approach include ALK$^+$ ALCL, CTCL, and very aggressive T-cell lymphomas (leukemic presentation). The overall prognosis of the ALK$^+$ ALCL subtype is substantially better than those of other T-cell lymphomas, with overall response rates to CHOP-based induction therapy of >90% and a 5-year OS of 70% [41]. AutoHCT in first CR has shown excellent results in ALK$^+$ ALCL patients, but superiority to standard chemotherapy is unproven. Additionally, newer agents targeting CD30 and ALK can provide excellent responses in the relapsed setting [42], thus eliminating the need for aggressive approaches in first CR. Very aggressive and leukemic variants of PTCL, including HSTCL,

Table 58.3 Prospective PTCL trials for autoHCT

Ref.	*n*	Histologies	Induction therapy	Conditioning regimen	Patients transplanted (%)	OS	PFS
d'Amore 2012 [45]	160	Includes ALK$^+$ ALCL, 1° leukemic	Age ≤60 CHOEP 14; age >60 CHOP 14	BEAM/BEAC	72	51% (5 yr)-	44% (5 yr)
Reimer 2009 [46]	83	No ALK$^+$ ALCL	CHOP × 4–6 cycles	TBI/CY	66	48% (3 yr)	36% (3 yr)
Corradini 2006 [47]	62	Includes ALK$^+$ ALCL (30%)	APO alternating with DHAP, or MACOP-B followed by mitoxantrone/Ara-C	BEAM	71	34% (12 yr)	30% (12 yr)
Ahn 2011 [49]	46	25% had NK/T No ALK$^+$ ALCL	CHOP, CHOP-like or non-anthracycline	BU/CY/VP-16	66	57% (5 yr)	55% (5 yr)
Mercadal 2008 [48]	41	No ALK$^+$ ALCL	High-dose CHOP alternating with ESHAP	BEAM/BEAC	41	39% (4 yr)	30% (4 yr)
Rodriguez 2007 [50]	26	No ALK$^+$ ALCL	Mega-CHOP, if PR or SD after 3 cycles, got IFE	BEAM	73	73% (3 yr)	53% (3 yr)

n, Number of patients; ALK, anaplastic lymphoma kinase; ALCL, anaplastic large-cell lymphoma; NK/T, NK/T-cell lymphoma; CHOEP, cyclophosphamide, doxorubicin, vincristine, etoposide, prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; APO, doxorubicin, vincristine, prednisone, DHAP, cisplatin, Ara-C, prednisone; MACOP-B, methotrexate with leucovorin rescue, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin; BEAM, BCNU, etoposide, Ara-C, melphalan; BEAC, BCNU, etoposide, Ara--, cyclophosphamide; TBI, total body irradiation; BU, busulfan, CY, cyclophosphamide; VP-16, etoposide; CR, complete response; PR, partial response; SD, stable disease; OS, overall survival; PFS, progression-free survival; yr, year(s).

HTLV-1-associated ATLL, T-cell prolymphocytic leukemia (T-PLL), and primary cutaneous γ/δ T-cell lymphoma, may not benefit from upfront autoHCT. These diseases have a very aggressive clinical course due to inherent chemoresistance and patients tend to experience multiple failures of primary therapy; hence autoHCT approaches have generally not produced long-term remissions in these patients [43,44]. Long-term remissions and improved survival have been seen in patients with these highly aggressive variants following allogeneic transplantation in first remission.

To date, six prospective trials have reported on the use of autoHCT performed in first CR, primarily for nodal PTCL. In all trials described in Table 58.3, patients were induced with chemotherapy, and those who achieved a CR or PR and maintained transplant eligibility received high-dose therapy and autoHCT.

The largest study in this category is a prospective multicenter trial by the Nordic Lymphoma group that took a dose-dense approach to the treatment of PTCL followed by autoHCT [45]. This study of 160 patients has a long median follow up of 5 years. The histological subtypes were distributed as follows: PTCL-nos ($n=62$), ALK$^-$ ALCL ($n=31$), AITL ($n=30$), EATL ($n=21$), panniculitis-like T-cell lymphoma ($n=6$) T/NK nasal-type ($n=5$), and HSTCL ($n=5$). Patients aged 60 years or younger received primary induction therapy with CHOEP-14 (doxorubicin, vincristine, etoposide, prednisone), and those over age 60 years received CHOP-14 (no etoposide). Of the 131 responders to initial therapy, 114 underwent BEAM/BEAC conditioning and autoHCT, after which 90 patients were in CR and nine in PR, at 3 months post-transplant. In the post-transplant setting there were 39 relapses, with 28 in the first 2 years and the remainder as late as 71 months post-transplant. In an analysis of the entire cohort, with a median follow-up of 60 months, the TRM was 4%, OS 51% and DFS 44%. Early failures occurred in 26% of patients. In subset analysis, the best outcomes were seen in patients with ALK$^-$ ALCL with a 5-year OS of 70% and a PFS of 61%; AITL, OS 52%, PFS 49%; PTCL-nos, OS 47%, PFS 38%; and EATL, OS 48%, DFS 38% (Figure 58.1). Female gender and ALCL histology had positive prognostic value, and IPI had a predictive value for OS in AITL and for PFS in AITL and PTCL-nos. Bone marrow involvement and increasing age were negative prognostic factors.

Several other centers have reported similar studies, as detailed in Table 58.3. In the various groups, 41–73% of the PTCL patients were able to maintain eligibility for, and receive, autoHCT [46–50]. The remaining patients were taken off study owing to progressive or unresponsive disease, inability to mobilize sufficient stem cells, or comorbidities that prohibited autoHCT. Follow-up was typically 3–5 years, with OS ranging from 34 to 73% and PFS from 30 to 53%. The best results were from studies including ALCL patients [45,51]. The Korean cohort [49] included 25% of patients with NK/T-cell lymphoma and resulted in 5-year OS and EFS of 57 and 55%, respectively The induction regimens varied between studies, so it is difficult to make specific recommendations. The TRM was below 5% for all, which is reasonable in this relatively young and healthy population of patients. Most studies used chemotherapy-based conditioning and all excluded patients with CNS involvement, and also leukemic variants of PTCL and ATLL.

These studies evaluate the efficacy of autoHCT as consolidation therapy for first remission and compar with published data for chemotherapy alone in PTCL. A large population-based study from Sweden has analyzed the outcomes of over 755 patients treated over a 9-year period, including the patients from the Nordic Lymphoma study group (discussed previously [45]). In a subgroup analysis of 252 transplant eligible patients, the OS and PFS were significantly higher in the 128 patients transplanted in CR1 vs. the 124 who did not receive an autoHCT in CR1 [52]. A meta-analysis of 21 studies evaluating the effects of high dose therapy and autoHCT as first-line treatment in PTCL showed a trend towards improved outcomes compared to non-transplant therapies alone [53]; but there was not sufficient data to make firm conclusions. In these studies, a significant fraction of patients failed initial therapy prior to receiving autoHCT, underscoring the need for more aggressive and targeted therapies to improve initial response rate and reduce the number of early relapses. Late treatment failure patterns support the need to define better those patients at risk for late relapse through biomarker and minimal residual disease (MRD) evaluation. This could define a subset of patients who would benefit from maintenance therapy.

Allogeneic HCT for PTCL

Allogeneic HCT (alloHCT) has been used extensively to salvage patients with NHL and is generally considered to be curative therapy as it provides long-term remission with eventual plateaus in OS and PFS. However, alloHCT is associated with a high risk of complications related to continued immunosuppression, graft-versus-host

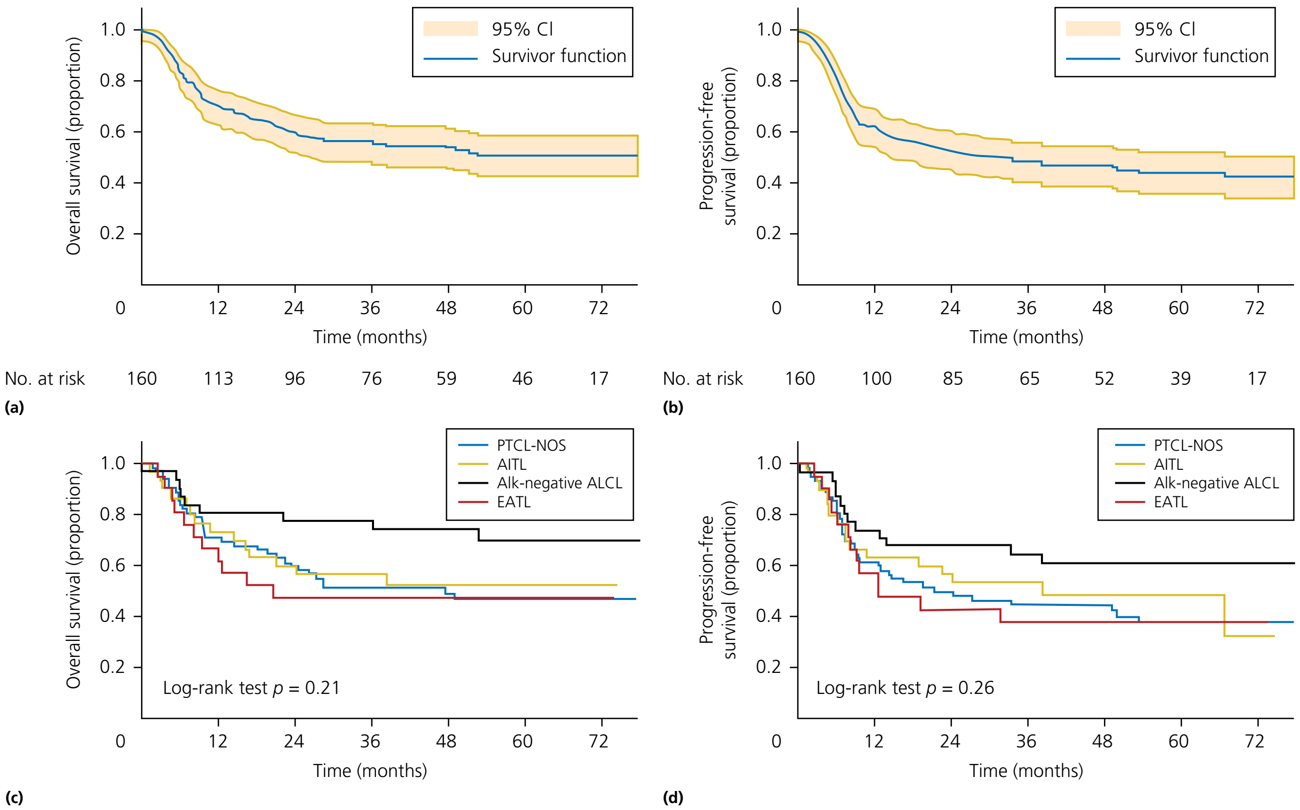

Figure 58.1 Kaplan–Meier estimates of (a) overall survival (OS) and (b) progression-free survival (PFS) for the entire NLG-T-01 cohort and (c) OS and (d) PFS for the four largest histologic subtypes. PTCL-NOS, peripheral T cell lymphoma, not otherwise specified; Alk-negative ALCL, anaplastic lymphoma kinase-negative anaplastic large-cell lymphoma; AITL, angioimmunoblastic T-cell lymphoma; EATL, enteropathy-associated T-cell lymphoma. Source: d'Amore et al. 2012 [45]. Reproduced with permission of the American Society of Clinical Oncology.

disease (GVHD), and the long-term toxicities of conditioning regimens. The indications for this therapy are highly dependent on the availability of a donor, comorbid conditions of the patient, and previous treatments. The chance of cure or long-term remission has to be weighed against the risk of morbidity and mortality associated with the transplant procedure. For PTCL, the rationale for allogeneic transplants has been borrowed from the paradigm of treating aggressive- and intermediate-grade B-cell lymphomas with the intent of invoking a graft-versus-lymphoma (GVL) effect to provide long-term disease control. AlloHCT has been used in the setting of relapsed or refractory T-cell NHL, although increasingly transplant physicians are using this modality in the upfront setting for particularly aggressive histologies such as the γ/δ T-cell lymphomas and extra-nasal NK/T-cell lymphomas [54].

A French study has reported on the feasibility and toxicity of upfront alloHCT in 49 newly diagnosed PTCL patients. Forty-two patients had an HLA-matched donor, but only 29 proceeded to transplant after achieving a remission with CHOP. For transplanted patients the 1- and 2-year OS was 76% and 72%, respectively. TRM after 1 year was 8%. Of note, the PFS of the patients who did not undergo a transplant was less than 30% [55]. Most other published studies describe outcomes for treatment of relapsed disease. There is one prospective phase II study of 17 PTCL patients with relapsed disease reported by Corradini et al. [56]. All patients received similar debulking chemotherapy with cisplatin, dexamethasone, and cytarabine for 4–6 cycles and underwent reduced-intensity allogeneic transplant following conditioning with fludarabine, thioTEPA (*N*-triethylenethiophosphoramide), and cyclophosphamide. This study was also the first to report on the use of reduced-intensity conditioning (RIC) to reduce high TRM (previously reported at ~30%). With this particular approach, after a median follow-up of 28 months, the NRM was only 6% and the 3-year OS and DFS were 81 and 62%, respectively. This study established that RIC could be used in PTCL with good long-term outcomes implicating a graft-versus-T-cell lymphoma effect. The patients in this study were heavily pretreated, including 8/17 patients with a prior autoHCT; however, 15/17 were chemosensitive, indicating that the adverse outcomes associated with T-cell lymphomas can be overcome by alloHCT, at least in chemosensitive patients.

All remaining studies are retrospective and consist of patients with non-uniform histologies, conditioning regimens, and GVHD prophylaxis. As these diseases are rare, initial reports consisted of small case series, but in the last 10 years international institutions and lymphoma groups have made a concentrated effort to describe the experience of allogeneic stem cell transplantation in PTCL. AlloHCT studies for PTCL are listed in Table 58.4. Most studies are focused on the common nodal histologic subtypes of PTCL, namely PTCL-nos, AITL, and ALCL, and exclude CTCL, HTLV-1-associated ATLL, and patients with CNS involvement. The role of transplantation in ATLL is described elsewhere (Chapter 69).

The largest PTCL alloHCT study to date included 77 patients from multiple centers in France and was reported by Le Gouill et al. [57]. This study investigated differences in the common histologic subtypes of PTCL in the context of allogeneic stem cell transplant. All patients had relapsed disease and about 25% of them had failed a prior transplant, indicating the heavily pretreated

Table 58.4 Allogeneic HCT for PTCL

Ref.	*n*	Histologies	Disease status at transplant	Regimen/graft source	GVHD	Mortality/ relapse	Survival outcomes
Loirat 2014 [55] Single Center	29	PTCL mixed	CR1 42%	RIC 83% SIB 45%, MUD 28% Hap 11%, CBU 17%	aGVHD 42% cGVHD 28%	TRM 7% (1 yr)	OS 72% (2 yr) PFS 65% (2 yr)
Goldberg 2012 [63] Single-center	34	PTCL mixed CTCL 9%	CR 47% Prior autoHCT 12%	MAC 62%, TBI-based 47%/ PBSC 71%, SIB 53%	aGVHD 41% cGVHD 25%	TRM 18% RPR 33% (6 mo)	OS 61% (2 yr) PFS 50% (2 yr)
Dodero 2012 [58] Multi-center	52	PTCL mixed	CR 40% Prior autoHCT 52%	All RIC PBSC 79%, SIB 63%, MUD 25%, Hap 12% 12 DLIs	aGVHD 22% cGVHD 27%	NRM 12% (5 yr) RPR 49% (5 yr)	OS 50% (5 yr) PFS 40% (5 yr) 8/12 DLI responses
Jacobsen 2011 [64] Single-center	52	PTCL mixed CTCL 15%	CR 42% Prior autoHCT 21%	MAC 60%, TBI-based 60% PBSC 73%, SIB 50%	aGVHD 21% cGVHD 37%	NRM 27% (3 yr) RPR 47% (3 yr)	OS 41% (3 yr) PFS 30% (3 yr)
Shustov 2010 [61] Single-center	17	PTCL mixed CTCL 6%, PLL 18%	CR 47% Prior autoHCT 41%	All RIC, TBI/Flu PBSC 100%, SIB 41%	aGVHD 65% cGVHD Ext 53%	NRM 19% (3 yr) RPR 26% (3 yr)	OS 59% (3 yr) PFS 53% (3 yr)
Zain 2011 [60] Single-center	38	PTCL mixed CTCL 35%	CR 18% Prior autoHCT 3%	MAC 35%, TBI-based 32% PBSC 84%, SIB 68%	aGVHD 51% cGVHD 82%	NRM 29% (5 yr) RPR 24% (5 yr)	OS 54% (5 yr) PFS 46% (5 yr)
Delioukina 2012 [62] Single-center	27	PTCL mixed CTCL 41%	CR 22% Prior autoHCT 4%	RIC only 89% PBSC, SIB 56%	aGVHD 48% cGVHD 71%	NRM 25% (2 yr) RPR 30% (2 yr)	OS 55% (2 yr) PFS 47% (2 yr)
Kyriakou 2009 [39] Multi-center	45	AILD only	CR 27% Prior autoHCT 33%	MAC 56%, TBI-based 44% PBSC 91%, SIB 60%	aGVHD 20% cGVHD 54%	NRM 25% (1 yr) RPR 20% (3 yr)	OS 64% (3 yr) PFS 53% (3 yr)
Le Gouill 2008 [57] Multi-center	77	PTCL mixed	CR 40% Prior autoHCT 25%	MAC 74%, TBI-based 66% PBSC 31%, SIB 78% 2 DLIs	Grade III/IV aGVHD 21%	TRM 34% (5 yr)	OS 57% (5 yr) EFS 53% (5 yr) 2/2 DLI responses
Hamadani 2008 [66] Single-center	14	PTCL mixed	CR 21% (no CR1) Prior autoHCT 14%	MAC 57% (no TBI), PBSC 86%, SIB 64% 4 DLIs	aGVHD 50 % cGVHD 50 %	NRM 57% (3 yr) RPR 69% (3 yr)	OS 35% (3 yr) PFS 31% (3 yr) 2/4 DLIs responses
de Lavallade 2008 [100]	7	PTCL nodal	CR 43% Prior autoHCT 86%	All RIC, SIB 100% 1 DLI	aGVHD 43% cGVHD 67%	TRM 0% RPR 29%	7 surviving, 6 in remission at 40 mo 1/1 DLI response
Woessmann 2006 [101] Multi-center	20	ALCL in pediatrics	CR 60% (no CR1) Prior autoHCT 25%	MAC 95%, TBI-based 75% PBSC 60%, SIB 40%, MUD 40%, Hap 20%	aGVHD 45 % cGVHD Ext 20%	TRM 15 % (3 yr) RPR 10% (3 yr)	EFS 75% (3 yr)
Murashige 2005 [65] Multi-center	28	NK/T-cell	CR 29% Prior autoHCT 25%	MAC 82%, TBI-based 71% PBSC 71%, SIB 78%	aGVHD 46% cGVHD 32%	TRM 29% RPR 29%	OS 40% (2 yr) PFS 34% (2 yr)
Suzuki 2006 [59] Multi-center	15	NK/T-cell	CR 40%	MAC 100%, TBI-based 100% PBSC 33%, SIB 93%		TRM 48% (4 yr) RPR 17% (4 yr)	OS 29% (4 yr)
Wulf 2005 [102] Single=center	10	PTCL mixed	CR 10% Prior autoHCT 20%	RIC preceded by alemtuzumab 100% PBSC 100%, SIB 40%	aGVHD 50% cGVHD 56%		8 surviving, 7 in remission at 7 mo
Corradini 2004 [56] Phase II multi-center	17	PTCL nodal	CR 12% Prior autoHCT 47%	RIC 100% SIB 94 % 2 DLIs	aGVHD 35% cGVHD 41%%	NRM 6% (2 yr)	OS 81% DFS 62% (3 yr) 2/2 DLI responses

n, Number of patients; PTCL, peripheral T-cell lymphoma; ALCL, anaplastic large cell lymphoma; AILD, angioimmunoblastic lymphoma; NK/T-cell, natural killer/T-cell lymphoma; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; TBI, total body irradiation; Hap, haploidentical donor; CBU, cord blood unit; PBSC, peripheral blood stem cells; SIB, sibling donor; DLI, donor lymphocyte infusion; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; TRM, transplant-related mortality; NRM, non-relapse mortality; RPR, relapse progression rate; CR, complete response; OS, overall survival; PFS progression-free survival; EFS, event-free survival; mo, months; yr, year(s).

nature of the population. The histologic distribution was as follows: 27 ALCL, 27 PTCL-nos, 11 AITL, and 12 others including aggressive histologies; 77% of the patients had chemosensitive disease at the time of transplant and 67% received ablative conditioning regimens. TRM was high at 33%, and the 5-year OS and DFS were 57 and 53%, respectively. This is the only study demonstrating differences in outcomes based on histology. At 5 years, OS and PFS were 80 and 80% for AITL, 63 and 58% for PTCL-nos, 55 and 48% for ALCL, and 33% for other histologies (Figure 58.2). Both the OS and PFS curves reached a plateau after 20 months. Grade III and IV acute GVHD and chemoresistant disease were negative prognostic factors by multivariate analysis. The 5-year OS of 29% for chemoresistant patients is encouraging and supports a GVL effect.

Most published studies have reported similar numbers at 3–5 years of follow-up, with OS between 50 and 70% and PFS up to 60%, with a plateau in relapse incidence within the first 2 years. Chemosensitive disease at the time of transplant results in better OS and PFS [39,57–59], indicating that achievement of disease control before allogeneic transplant is crucial to outcome. In one of the larger studies of 52 PTCL patients, Dodero et al. reported superior 5-year relapse for patients allografted in CR compared with PR (24% vs. 54%), and for those with chemosensitive compared with refractory disease (40% vs. 77%) [58]. From a City of Hope study, the 5-year OS for patients in CR/PR compared with those with active disease was 72.9% versus 43.2% [60].

The effect of conditioning regimen is unclear, but there has been an increasing shift towards RIC in recent years due to the high TRM reported in earlier studies. TRM after alloHCT for PTCL has been reported to be as high as 30%, likely due to advanced-stage disease and poor performance status. Several centers have reported on the results of RIC [61,62] and there appears to be a decrease in TRM to around 19–20%. A study from City of Hope compared outcomes for RIC and fully ablative conditioning and found no differences in OS and DFS [60].

The Memorial Sloan Kettering Cancer center used T-cell depletion in 47 patients, resulting in a 25% incidence of chronic GVHD (compared with 60% and above in other series) and a TRM of 18% at 45 months [63]. They also examined pre-transplant Ki-67 immunohistochemistry as a biological marker in PTCL and found that Ki-67 nuclear expression in ≤25% cells, indicating a more indolent histology, correlated with better OS. Differences in OS and PFS for various histologies were reported for the 115 patients in the study by Le Gouill et al. mentioned above. The City of Hope series [60,62] compared CTCL and PTCL histologies and found similar outcomes for allogeneic transplants in the two groups. The EBMT Registry reported outcomes for AITL alone [39], with a 3-year OS of 64% and PFS of 53%, similar to those seen in other studies. Jacobsen et al. reported no difference in outcomes when histologies were stratified as nodal versus extranodal [64].The series from Japan focused on NK/T-cell lymphoma, based on its high prevalence in Japan, and reported inferior 2-year OS and PFS of 30–40% compared with 70% for PTCL from the same registry [59]. Nonetheless, patients undergoing either autoHCT or alloHCT for NK/T-cell lymphoma showed better outcomes than those without HCT [59,65].

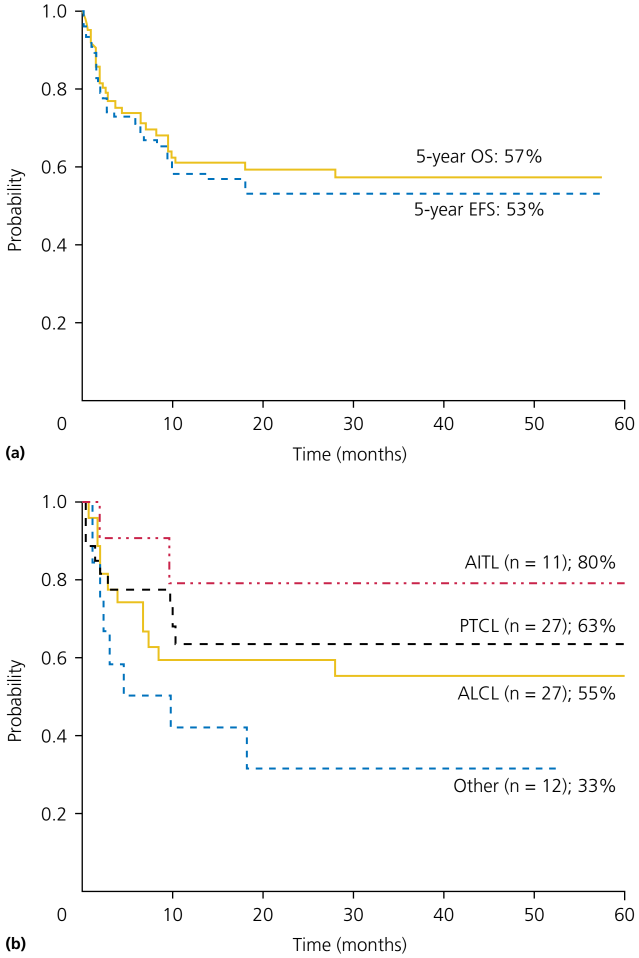

Figure 58.2 (a) Overall survival (OS) and event-free survival (EFS) after transplantation for all 77 patients. (b) Five-year OS according to histopathologic subtypes. PTCL, peripheral T-cell lymphoma; AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large-cell lymphoma; other histopathologic subtypes are classified as “other.” Source: Le Gouill et al. 2008 [57]. Reproduced with permission of the American Society of Clinical Oncology.

The use of donor lymphocyte infusion (DLI) to induce remission for relapsed disease after allogeneic transplant is considered validation of a graft-versus-disease effect and there are reports in the literature documenting response to DLI for PTCL. The largest experience is from Dodero et al., where 8/12 patients with documented relapse responded to DLI and achieved a state of remission [58]. There were two patients in the Le Gouill series, two in the Corradini series [56], two in the Hamadani series [66], and one of two in the Goldberg series [63] who responded to DLI after disease relapse and had long remissions.

In conclusion, the use of alloHCT, at least in some of the common nodal histologies of PTCL, can provide long-term disease control for patients with chemosensitive disease, with most relapses occurring within the first 2 years. The risk of acute and chronic GVHD remains high, but TRM can be reduced using RIC to minimize regimen-related toxicities. A GVL effect is implicated by the success of RIC regimens and use of post-transplantation DLI to induce remission. However, disease chemosensitivity and optimal disease control are crucial for the best outcomes with this modality. The use of alloHCT in the upfront setting is still being explored and early data is encouraging. Since most relapses occur within the first 2 years after transplant, consideration should be given to maintenance therapies using targeted agents to reduce residual disease and improve outcomes following allogeneic transplantation for PTCL.

HCT for cutaneous T-cell lymphoma (CTCL)

Autologous HCT

For chemosensitive relapsed aggressive lymphomas, autoHCT is the standard of care. CTCL outcomes for this approach have paralleled those of other low-grade lymphomas, resulting in frequent relapses. Even though data are limited, a few small series have shown high response rates, but with early relapses, typically within a year (Table 58.5). These series reports of up to 10 patients indicate that high-dose therapy and TBI-based regimens can be delivered to CTCL patients without an increased incidence of transplant-related complications. T-cell depletion or CD34 selection do not appear to improve relapse incidence [67–73]. The use of autoHCT has not shown promising results in the relapsed setting of advanced CTCL, due mainly to the inherent chemoresistance of these tumor types.

Allogeneic HCT

In an attempt to improve the outcomes of high-dose therapy and provide better disease control, alloHCT has been performed in select patients with advanced disease, invoking the GVL effect. Owing to the general immunosuppressive state and the broken skin

Table 58.5 Autologous HCT for CTCL

Ref.	*n*	Regimen	TSEB/TBI	Response	Relapse	Survival
Ingen-Housz-Oro 2004 [68]	10	Varied	TBI based	7/10 CR	7/10, 6 in ≤4 mo	Not reported
Olavarria 2001 [70]	9	T-depleted/CD34$^+$ selected	4 TSEB/2 TBI	100%	7/9 in 7 mo	Median 11 mo
Ferra 1999 [73]	1	CY/CAR	TBI	CR	2 mo	22 mo
Sterling 1995 [72]	1	CY/prednisone	TBI	CR	3 mo	15 mo
Moreau 1994 [69]	2	2 CY/CAR/VP16	TBI	2 CR	None	44 and 51 mo
Bigler 1991 [67]	6	Varied	4 TSEB/3 TBI	5/6 CR	3/6 in 100 days	Median 21 mo

n, Number of patients; CY, cyclophosphamide; CAR, carmustine; VP16, etoposide; CIS, cisplatin; TSEB, total skin electron beam therapy; TBI, total body irradiation; CR, complete response; mo, months; yr, year(s).

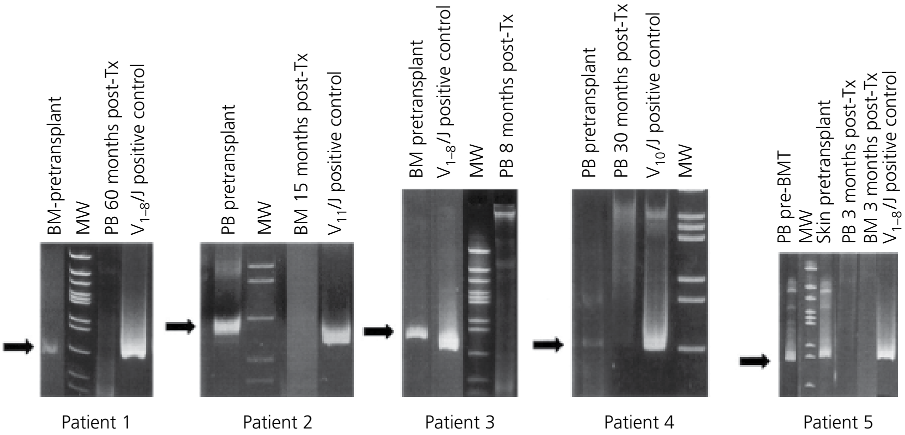

Figure 58.3 Clonal T *TCRγ* gene rearrangements are eradicated after alloHTC. Pre-therapy bone marrow (BM), peripheral blood (PB), and skin (patient 5) demonstrated TCRγ rearrangements while all post-therapy (Tx) specimens are negative. Arrows indicate the size of the expected positive polymerase chain reaction product. MW, molecular weight marker. Source: Molina et al. 2005 [77]. Reproduced with permission of the American Society of Clinical Oncology.

barrier, infectious complications remain a major challenge in the management of CTCL patients. Even though early studies were small and retrospective, complication rates were acceptable, and supported an alloHCT approach [74–81].

To date there are approximately 30 articles in PubMed concerning the use of allogeneic transplant in mycosis fungoides or Sézary syndrome. Earlier studies were case reports of 1–3 patients with considerable heterogeneity in disease status, source of stem cells, conditioning regimens, and so on. These studies indicate that in spite of a broken skin barrier and a depressed immune system, allogeneic transplantation can be performed in CTCL patients with no apparent disease-specific complications. Despite the salvage setting, long-term disease control and survival can be demonstrated based on clearance of clinical symptoms and also molecular evidence of disease by TCR gene rearrangements [77–79,82–85] (Figure 58.3). GVL effects are evident from the successful use of RIC regimens, remission of relapse after withdrawal of immunosuppression and donor lymphocyte infusions [79], and an association of disease remission with GVHD [83]. A summary of these case reports is presented in Table 58.6.

With advances in the field of allogeneic transplants, there are now data regarding the use of cord blood as a stem-cell source for these patients [86,87]. A review of these cases in detail indicates that an allogeneic transplant can achieve a CR even in the most refractory cases, as shown in Figure 58.4, and RIC appears to be just as effective and less toxic. There may be increased skin toxicity and flaring associated with TBI-containing regimens. Disease recurrence in the skin can occur after alloHCT, but seems to respond well to adjustment of immunosuppression, DLI infusion, or mild treatments for the skin. There appear to be few blood or systemic recurrences. The numbers are too small to conclude whether recurrences are more common after RIC as opposed to fully ablative conditioning, but there seems to be a trend among physicians towards the use of RIC regimens.

There is at least one reported case of autoHCT followed by alloHCT from a human leukocyte antigen (HLA)-matched sibling for a CD8$^+$ mycosis fungoides patient with tumor stage disease and blood involvement, although it was not performed in a planned tandem fashion [88]. The patient was conditioned with alemtuzumab, fludarabine, and cyclophosphamide, with resolution of disease in the lungs and skin by day 17. A recurrence at day 18 was treated with withdrawal of immunosuppression with good results, although the patient died from sepsis at day 256.

Based on these case reports, several transplant centers have performed retrospective database analyses for CTCL patients who have undergone allogeneic transplantation, in an attempt to identify factors that may refine the use of alloHCT in the

Table 58.6 Retrospective Reports on Allogeneic HCT for CTCL

Ref.	*n*	Disease stage at transplant	Regimen/donor	GVHD	Survival
Lechwicz 2014 [89] CIBMTR	129	Relapsed/ refractory	RIC 64%, MAC 36%	aGVHD 41% cGVHD 43% at 2 yrs	5-yr OS 32% 5-yr PFS 17% 2-yr NRM 22%
Masson 2013 [106]	37	Advanced stage	RIC 68%, MAC 32% rATG 43%	aGVHD 70% cGVHD 44%	2-yr OS 57% 2-yr PFS 31% +ATG vs no ATG ↑ PFS (HR 2.9, p=0.01)
Duvic 2010 [76]	19	Advanced stage	TSEB + RIC	aGVHD 62% cGVHD 67%	2-yr OS 79% 2-yr PFS 53% 2nd malignancies 21%
Zain 2011 [60]	13	8% in CR Advanced stage	RIC 77% MAC 23%	aGVHD 53.9% cGVHD 53.9%	5-yr OS 38% 5-yr PFS 38%
Paralkar 2012 [81]	12	Advanced stage 25% in CR	MUD 42% RIC 83% TBI/CY 17%	aGVHD 75% cGVHD 8.3%	OS 58% at 2 yr Median EFS 5.3 mo
Duarte 2010 [90]	60	≥Stage IIB 47% chemoresistant 17% in CR	RIC 73%, MAC 27% TCD 42%	aGVHD 40% cGVHD 32%	3-yr OS 54%: 63% RIC, 29% MAC; 3-yr PFS 34%: 39% RIC, 22% MAC
Tsuji 2010 [87]	2	Tumor stage – refractory	TSEB, hyperCVAD + TBI/cord blood	Pt. 1: died early Pt. 2: skin cGVHD	Pt. 1: 23 days pneumonia Pt. 2: died at 17 mo on day 23 of 2nd HCT
Kahata 2008 [85]	1	Sézary IVB T4N3M1 refractory	After Flu + Mel + low-dose TBI/MUD	aGVHD cGVHD	36 mo
Fukushima 2008 [86]	1	Refractory stage IV MF with node and BM involvement	MUD graft rejected, then TBI + CY/cord blood	No GVHD	23 mo
Introcaso 2008 [84]	1	97% blood involvement	Low-dose TBI + Flu + CY	aGVHD grade I cGVHD of liver/gut	40 mo cGVHD resolved
Molina 2005 [77]	8	4 Sézary, 4 advanced MF all T3 or T4	TBI + CY = 3, BU/CY = 1 RIC Flu + Mel = 4 4 SIBS, 4 MUDS	7 aGVHD 4 limited cGVHD 3 extensive cGVHD	2 deaths in remission 6 alive in remission Median FU 56 mo
Herbert 2004 [103]	3	Pt. 1: extensive refractory MF Pt. 2: Sézary in bone marrow Pt. 3: MF transformed	RIC, Flu + Mel, SIB 2 BM, 1 PBSC (pt. 3)	aGVHD, cGVHD aGVHD, cGVHD cGVHD	95 mo[a] 11 mo (infection/cGVHD) 17 mo[a]
Soligo 2003 [83]	3	MF stage IIIB to IVB	RIC, Flu + CY+ 200cGy TBI/SIB PBSC	Pt. 1: skin aGVHD grade III Pt. 2: aGVHD and cGVHD Pt. 3: aGVHD	Pt. 1: 2 yr Pt. 2: 18 mo Pt. 3: 73 days (infection)
Guitart 2002 [79]	3	Pt. 1: tumor lesions Pt. 2: stage IVA with Sézary Pt. 3: stage IVA	Pt. 1: CY + TBI ± VP16 Pt. 2: SIB BM with added Pt. 3: CD34-enriched PBSC	All 3 aGVHD Pt. 3: also cGVHD	Pt. 1: alive in CR 4.5 yr Pt. 2: alive in CR 15 mo Pt. 3: alive in PR at 5 yr
Masood 2002 [82]	1	Stage IV, T3N3M0 Therapy resistant	TSEB, TBI + CY/SIB PBSC	Skin, gut GVHD	Alive at 2 yr
Burt 2000 [104]	1	Refractory disease Skin, node, and blood	TBI + CY	Skin cGVHD After ↓IST	Alive at 17 mo
Molina 1999 [78]	1	Skin, blood, and marrow	TBI + CY/MUD BM	Skin cGVHD	Alive at 36 mo
Koeppel 1994 [105]	1	MF stage IVA	TBI + CY/SIB BM	Skin cGVHD	Alive at 72 mo

n, Number of patients; SIB, sibling donor; MUD, matched unrelated donor; rATG, rabbit anti-thymocyte globulin; HR, hazard ratio BM, bone marrow; PBSC, peripheral blood stem cells; TSEB, total skin electron beam therapy; TBI, total body irradiation; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; RIC, reduced-intensity conditioning; MAC, myeloablative conditioning; TCD, T-cell depletion; FU, follow-up; DLI, donor lymphocyte infusion; OS, overall survival; PFS, progression-free survival; mo, months; yr, year(s).

[a] Extended follow-up data from [80].

management of these diseases. To date there have been no prospective studies on this topic. The series are focused on mycosis fungoides (MF) and Sézary syndrome (SS) and most physicians use these data to extrapolate for other types of CTCL. In the largest series of 129 patients with MF/SS, reported by the Center for International Blood and Marrow Transplant Research (CIBMTR), 64% of patients received RIC. The OS at 1 and 5 years was 54% and 32%, respectively, while the PFS at 1 and 5 years was 31% and 17%. The NRM at 1 and 5 years was 19% and 22%, respectively [89]. A retrospective analysis of 60 patients from the EBMT database included 36 patients with MF and 24 with SS; 44 patients received RIC conditioning and 25 underwent T-cell depletion. The OS in this series was 54% at 3 years. RIC yielded decreased NRM without increased relapse, resulting in improved OS, whereas T-cell depletion increased the relapse risk. DLI was used successfully to treat relapsed disease [90]. A meta-analysis of 39 cases of advanced MF/SS confirmed that at 5 years, alloHCT resulted in a better outcome (OS 80%, *n* = 20) compared with autoHCT (OS 23%, *n* = 19) [80]. The incidence of GVHD was reported to be as high as 90%. Patients with SS also showed encouraging results from alloHCT although they have rarely been reported. The results of alloHCT for dedicated CTCL series are summarized in Table 58.6, including a series that reports a subanalysis of outcomes for CTCL/MF/SS patients within a larger study of mature T-cell NHL [60].

Conclusion and future directions

The recent approval of targeted therapies for PTCL and CTCL is changing the landscape of these diseases. For patients with relapsed refractory NK/T-cell NHL, single-agent treatment with pralatrexate [91], vorinostat [92], romidepsin [93], or brentuximab vedotin [42] can serve as a bridge therapy prior to transplant, which remains the best option for long-term cure of these patients. With ongoing trials of novel agents singly and in combination, it is becoming

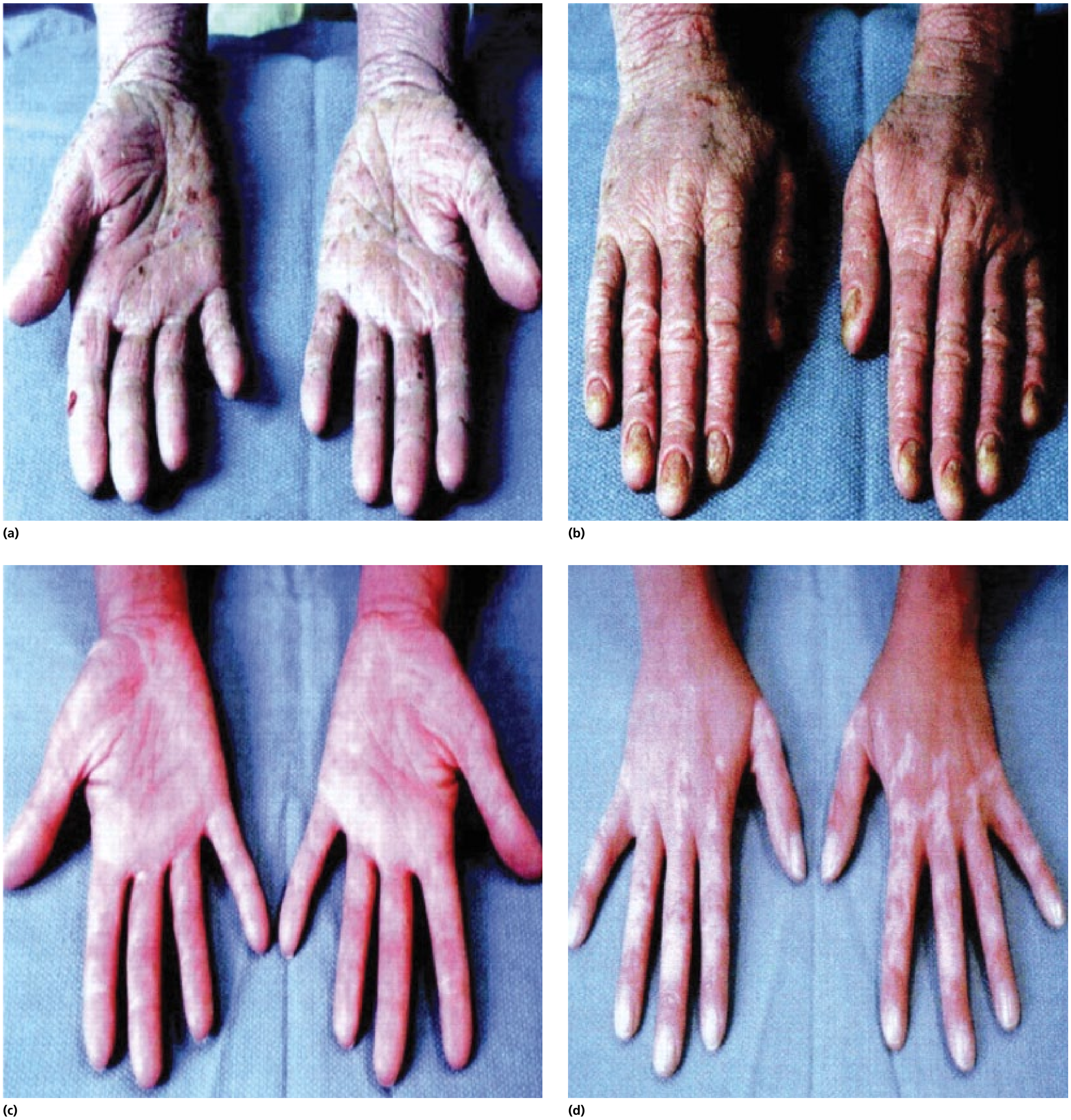

Figure 58.4 Patient with Sézary syndrome with generalized erythroderma and circulating (Sézary) cells. (a, b) Before alloHCT, the hands are affected with fissuring, cracking, and nail destruction. (c, d) One year after transplantation, there was regression of skin involvement and normalization of nails. Source: Molina et al. 2005 [77]. Reproduced with permission of the American Society of Clinical Oncology.

increasingly possible to depart from the B-cell lymphoma paradigm of toxic multiagent chemotherapy. The future will focus on more disease-specific, low-toxicity agents to treat NK/T-cell NHL and its various subtypes, in both the upfront and relapsed settings, to overcome the inherent chemoresistance of these tumors. We also expect that in time, novel agents will be approved for use in conditioning regimens and be considered for maintenance therapy in patients harboring residual disease post-transplantation.

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CHAPTER 59

Hematopoietic Cell Transplantation for Chronic Lymphocytic Leukemia

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Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults, occurring in roughly 1 of 200 individuals surviving into their seventh decade. In spite of significant progress, CLL remains an incurable malignancy with conventional chemo-immunotherapy. Given the variable clinical course that patients experience, management of CLL has required the development of robust prognostic algorithms to determine which patients require therapy and, more specifically, which may benefit from aggressive therapies including hematopoietic cell transplantation (HCT). Additionally, treatment and transplant approaches suitable for patients with comorbidities have become increasingly important. Promising developments in the management of CLL that have recently emerged include the advent of small molecular inhibitors of B-cell receptor (BCR) and other signaling pathways, and it is probable these therapies ultimately will alter the landscape for management of CLL. Additionally, manipulated cell therapies such as chimeric antigen receptor (CAR)-expressing T cells hold the promise of targeting cell-mediated immunity for long-term disease control. At present, however, allogeneic HCT remains the only well-supported approach for achieving long-term disease control in high-risk CLL. Approximately 50% of high-risk CLL patients undergoing reduced-intensity conditioning (RIC) allogeneic HCT achieve durable complete remissions lasting 5 years or longer – indeed, many of these patients are likely cured. Such results will remain the benchmark for success for future therapeutic advances in the management of CLL.

Epidemiology

United States registry data from the Surveillance Epidemiology and End Results (SEER) program of the National Cancer Institute estimate that just over 16,000 men and women are diagnosed with CLL annually [1]. The per-capita incidence of CLL appears to be relatively stable (roughly 4.5–5 cases per 100,000), which contrasts with a documented increase in the incidence of non-Hodgkin lymphomas in general since the 1970s and 1980s (roughly 11 per 100,000 in 1975 up to 20.2 per 100,000 in 2009). The median age at diagnosis of CLL is 72 years, with roughly one-third of patients diagnosed being under 65 years of age. Roughly 10% are under 55 years of age at diagnosis, and less than 2% are younger than 45 years of age. Thus, although the relative incidence in young patients under the age of 65 years is low, the high incidence of CLL leads to a substantial population of transplant-eligible patients in need of curative therapy.

Etiology

In most, if not all, cases of CLL, the malignant clone may pass through a premalignant phase of accumulation termed monoclonal B-cell lymphocytosis (MBL) [2]. The rate of progression from MBL to CLL appears to be low, however, since 3–6% of adults with normal blood counts have detectable $CD19^+CD5^+CD23^+$ CLL-phenotype MBL. The incidence of MBL is likely 100 or more times higher than that of CLL, suggesting that monoclonal proliferation of B cells is only rarely associated with transition to malignancy in the form of CLL, with a roughly 1.1–1.4% annual risk of progression [3].

CLL is characterized by the progressive accumulation of morphologically mature-appearing small B lymphocytes, but their immature immunophenotype ($CD19^+CD5^+CD23^+$) and heterogeneous immunoglobulin heavy chain (IGH) rearrangements have not supported a unifying etiology thus far. Patients with CLL cells bearing somatically hypermutated (SHM) IGH loci, which suggests antigen-induced maturation in the germinal center, experience dramatically different clinical courses compared with those in whom IGH rearrangements lack SHM. This suggests that CLL may derive from two different etiologic pathways. In general, however, the CLL cells observable in the peripheral blood and bone marrow of patients are non-proliferative cells arrested in G0/G1, but are resistant to apoptosis. CLL cells overexpress the anti-apoptotic protein BCL2, suggesting that this leads to accumulation of cells otherwise destined to undergo programmed cell death [4]. Necessarily, there is some element of proliferative drive leading to the markedly elevated absolute lymphocyte counts seen in some patients with CLL – in some cases higher than 200,000–500,000/µL. The molecular drivers underpinning the proliferative drive in CLL remain to be better elucidated.

Although many acute leukemias and other hematolymphoid malignancies arise in association with chromosomal translocations leading, generally, to the dysregulated expression of proto-oncogenes, CLL has not demonstrated an association with chromosomal translocations. Rather, the primary pattern appears to be loss of cell cycle regulators, including proteins such as Ataxia Telangiectasia Mutated (ATM) at 11q22-23 and tumor suppressor p53 at 17p13, but also micro-RNAs (miRNAs) that may serve regulatory roles [5]. The most

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common cytogenetic abnormality associated with CLL is a deletion at 13q14.3 that develops in more than 50% of cases followed longitudinally [6]. Two miRNAs, *MIRN15A* and *MIRN16-1*, cluster within 200kb at 13q14.3 [7]. They are ubiquitously expressed as non-coding RNAs with highest expression in normal CD5$^+$ lymphocytes. The majority of CLL patients have decreased *MIRN15A* and *MIRN16-1* expression that correlates with loss of heterozygosity (LOH) in 68% of informative cases.

Insights from studies using recently developed genomic technologies, including gene expression arrays and next-generation sequencing, have revealed extensive molecular heterogeneity in CLL. In 2011, several large-scale whole genome sequencing (WGS) and/or whole exome sequencing (WES) efforts were reported, revealing consistent identification of frequently mutated genes in CLL [8–10]. Puente et al. performed WGS on four patients with CLL to identify candidate mutated genes, which were then analyzed in 363 additional patients. Recurrent mutations in *NOTCH1*, *XPO1*, *MYD88*, and *KLHL6*, with the first two clustering with cases of IGH unmutated CLL and the latter two clustering in patients with IGH mutated CLL [8]. Quesada et al. completed WES on 105 patients with CLL, identifying 78 genes with predictable functional alteration and occurrence in at least two patient tumors [10]. Among the most commonly mutated proteins was SF3B1, a subunit of the spliceosome, mutation of which was associated with poor overall survival [10]. Using WES and WGS on CLL cells, Wang et al. studied 91 individuals and identified nine genes mutated with significant frequency in CLL patients, including *TP53*, *ATM*, *MYD88*, *NOTCH1*, *SF3B1*, *ZMYM3*, *MAPK1*, *FBXW7*, and *DDX3X* [9]. These nine genes implicated five core signaling pathways in the pathogenesis of CLL, including DNA damage and cell cycle control, Notch signaling, inflammatory pathways, Wnt signaling, and RNA splicing and processing, thus providing new directions for the development of targeted therapies. Schuh et al. used WGS to demonstrate intratumoral heterogeneity in the form of subclonal populations in three IGH unmutated CLL patients sampled at five time points for 7 years [11]. Increasingly aggressive clones bearing increasing mutational burdens appear to be selected for with successive rounds of treatment, suggesting that this analytic approach will become vital for targeting emergent, treatment-resistant clones.

Clinical presentation, diagnosis, and staging

CLL is frequently diagnosed in asymptomatic individuals in whom routine blood count testing reveals absolute lymphocytosis. Some patients, however, present with profound lymphadenopathy or constitutional symptoms associated with rapidly expanding malignant cells, i.e., fever, night sweats, and weight loss. Other patients present with fatigue secondary to anemia resulting from either bone marrow infiltration with CLL cells or paraneoplastic autoimmune hemolytic anemia.

The diagnosis of CLL is suggested by peripheral blood lymphocytosis and confirmed by flow cytometry of blood or marrow revealing a clonal B-cell population expressing mature B-cell marker CD19, low-level CD20, and high-level CD5 and CD23 [12,13]. In the WHO classification, CLL is always a neoplastic B-cell disease, while the entity previously referred to as T-CLL is now called T-cell prolymphocytic leukemia (T-PLL) [14]. CLL cells expresses low-level immunoglobulin with evidence of either lambda or kappa light-chain restriction. The National Cancer Institute – Working Group (NCI-WG) CLL diagnostic criteria [15] were established in 1988, with revisions in 1996 and 2008, and include [15,16]:

1. A lymphocyte count >5 × 10^9/L with small mature lymphocytes in the peripheral blood for more than 1 month.
2. Immunophenotype as detected by flow cytometry showing:
   - **a** light chain restriction;
   - **b** coexpression of CD19 and CD5 together with CD23 expression;
   - **c** low expression of surface immunoglobulin and the absence or low expression of CD79b.

A bone marrow biopsy is not required for diagnosis, but is recommended before initiating therapy [16]. A bone marrow biopsy, however, is required to assess a complete remission and is often useful when evaluating factors that may contribute to cytopenias including autoimmune responses, leukemia infiltration, or therapy-related causes. Computed tomography (CT) and/or positron emission tomography (PET) scans are not required for diagnosis, and staging relies on physical examination and blood counts only. CT ± PET scans may be useful in select patients with clinically problematic lymphadenopathy.

Two staging systems for defining degree of CLL disease involvement have been validated, the modified Rai staging system [17,18] and the Binet staging system [19] (Table 59.1). Both systems signify advancing degrees of CLL involvement based on the presence of lymphadenopathy, hepatosplenomegaly, and degree of marrow compromise.

Table 59.1 Modified Rai and Binet staging systems for CLL

Stage	Findings	Median OS (months)
Rai [17,18]		
0	Lymphocytosis only	>120
I	Lymphocytosis + lymphadenopathy	95
II	Lymphocytosis + hepatosplenomegaly	72
III	Lymphocytosis + Hb <11g/dL	30
IV	Lymphocytosis + Plt <100 × 10^9/L	30
Binet [19][a]		
A	Hb >10g/dL, Plt >100 × 10^9/L, <3 enlarged areas	>120
B	Hb >10g/dL, Plt >100 × 10^9/L, ≥3 enlarged areas	61
C	Hb <10g/dL or Plt <100 × 10^9/L	32

Hb, hemoglobin; Plt = platelets.

[a] Binet areas include cervical, axillary, or inguinal (each unilateral or bilateral), spleen and liver, with number of enlarged areas ranging from 0 to 5.

Prognostic markers

The natural history of CLL is heterogeneous, with survival ranging from months to decades, and overall survival is grossly predicted by the Binet and Rai staging systems (Table 59.1). Patients with low-risk disease have a median survival of 15 years, patients with intermediate-risk disease survive 5–7 years, and patients with high-risk disease have a life expectancy of less than 3–4 years [20]. Amongst low-risk patients, 50% ultimately require treatment and over 25% die of causes related to CLL, demonstrating the need to use additional methods for prognosticating patient outcomes [21,22]. Additionally, these clinical staging systems do not predict an individual's rate of clinical progression when diagnosed with early-stage disease, or the likelihood of response to treatment in a patient with advanced-stage disease.

Markers of CLL tumor burden

Lymphocyte doubling time and the bone marrow infiltration pattern are easily assessable and have prognostic relevance in CLL. A lymphocyte doubling time of >12 months predicts long treatment-free survival and prolonged overall survival [23]. Bone marrow CLL histology demonstrating diffuse infiltration conveys a worse prognosis than nodular infiltrates [24]. Serologic measurements that correlate with tumor burden include β_2-microglobulin and soluble CD23. β_2-Microglobulin is an extracellular protein that associates with the α-chain of class I major histocompatibility complex (MHC), and high values predict poor responses to chemotherapy and shortened survival [25]. Elevated soluble CD23 at initial diagnosis also predicts early disease progression [26].

Cytogenetic prognostic markers

Approximately 80% of CLL patients have cytogenetic abnormalities detected by fluorescence in situ hybridization (FISH) [6]. These genomic abnormalities provide pathogenic insights as they identify candidate genes and have prognostic significance, as shown in Table 59.2. The median survival times for the 17p deletion, 11q deletion, 12q trisomy, normal karyotype, and 13q deletion groups were shown by Döhner et al.'s study to be 32, 79, 114, 111, and 133 months, respectively [6], and have been independently confirmed by other groups [27,28]. Interphase cytogenetic abnormalities, especially detection of 17p deletion and 11q deletion, have been confirmed as high-risk CLL features predicting shortened progression-free survival and overall survival for untreated CLL patients who receive combined fludarabine and rituximab [29] or fludarabine and cyclophosphamide treatment [30]. In these studies, high-risk cytogenetic features did not identify which patients respond to these initial therapies, but predicted who would experience disease progression more quickly and have shortened survival.

Detection of high-risk cytogenetic features could influence initial treatment decisions because CLL patients who have CLL with deletion of chromosome 17p respond poorly to fludarabine-based therapy, and may benefit from use of alemtuzumab [31–33]. Initial studies with the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib (PCI-32765) also showed promising results with 17p deleted CLL [34]. Because cytogenetic markers have proven to be so powerful for prognosis in CLL, it is recommended that interphase cytogenetic assessment, including FISH analysis for chromosome 11 and 17 abnormalities, be performed on all CLL patients needing treatment, and it is imperative that these data be acquired for patients treated in clinical trials and those being considered for HCT [16]. Repeating cytogenetic and FISH studies upon relapse may reveal clonal evolution and acquisition of clinically relevant poor prognostic markers such as 17p deletion [35].

Table 59.2 CLL prognostic markers

Group [6]	Frequency (%)	Median OS (months)
FISH cytogenetics		
del 13q (heterozygous)	55	133
Normal karyotype	18	111
Trisomy 12	16	114
del 11q	13	79
del 17p	9	32
IGH mutation [40]		
Somatically mutated	44	152
Unmutated	56	79
V3–21	11	63
Zap-70 expression [45]		
<20%	50	NR
>20%	50	29
CD38 [47]		
<30%	58	293
>30%	42	109

FISH, fluorescence in situ hybridization; del, deletion; IGH, immunoglobulin heavy chain; NR, not reached; OS, overall survival.

IGH gene mutation status

One of the most useful prognostic indicators for CLL is the IGH gene mutation status [36,37]. CLL appears to arise from a stage of B-cell ontogeny bridging the point at which antigen-induced affinity maturation may occur in the germinal center. As such, SHM at the IGH locus may reveal important underpinnings of two subsets of CLL that appear to have very different natural histories – somatically mutated versus unmutated CLL. The IGH locus is considered unmutated when the V, D, and J segments remain >98% identical with germline IGH gene segments published by the International ImMunoGenetics (IMGT) Information System [38,39]. Approximately half of CLL patients possess clones with unmutated IGH genes, and these patients have relatively poor clinical outcomes with rapid disease progression, shortened time to treatment, and shortened overall survival. In a study of 300 CLL patients stratified by IGH mutation status, the median survival times were 79 months for the IGH unmutated group and nearly double that at 152 months for the mutated group (Table 59.2) [40]. Interestingly, a prospective analysis of 64 CLL patients over a minimum period of 3 years identified 11 patients undergoing cytogenetic clonal evolution, and all 11 had unmutated IGH gene status, suggesting that unmutated IGH CLL may have chromosome instability [35]. We recommend obtaining the IGH mutation status on all CLL patients, both for prognostic reasons and for potential use as a molecular minimal residual disease (MRD) marker (discussed below). Another benefit to sequencing IGH is the possibility of revealing high-risk V segment usage, such as V3–21, which conveys a poor prognosis in spite of SHM [41,42].

CD38 and ZAP-70 expression

IGH mutational status has historically been difficult to determine for some institutions, but the availability of this assay has recently improved and should be available to most patients. Cell surface markers assessable by flow cytometry have been evaluated for their correlation with IGH mutation status to allow prognostication when IGH mutation status was unavailable. CD38 expression on CLL cells was the first of these markers demonstrated to correlate with IGH mutation status – higher level CD38 (>30%) is more frequently found on IGH unmutated CLL and correlates with a poor prognosis [36]. CD38 expression is dynamic and may correlate directly with activation and proliferative status; however, there is no established role for serial assessment of CD38 expression [43]. Gene expression profiling subsequently demonstrated a close association between expression of zeta-associated protein (ZAP-70) and IGH unmutated CLL [44]. ZAP-70 is highly expressed in natural killer (NK) and T cells whereas normal B cells have low or no ZAP-70 expression. ZAP-70 expression on >20% of CLL phenotype cells is associated with worse prognosis and there is a strong correlation between IGH unmutated status and ZAP-70 positivity. Additional studies have shown, however, that approximately 10–30% of CLL clones are discordant between ZAP-70 and IGH mutation status [40,42,45–47]. Together, these data suggest that a prognostic model for CLL can be based on either genomic alterations and ZAP-70 expression or IGH mutation status, high-risk genomic aberrations, and canonical high-risk V-segment usage (e.g., V3–21) [42]. This need for multiple independent prognostic predictors suggests again that CLL is a heterogeneous disease with multiple pathogenic mechanisms.

Response criteria

Assessment of response includes a careful physical examination and evaluation of blood and marrow. According to the revised NCI-WG guidelines, a complete response requires a normal physical examination, lymphocytosis ≤ 5×10^9/L, the absence of clonal lymphocytes, hemoglobin >11g/dL, and platelets >100×10^9/L. Patients with residual CLL cells by conventional flow cytometry or immunohistochemistry are defined as having partial response (PR) [16].

Minimal residual disease – methods for monitoring depth of treatment response

Chronic lymphocytic leukemia is readily amenable to detection and quantification of MRD using either multiparameter flow cytometry (MPFC) or molecular methods based on detecting clonal immunoglobulin gene rearrangements specific to the malignant clone using allele-specific oligonucleotide polymerase chain reaction (ASO-PCR)- or high-throughput sequencing (HTS)-based approaches. These methods have become increasingly relevant as the efficacy of therapy has improved and a larger proportion of patients are achieving deep remissions. It remains equally important, however, that as the ability to identify high-risk CLL patients has improved, to develop methods to monitor early loss of response to therapy in patients who ultimately fare poorly with successive relapses.

The European Research Initiative in CLL (ERIC) group has mounted an impressive effort to establish an internationally harmonized method for quantifying CLL MRD using four- or six-color MPFC [48,49]. In 2007, this group identified four-color antibody panels consisting of CD5/CD19 with CD20/38, CD81/22, and CD79b/CD43 that performed well across different laboratories with >95% accuracy and 95% concordance with ASO-PCR when disease burden was above 1:10,000 [48]. In an attempt both to simplify the standardized work flow for MPFC MRD detection and to provide the possibility of deeper sensitivity, the ERIC group subsequently demonstrated that a preliminary assessment using CD5/CD19/κ/λ was adequate for identifying samples with high probability of containing residual disease. The panel provided a 100% positive predictive value when samples contained more than 82% of B cells with CD5 expression and skewed light-chain ratio (κ:λ <0.05:1 or >32:1), which was adequate, but only qualitative, for about half of the 784 samples they evaluated from CLL patients during and after therapy [48]. The authors noted, however, that the expression of CD5 on regenerating B cells following allogeneic transplant may confound this analytic algorithm, which may be particularly relevant following HCT [49–51]. For samples in which CD5/CD19 and light-chain clonality were insufficient to identify residual disease, the group recommended analysis using six-color antibody panels consisting of CD5/CD19/CD20 with CD3/CD38/CD79b and CD81/CD22/CD43. This approach demonstrated 100% concordance with the previously standardized four-color panels using the same antigens, and because of the additional channels available with each assay on six-color cytometers, fewer tubes per patient need be analyzed, thus improving time efficiency. At least 50 events with the CLL immunophenotype are required to identify MRD confidently, thus necessitating the collection of a minimum of 500,000 leukocyte events on the cytometer for a 1:10,000 MRD detection threshold.

A frequently used alternative to immunophenotyping-based MRD detection is the use of molecular approaches for detection and quantification of CLL-specific IGH rearrangements in genomic DNA isolated from blood or bone marrow samples. Most of this work has been achieved using ASO-PCR, in which one or more patient-specific ££PCR primers and quantitative PCR probe are generated to make patient-specific assays [52,53]. This approach, although offering improved sensitivity (1:100,000 routinely), is not available outside a small number of academic centers with the resources to maintain the expertise for patient-specific assay development and validation.

We recently demonstrated a method for MRD quantification in CLL using a platform comprised of consensus IGHV and IGHJ primers and next-generation sequencing. Using 454 pyrosequencing and BIOMED-2 primers [54,55], a pilot study of six post-transplant CLL patients demonstrated accurate MRD quantification to a threshold of 1:100,000, with good correlation with ASO-PCR [56]. This study also identified some of the bioinformatic hurdles that must be overcome for accurate disease quantification using a sequencing-based approach. In a larger study with 40 patients and using an Illumina HTS-based MRD quantification approach called Sequenta LymphoSIGHT, MRD was quantified in all patients using consensus primers with a detection threshold of 1:1,000,000 [57]. The greatest advantage of this approach is the ability to assay all CLL patients for MRD without generating allele-specific primers or probes, as is necessary for ASO-PCR. Added sensitivity through sequencing depth (e.g., 1:1,000,000 detection) is a feature not easily achievable with MPFC or ASO-PCR, the benefit of which remains to be determined in larger studies. We have demonstrated that the IGH sequence data generated for the purpose of MRD quantification in CLL can also be use to profile the immunoglobulin repertoire, which may have importance in the post-transplant setting for monitoring the effects of graft-versus-host disease (GVHD) and its treatment on B-cell reconstitution [58].

Initial CLL therapy

Although the comprehensive description of treatment options for CLL patients is beyond the scope of this chapter, Figure 59.1 shows a schema for CLL diagnosis, prognostic evaluation, and therapeutic options. For those with low-risk CLL (Rai stage 0–1/Binet A, 13q14 cytogenetics, and mutated IGH gene status), observation with clinical follow up every 3–6 months is usually acceptable. Symptomatic disease progression or intermediate-risk disease at diagnosis may be managed with standard therapy. For decades, the standard therapy was chlorambucil, but this approach is now primarily used in patients with comorbidities. Three randomized trials have demonstrated significantly higher complete response (CR) rates and longer progression-free survival (PFS) using fludarabine-based therapy compared with alkylator-based therapies [59–61]. A subsequent randomized trial demonstrated that fludarabine plus cyclophosphamide combined therapy is superior to single-agent fludarabine as initial therapy, providing improved overall response rate (ORR), CR, and PFS [62].

The addition of monoclonal antibodies directed at B-cell surface antigens further improves the therapeutic benefit of chemotherapy. Rituximab, a chimeric humanized mouse anti-human CD20 antibody, and alemtuzumab, a humanized rat anti-human CD52 antibody, are effective as single agents or in combination therapy. Combining purine analogs with an alkylating agent and rituximab [i.e., fludarabine, cyclophosphamide, and rituximab (FCR)] has been reported to provide overall response rates of 95% and complete response rates of 70% [63]. In addition, these combination regimens can achieve molecular remissions; however, higher response rates have not yet demonstrated improved overall survival, and most patients treated with FCR will eventually relapse.

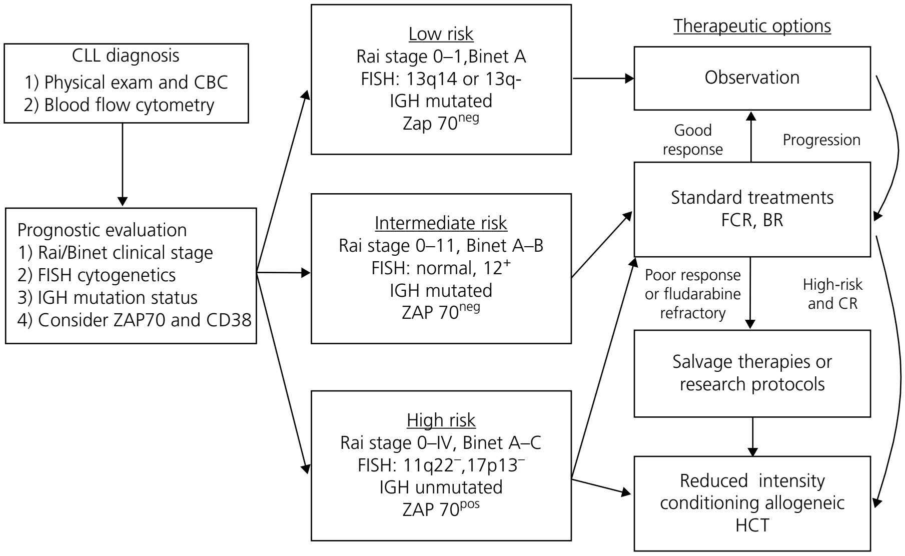
CLL diagnosis
1) Physical exam and CBC
2) Blood flow cytometry
Prognostic evaluation
1) Rai/Binet clinical stage
2) FISH cytogenetics
3) IGH mutation status
4) Consider ZAP70 and CD38
Low risk
Rai stage 0–1,Binet A
FISH: 13q14 or 13q-
IGH mutated
Zap 70neg
Intermediate risk
Rai stage 0–11, Binet A–B
FISH: normal, 12+
IGH mutated
ZAP 70neg
High risk
Rai stage 0–IV, Binet A–C
FISH: 11q22−,17p13−
IGH unmutated
ZAP 70pos
Therapeutic options
Observation
Good response
Progression
Standard treatments
FCR, BR
Poor response or fludarabine refractory
High-risk and CR
Salvage therapies or research protocols
Reduced intensity conditioning allogeneic HCT

Figure 59.1 CLL diagnosis, prognostic evaluation, and treatment options. Risk-stratified CLL disease assessment guides treatment. High-risk patients are predicted by cytogenetic abnormalities 11q⁻ and 17p⁻, unmutated IGH gene status, and IGH V3–21 usage. High-risk patients follow a treatment course that begins with standard combination therapy to cytoreduce their disease, and once they achieve adequate disease control they should be considered for RIC allogeneic HCT.

A recent alternative to FCR favored by some for its improved tolerability is bendamustine with rituximab (BR). Although bendamustine originally acquired its US Food and Drug Administration (FDA) label for indolent NHL, it has proven to be useful in both front-line and salvage therapy in CLL. The German CLL Study Group performed a phase II trial using BR in front-line therapy and demonstrated an 88% overall response rate with 23% CR and 65% PR. Response rates were favorable in IGH unmutated and 11q deleted CLL, but only 38% of those with 17p deletions achieved a PR and none achieved a CR [64].

Clinical challenges in advanced and high-risk CLL

Although most patients with CLL are elderly and half of CLL patients may not require treatment, there is a substantial proportion of young patients diagnosed with CLL bearing high-risk features, including IGH unmutated status, 17p or 11q deletions, and/or high ZAP-70 expression. It is these patients for whom it is valuable to consider the role for HCT. Recommendations regarding the use of allogeneic HCT in first CR in specific circumstances, or at relapse, are likely to change over the coming years if preliminary results with targeted therapies are confirmed. Nevertheless, at present, allogeneic HCT remains the only known modality offering long-term disease-free survival to patients. It is important to have a framework within which to guide patients in treatment decisions involving HCT therapy. A reasonable part of discussion for patients with high-risk CLL is the rationale for allogeneic HCT instead of autologous HCT, as the latter is typically recommended for relapsed non-Hodgkin lymphomas, but currently has a limited role in the management of CLL.

Historical experience with autologous transplantation

High-dose chemotherapy with autologous HCT for CLL was explored in the 1980s–1990s, but this approach has not been compared with conventional chemo-immunotherapy or allogeneic HCT in randomized controlled trials. Nonetheless, clinical and molecular remissions have been reported using autologous HCT, including in patients with poor prognostic features [65,66], so these results may yet be instructive.

Relative to other hematologic malignancies, autologous HCT was introduced to CLL care relatively late, presumably due to concern that persistent CLL would contaminate the graft. To address this concern, the first clinical trial of autologous HCT for CLL employed in vitro “purging” of the bone marrow graft with anti-CD20, anti-CD10, and B5 monoclonal antibodies via complement-mediated cell lysis [67]. All 12 patients in this pilot study achieved timely hematologic reconstitution and 11 achieved clinical CR. Six of the 12 obtained molecular remissions by clonal IGH gene rearrangement PCR detection studies. Khouri et al. reported a similar 11-patient early feasibility study using total body irradiation (TBI) and cyclophosphamide (CY) conditioning with anti-CD19 immunomagnetic bone marrow graft purging [68]. Six patients achieved CR and five had no detectable MRD by IGH PCR analysis. In all, at least six single-center phase I or retrospective studies with fewer than 20 patients each investigated autologous HCT for CLL [69–74].

Subsequently, three phase II and three phase III studies of autologous HCT in CLL have been reported (Table 59.3). Of note, all of these trials were initiated prior to the availability of rituxumab, and the main impetus for these studies was the poor success of conventional treatment regimens at the time (chlorambucil alone, fludarabine alone, or fludarabine with an alkylator). The Dana-Farber Cancer Institute (DFCI) reported experience using autologous HCT with B-cell-depleted marrow after TBI/CY conditioning in 137 patients with high-risk CLL between 1989 and 1999 [75]. Patients in this trial were a mix of relapsed CLL and high-risk CLL immediately following front-line therapy. With a median follow-up of 6.5 years, the PFS was 30% and overall survival (OS) was 58%, notably without evidence of a survival plateau. A high rate of secondary neoplasms, including therapy-related myeloid neoplasia (12%), was observed [75].

The UK Medical Research Council (MRC) completed a trial in which 115 patients were deemed eligible for autologous transplantation

Table 59.3 Selected studies of autologous HCT for patients with CLL

Study	*n*	Median age (years)	Conditioning regimen	Graft Purge	NRM	CR	Minimal residual disease	PFS (median f/u)	OS (median f/u)	Second malignancy incidence (%)
Gribben 2005 [75] (DFCI single-center phase 2)	137	51	TBI/CY	Bone marrow B-cell depleted	4% (day 100) 17% (overall)	NR	NR	30% (6.5 yr)	58% (6.5 yr)	12
Milligan 2005 [76] (MRC phase 2)	65/117	49	49 TBI/CY 11 BEAM 1 BU/CY	PBSC CD34 selected	1.5%	74%	16/20 MRD neg	51.5% (5 yr)	77.5% (5 yr)	12.4
Dreger 2012 [77] (German CLL Study Group, phase 2)	131	51	CHOP Fludarabine Flu/CY	PBSC B-cell depleted	4% (2 yr) 14% (10 yr)	NR	Few/52 MRD neg	5.7 yr (median)	11.3 yr (median)	19
Sutton 2011 [79] (French CLL Group, phase 3)	CR: *n* = 52 (ASCT) *n* = 53 (Obs) Non-CR: *n* = 46 (ASCT) *n* = 48 (FC)	57 56 54 57	TBI/CY	PBSC Unmanipulated	2% 0% 4% 6%	96% 92% 59% 54%	MRD neg 37.5% 15% 32% 47%	79.8% 35.5% (3 yr) 45% 45% (3 yr)	95.7% 97.8% (3 yr) 82% 87% (3 yr)	4 0 4 6
Michallet 2011 [80] (EBMT CLL Working Party,phase 3)	*n* = 112 (ASCT) *n* = 111 (Obs)	54 53	TBI/CY	PBSC Unmanipulated	4% 0%	NR	NR	42% vs. 24% (5 yr)	85.5% vs. 84.3% (5 yr)	NR
Brion 2012 [81] (GOELAMS LLC 98, phase 3)	*n* = 43 (ASCT) *n* = 39 (Conv. Chemo)	54 55	TBI/CY CHOP	PBSC CD34 selected	0% 0%	64% 44%	NR	53 mo vs. 22 mo (median)	107.4 mo vs. 104.7 mo (median)	18.6 vs. 20.5

n, Number of patients; ASCT, autologous stem cell transplant; BEAM, BCNU, etoposide, Ara-C, melphalan; BU, busulfan; CY, cyclophosphamide; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; FC, fludarabine, cyclophosphamide; NR, not reported; NRM, non-relapse mortality; OS, overall survival; PBSC, peripheral blood stem cells; CR, complete response; PFS, progression-free survival; TBI, total body irradiation; f/u, follow-up; mo, months; yr, year(s).

after front-line fludarabine therapy from 1999 to 2004 [76]. Importantly, this trial identified a high rate of failed hematopoietic stem-cell mobilizations following fludarabine, with only 67% of patients collecting an adequate graft. With 65 patients ultimately undergoing TBI/CY or BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea], etoposide, Ara-C (cytosine arabinoside), and melphalan (BEAM) conditioning with autologous CD34-selected (i.e., T- and B-cell depleted) peripheral blood progenitor cell (PBPC) transplant, the 5-year PFS was 51.5% with 77.5% OS. Complete remissions increased from 37% pre-HCT to 74% after HCT. Sixteen of 20 evaluable patients achieved MRD negativity as assessed by IGH PCR, and the presence of MRD after autologous HCT predicted subsequent relapse [76]. The rate of therapy-related myeloid neoplasia was similar to the DFCI study at 12.4%.

Lastly, the German CLL Study Group recently published data from their CLL3 trial in which autologous HCT was included as part of front-line therapy [77]. Between 1996 and 2002, a total of 131 patients received ex vivo B-cell-depleted autologous PBPC after induction chemotherapy [cyclophosphamide, hydroxydaunomycin, vincristine (Oncovin), prednisone, and prednisone (CHOP), fludarabine, or fludarabine with cyclophosphamide], followed by a dexamethasone (Dexa)-BEAM mobilization prior to stem-cell collection, followed by a TBI/CY preparative regimen. After a median follow-up of nearly 9 years, the median PFS and OS were 5.7 and 11.3 years, respectively. This trial retrospectively evaluated diagnostic samples from all patients with cytogenetic and IGH mutation studies and determined that those with 17p deletions [PFS hazard ratio (HR) 19.08, $p < 0.001$; OS HR 8.35, $p < 0.001$] and IGH unmutated status (PFS HR 3.35, $p < 0.001$; OS HR 3.11, $p = 0.006$) did poorly after autologous HCT. Molecular MRD was evaluated in 52 patients and although there was a median MRD reduction of >3 logs, few achieved MRD negativity. The risk of secondary malignancy in this trial was 19% at 10 years of follow-up. Within the above report, Dreger et al. also presented a retrospective comparison between the autologous HCT patients on the CLL3 trial and 126 patients receiving FCR on the CLL8 trial [78]; between 2002 and 2006, time to retreatment and OS did not differ between the two groups, suggesting no benefit for high-dose therapy in the rituximab era.

Three randomized trials involving autologous HCT for CLL have been conducted. From 2001 to 2007, the French CLL group conducted a trial in which 241 patients with advanced CLL (Binet B or C) received three cycles of mini-CHOP, then three cycles of fludarabine, and those who were in CR after these therapies were randomized to autologous HCT or observation [79]. Of 105 who achieved CR, 52 underwent autologous HCT and 53 were observed expectantly. The 3-year event-free survival (EFS) for the two groups was 79.8 and 35.5%, respectively, with similar OS rates above 95%. Patients who did not enter CR with the induction regimen were randomized to autologous HCT or fludarabine plus CY and, importantly, these two groups had similar 3-year EFS of roughly 45% without a difference in OS (~85% in both).

The EBMT Chronic Leukemia Working party randomized 223 patients with response to first- or second-line treatment to autologous HCT or observation [80]. In 112 autografted patients, the median EFS was 51.2 months with a 5-year EFS of 42%. In 111 patients managed expectantly, the median EFS was 24.4 months with a 5-year EFS of 24%. OS, however, was not significantly different at 85.5 and 84.3%, respectively. Of note, this trial included very few patients with 17p deletions and roughly 13% of patients had Binet A disease with progression. These lower risk features may account for the relatively high 5-year OS. This trial again identified a high rate of collection failures, with roughly 10% being unable to proceed to autograft due to inadequate stem-cell yield.

Recently, Brion et al. reported results from the GOELAMS LLC 98 trial, in which 86 patients received either six cycles of monthly CHOP, followed by CHOP maintenance every 3 months for six additional cycles for those achieving only PR, versus three cycles of monthly CHOP followed by CY mobilization of PBSC then a CD34 selected autograft following conditioning with TBI/CY [81]. Those in PR after collection were given a supplementary three cycles of fludarabine prior to the autograft. Although the PFS was longer with up-front autograft (53 months versus 22 months, $p < 0.0001$), there was no significant difference in median survival of ~105 months.

In summary, although autologous HCT provides benefit for disease control in patients receiving only chemotherapy without rituximab, it likely does not improve response rates in the rituximab era. The high rate of treatment-related MDS/AML after TBI-based preparative regimens (10% or higher) is a significant concern, and it remains unknown whether autologous HCT for CLL after chemotherapy-based conditioning would have a more favorable risk profile, but it is unlikely that such studies will be carried out, as the likelihood of improving PFS rates is low.

Allogeneic transplantation

Because autologous HCT fails to achieve a plateau in relapse and risk of death, and is associated with a high risk of secondary malignancy, allogeneic HCT has become generally strongly favored over high-dose chemotherapy with autologous HCT for patients with relapsed or high-risk CLL. The prevailing preference for allografting in CLL is based on the fact that CLL is a malignancy with a well-established responsiveness to graft-versus-leukemia (GVL) effects [75,82–85]. An important question, then, is whether conditioning intensity is important to convey adequate disease control, or it is sufficient to achieve adequate engraftment with RIC approaches and permit GVL effects to control the disease. Early experience with myeloablative allogeneic HCT for patients with CLL revealed GVL activity, but overall outcomes were unsatisfactory, owing to high non-relapse mortality (NRM) or treatment-related mortality (TRM), as discussed below, and RIC regimens have rapidly become the favored approach for allogeneic HCT.

Special donor considerations in allogeneic transplantation for CLL

Siblings have been noted to have clonal B-cell proliferations in 8–10% of CLL patients' families [86–88]. The overall risk for developing CLL is two to seven times higher for first-degree relatives than in the general population [89]. This has special significance in allogeneic HCT when siblings may be used as donors, and supports screening of HLA-identical siblings using peripheral blood flow cytometry to assess for CLL-phenotype monoclonal B-cell lymphocytosis ($CD19^+CD5^+CD23^+$). Identification of a clonal B-cell population in a prospective donor carries bioethical considerations, as discussed by Hardy et al. [90], and it is our practice instead to select a matched unrelated donor when possible. The basis for germline transmission of CLL risk remains to be better elucidated.

Allogeneic HCT with myeloablative conditioning

Myeloablative allotransplants for CLL were evaluated in trials during the 1980s and 1990s using TBI/CY conditioning regimens similar to those used in autologous HCT at the time. An early EBMT registry study of 54 patients, median age 41 years, with CLL reported a transplant-related mortality of 46% and a 3-year probability of survival of 46%. Nearly all the patients received TBI/CY conditioning and HLA-identical sibling bone marrow grafts. Ten-year clinical outcomes were reported for the entire cohort, demonstrating 41.2% OS and 36.6% leukemia-free survival [91]. This first long-term registry study suggested that CLL can be cured by conventional allogeneic HCT with long-term continuous complete remissions observed up to 16 years after transplantation. Table 59.4 summarizes a number of single-institution CLL allotransplant studies using myeloablative conditioning [91–100]. Most studies involved fewer than 30 patients. These studies, in aggregate, demonstrate

Table 59.4 Selected studies of myeloablative allogeneic HCT for patients with CLL

Study	*n*	Median age (years)	Refractory patients (%)	Conditioning regimen	Graft source (%)	NRM (%)	Acute GVHD grade ≥2 (%)	Chronic GVHD (%)	PFS (%) (years of projection)	OS (%) (years of projection)
Michallet 1996 [91] (EBMT registry study)	54	41	39	51 TBI/CY 3 BU/CY	Sib: 100 BM: 100	50	31	31	41 (10 yr)	37 (10 yr)
Pavletic 2000 [92] (Omaha, single-institution series)	23	46	61	16 TBI/VP/CY 6 TBI/CY	Sib: 88 BM: 70	35	54	68	65 (5 yr)	62 (5 yr)
Horowitz 2000 [93] (IBMTR registry study)	242	47	50	42% TBI/CY 33% TBI/CY	Sib: 78 BM: 75	30	NR	NR	44 (3 yr)	45 (3 yr)
Doney 2002 [94] (Seattle, single-institution series)	25	47	64	17 TBI/CY 7 BU/CY	Sib:100 BM: 88	40	56	40	NR	32 (5 yr)
Khouri 2002 [95] (Houston, single-institution series)	28	43	68	27 TBI/CY 1 BEAM	Sib: 79 BM: 82	32	49	64	45 (5 yr)	42 (5 yr)
Toze 2005 [96] (Canadian, two-institution series)	30	48	47	15 TBI/CY 15 BU/CY	Sib: 79 BM: 82	47	52	65	39 (5 yr)	39 (5 yr)
Gribben 2005 [75] (Boston, single-institution series)	25	47	NR	25 TBI/CY	Sib: 100 BM: 100	24	NR	NR	24 (6 yr)	55 (6 yr)
Pavletic 2005 [97] (NMDP/IBMTR registry study)	38	45	55	29 TBI/CY 6 TBI + other	Sib: 0 BM: 100	38	45	85	30 (5 yr)	33 (5 yr)
Sorror 2008 [98] (Seattle, single-institution series)	68	46	78	59 TBI/CY 9 BU/CY	Sib: 72 BM: 31	35	NR	NR	NR	45 (3 yr)
Michallet 2010 [99] (EBMT registry study)	102	53	NR	Multiple	Sib: 60 BM: 25	20–35[a]	34[a]	35[a]	NR	49* (5 yr)
Brown 2013 [100] (Boston, single-institution series)	32	48	69	TBI/CY	Sib: 59 BM: 44	48	50	51	36 (5 yr)	49 (5 yr)

n, Number of patients; BEAM, BCNU, etoposide, Ara-C, melphalan; BM, bone marrow; BU, busulfan; CY, cyclophosphamide; GVHD, graft-versus-host disease; NR, not reported; NRM, non-relapse mortality; PFS, progression-free survival; OS, overall survival; Sib, sibling; TBI, total body irradiation; yr, year.

[a] Data for this study were not reported by type of conditioning (e.g., ablative vs. non-ablative). These results combine conditioning types.

that myeloablative allogeneic HCT for CLL using TBI/CY or CY with busulfan (BU/CY) is associated with high rates of TRM (range 24–50%). Mature OS rates at 5 years or beyond were generally around 40% (range 33–62%).

Because of the rapid adoption of reduced-intensity allogeneic HCT for CLL during the late 1990s and early 2000s, no large-scale studies of myeloablative HCT in CLL have been conducted. Sorror et al. at the Fred Hutchinson Cancer Research Center published their experience with both myeloablative and non-myeloablative allogeneic HCT for CLL and other lymphomas between 1997 and 2005 [98]; however, only 10 of 68 patients undergoing myeloablative conditioning were treated for CLL, so this study provides little additional insight into outcomes with intensive conditioning for CLL allotransplant.

Michallet et al. reported outcomes for 102 patients undergoing myeloablative and 266 non-myeloablative allogeneic HCT for CLL between 1995 and 2007 [99]. Several different conditioning regimens were used. Unfortunately, results were published with stratification by degree of donor matching (sibling, fully matched unrelated donor, and partially mismatched unrelated donor), not by type of conditioning regimen. Interestingly, however, it was reported that a multivariate analysis failed to identify conditioning intensity as an independent predictor of OS, relapse, PFS, or TRM, possibly suggesting that outcomes with myeloablative conditioning may have improved to some extent in parallel with the adoption of RIC regimens. Alternatively, a study by Brown et al. in which myeloablative and reduced-intensity regimens were analyzed by treatment era (pre- and post-2004) suggested that outcomes between myeloablative conditioning and RIC regimens were similar before 2004, but that in more recent years the outcomes achieved with RIC regimens have improved with respect to both OS and TRM [100]. Because increased conditioning intensity appears not to offer additional survival benefit, it is likely not justifiable to pursue myeloablative allogeneic HCT in patients with CLL. Similarly, a limited registry study showed that patients with CLL transformation to prolymphocytic leukemia achieved similar outcomes using ablative conditioning or RIC [101].

Reduced-intensity conditioning for CLL allogeneic HCT

Remarkable improvements in patient outcomes have been achieved through the implementation of RIC regimens for patients with CLL. This sea change in practice patterns, elicited by the recognition that engraftment and GVL effects can be robust without intensive conditioning of the recipient, has occurred fairly rapidly within the past 15 years [102].

In 1998, Khouri et al. first reported the feasibility of using fludarabine and CY-based RIC allogeneic HCT for eight patients with advanced CLL, including two patients with Richter's transformation [85]. GVL activity was demonstrated by achievement of complete responses in several patients, and also demonstration of disease clearance following a donor lymphocyte infusion (DLI) given for persistent mixed chimerism. Further early evidence of GVL in CLL following RIC conditioning included a report from Dreger et al. in which the onset of chronic GVHD following RIC allotransplant was tightly correlated with conversion from MRD detectable to undetectable status using IGH ASO-PCR [103].

Adequate engraftment and robust anti-CLL GVL activity have since been verified in many studies using a wide array of RIC conditioning regimens. Table 59.5 reviews results of a selection of RIC allogeneic HCT trials in CLL [98,100,104–112]. Most strikingly apparent is the decrease in NRM afforded by adopting regimens with less up-front toxicity. Indeed, the roughly 15–20% NRM rates in early studies represents a vast improvement over the roughly 40% NRM experienced with myeloablative regimens. Equally as important, early studies verified the presence of GVL activity in patients with even refractory CLL and those with high-risk cytogenetic changes. For the most part, equivalent outcomes have been achieved

Table 59.5 Selected studies of reduced intensity conditioning (RIC) allogeneic HCT for patients with CLL

Study	*n*	Median age (years)	Refractory patients (%)	Conditioning regimen	Graft source (%)	NRM (%) (2 yr)	Acute GVHD grade ≥2 (%)	Chronic GVHD (%)	PFS (%) (2 yr)	OS (%) (2 yr)
Schetelig 2003 [104] (Germany, multi-institution study)	30	50	46	Flu/BU/ATG	Sib: 50 PBSC: 90	15	56	75	67	72
Dreger 2003 [105] (EBMT registry study)	77	54	33	Mixed	Sib: 81 PBSC: 91	18	34	57	56	72
Khouri 2004 [106] (Houston, single-institution series)	17	54	53	7 FC 10 FCR	Sib: 100 PBSC: 94	22	29	60	60	80
Sorror 2005 [107] (Seattle, single-institution study)	64	56	53	2Gy TBI/Flu	Sib: 69 PBSC: 100	22	55	50	52	60
Delgado 2006 [108] (London, multi-institution study)	41	54	31	Alemtuzumab Flu/Mel	Sib: 59 PBSC: 85	26	41 (any grade)	33	45	51
Brown 2006 [109] (Boston, single-institution study)	46	53	57	Flu/BU	Sib: 33 PBSC: 98	17	34	53	34	54
Update of Sorror 2008 [98] (Seattle, single-institution study)	82	56	87	2Gy TBI/Flu as above	Sib: 69 PBSC: 100	23 (5 yr)	55	50	39 (5 yr)	50 (5 yr)
Dreger 2010 [110] (Germany, multi-institution study)	90	53	47	Flu/CY ± ATG	Sib: 40 PBSC: 100	23 (4 yr)	45	73	42 (4 yr)	65 (4 yr)
Khouri 2011 [111] (Houston, single-institution, multi-study)	86	58	83	78 FCR 6 Flu/Mel/Ritux 2 other	Sib: 50 PBSC: 100	17	37	56	36 (5 yr)	51 (5 yr)
Brown 2013 [100] (Boston, single-center study)	76	55	55	Flu/BU	Sib: 37 PBSC: 99	16	30	65	43 (5 yr)	63 (5 yr)
Arai 2012 [112] (Stanford, single-center study)	22	55	73	TLI/ATG ± post-HCT Ritux	Sib: 54 PBSC: 100	3	6	20	47 (4 yr)	73 (4 yr)

n, Number of patients; ATG, antithymocyte globulin; BU, busulfan; CY, cyclophosphamide; FC, fludarabine, cyclophosphamide; FCR, fludarabine, cyclophosphamide, rituximab; Flu, fludarabine; GVHD, graft-versus-host disease; NRM, non-relapse mortality; PFS, progression-free survival; OS, overall survival; PBSC, peripheral blood stem cells; Ritux, rituximab; Sib, sibling; TBI, total body irradiation; yr, year.

with different RIC regimens. Schetelig et al. used fludarabine, busulfan, and antithymocyte globulin (ATG) [104], whereas the M.D. Anderson group developed FCR conditioning [106], and the Seattle group pioneered the use of low-dose TBI (2Gy) with fludarabine [107]. These different approaches yielded similar PFS of ~60% and 2-year survival rates of ~70% in early studies (Table 59.5). The 5-year follow-up from the Seattle group was published by Sorror et al. in 2008, demonstrating NRM 23%, PFS 39%, and OS 50% in 82 patients [98]. Outcomes were not affected by adverse cytogenetics and only lymphadenopathy >5cm at the time of conditioning was independently associated with treatment failure.

Similar results were reported by Dreger et al. in the German CLL Study Group in 90 patients conditioned with fludarabine and cyclophosphamide (with ATG for unrelated donors), with 4-year outcomes including NRM 23%, EFS 42%, and OS 65% [110]. Among patients with undetectable MRD at 1 year post-HCT, the risk of relapse was significantly lower [HR 0.09, 95% confidence interval (CI) 0.02–0.49, $p = 0.0052$] in comparison with those with detectable MRD. Khouri et al. at the M.D. Anderson Research Center similarly reviewed the 5-year follow-up on 86 patients undergoing RIC allogeneic HCT for CLL and reported NRM 17%, PFS 36%, and OS 51% [111].

Brown et al. at DFCI recently reported their long-term experience using fludarabine and busulfan RIC allogeneic HCT for patients with CLL [100]. Amongst 76 patients allografted between 1998 and 2009, the NRM was 16%, with PFS 43% and OS 63% at 5 years. Interestingly, this study found improved outcomes amongst the 42 patients transplanted since 2004, with just a 9.5% NRM, and an impressive 64% PFS and 83% OS at 5 years, suggesting that increasing familiarity with RIC allogeneic HCT at active transplant centers may be associated with improved outcomes over time.

Although single-center NRM decreased by more than 50% by adapting from myeloablative to RIC regimens as reported by Brown et al. [100], there remains a need to decrease NRM risk even further. To that end, the Stanford group developed a novel RIC regimen consisting of total lymphoid irradiation (TLI) and ATG, which is associated with low rates of acute and chronic GVHD, and very low rates (<5%) of NRM [113,114]. This regimen performs particularly well in patients with CLL and mantle cell lymphoma, with 4-year PFS and OS of 47 and 73%, respectively [112]. Using high-throughput sequencing-based MRD quantification, we demonstrated in a series of 40 consecutive high-risk CLL patients receiving allografts following TLI/ATG conditioning that sustained, deep remissions with no disease detectable at a 1:1,000,000 detection limit can be achieved [57].

Allogeneic HCT for CLL: patient selection

This chapter has reviewed CLL response to autologous and allogeneic HCT. Clinical prognostic criteria, including advanced Rai and Binet stage, and discrete molecular alterations, including cytogenetic abnormalities (17p and 11q deletions), unmutated IGH status, and specific immunophenotypic markers, including elevated ZAP-70 and CD38 expression, are associated with poor outcome following conventional chemotherapy treatment. Figure 59.1 presents a flow diagram incorporating clinical and molecular prognostic evaluation to guide risk-stratified treatment recommendations. It is clear that GVL alloimmunity following RIC allogeneic HCT can provide durable disease control, even for patients with poor-risk CLL, and RIC allogeneic HCT potentially cures CLL. Thus, identifying patients who warrant this approach is important so that appropriate escalation of care can commence in a timely manner.

Low risk patients have early-stage disease, with good prognosis cytogenetic findings ($13q^-$ without other changes) and mutated IGH status. At the time of diagnosis, the majority of patients have low-risk disease, and can be reassured that they are likely to have an initial asymptomatic period, and can be expectantly observed off therapy. They should be educated to recognize signs of disease progression, including lymph node enlargement, constitutional symptoms, and rapidly increasing lymphocytosis. Their clinical evaluations, including complete blood count analyses, should be obtained every 3–6 months with repeat prognostic evaluations performed when disease progresses clinically. Progressive alterations of cytogenetic and immunophenotypic characteristics suggest that CLL undergoes clonal evolution in association with more aggressive disease behavior, and supports repeating CLL molecular assessment when disease progression or treatment failure necessitates new therapy.

Intermediate-risk patients have normal cytogenetics or trisomy 12, in addition to mutated IGH gene status. Intermediate-risk disease is also defined by clinical progression scored by the Rai and Binet intermediate stages and their symptomatic problems warrant standard combination chemo-immunotherapy treatments, including purine analog therapy combined with anti-CD20 monoclonal antibodies, with front-line preference for the FCR regimen, or possibly BR in selected patients unable to tolerate FCR. The goal of this combination therapy is symptomatic relief. Those patients with persistent CLL or who have progression within 2 years of fludarabine-based therapy should be considered for more aggressive salvage therapies, clinical trial protocols, or RIC allogeneic HCT, since fludarabine-refractory patients have a 10-month median survival and only 22% respond to first-line salvage chemotherapy [115]. Once a CLL patient achieves CR, they should be closely followed with routine clinical and CBC assessment for evidence of disease progression every 3 months.

High-risk patients are not defined by their clinical stage, but are strongly predicted by cytogenetic abnormalities $11q^-$ and $17p^-$, unmutated IGH gene status, and IGH V3–21 usage. Such CLL patients may demonstrate additional poor prognostic factors defined by their clinical course: resistance or incomplete response to fludarabine [63,115] and short time from diagnosis to first therapy (<12 months) [116]. Thus, in Figure 59.1, high-risk patients follow a treatment course that begins with standard combination therapy to cytoreduce their disease, and once they achieve adequate disease control, they should immediately be considered for RIC allogeneic HCT. RIC allogeneic HCT does not include high-dose chemotherapy and high-risk patients who do not achieve adequate disease control (PR or better with lymphadenopathy <5cm in largest dimension) should proceed to additional salvage therapies, including alemtuzumab containing [e.g., cyclophosphamide, fludarabine, alemtuzumab, and rituximab (CFAR)] [117] or platinum doublet-containing regimens [e.g., oxaliplatin, fludarabine, cytarabine, and rituximab (OFAR)] [118], novel agents, or clinical trials to gain better disease control before proceeding to RIC allogeneic HCT.

Treatment of post-transplant relapse

Although overall outcomes have improved significantly with the use of RIC allogeneic HCT for high-risk CLL patients, 35–50% of patients will still experience disease relapse following transplant. Post-transplant relapse may be successfully managed using immunomodulation strategies, including immune suppression withdrawal (ISW), donor lymphocyte infusion (DLI), chemotherapy and/or antibody therapy followed by DLI, or a clinical trial. Rondón et al.

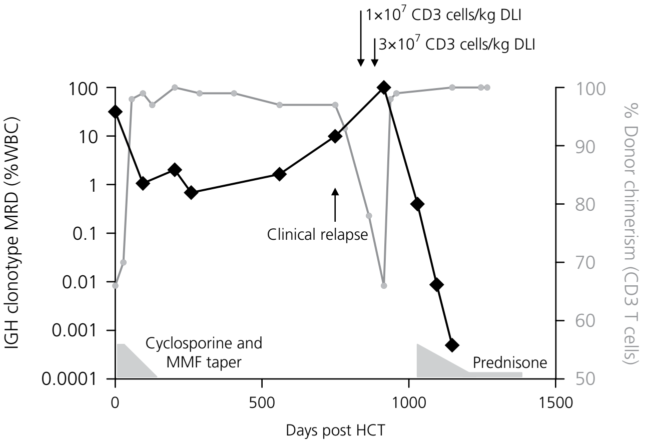

Figure 59.2 GVL effect. This graph shows the course of a 60-year-old man diagnosed with Rai stage III CLL with 17p deletion and unmutated IGH, who was treated with several chemotherapy regimens (FCR, R-CHOP, CFAR, OFAR), achieving only short-lived PRs. He proceeded to RIC allogeneic HCT using TLI/ATG conditioning and a matched unrelated donor. At the time of conditioning, he had persistent CLL, including bulky lymphadenopathy and 20% bone marrow infiltration. CR was achieved for roughly 2 years. Subsequent relapse was effectively controlled with two DLIs.

reported a case in 1996 in which post-allograft CLL relapse went into complete remission following a DLI 87 days after transplant [119]. Español et al. demonstrated in 2003 that even Richter's transformation occurring after allogeneic HCT can be effectively treated with ISW and DLI [120]. An example of this GVL effect is shown in Figure 59.2. Since these early reports, many of the studies discussed above have reported the use of immunomodulation and DLI to achieve durable complete remissions in CLL patients relapsing after allogeneic HCT. The roughly 20–25% gap between 4–5-year PFS and OS observed in several studies is due, in part, to the ability to salvage a substantial proportion of relapsed CLL patients using DLI. In fact, in a meta-analysis of DLI activity in different hematologic malignancies, CLL had the highest likelihood of response (55%) in comparison with non-Hodgkin lymphoma (52%), Hodgkin lymphoma (37%), acute lymphoblastic leukemia (27%), and multiple myeloma (26%) [121]. Khouri et al. treated half of the 86 patients in their study with post-HCT ISW and DLI, with a median number of additional cell infusions of two (range 1–8) starting roughly 6 months (range 63–909 days) after HCT [111]. Of these patients, 47% subsequently achieved a CR and the median survival for this group had not yet been met with 47 months of follow-up (range 5.4–110.2 months). In contrast, the median survival for those not achieving CR with DLI was 35 months. The most important determinants associated with favorable response to immunomodulation were achievement of complete donor chimerism at day 90 post-HCT and being HLA-A1 positive/HLA-A2 negative/HLA-B44 negative, suggesting that more studies are needed to understand better the parameters affecting GVL alloimmunity to make more effective use of this treatment strategy.

Novel agents in CLL

Post-transplant relapse is a scenario in which participation in a clinical trial or use of novel therapies that have become available is warranted. Several recent treatment innovations are likely to become more widely available in the near future and will be relevant considerations for salvage therapy following CLL relapse post-HCT. Ofatumumab, a humanized anti-human CD20 antibody, has activity in rituximab- and alemtuzumab-refractory CLL and may be a reasonable consideration [122], although there are not yet any published data in the post-allogeneic HCT setting. Lenalidomide, when used in conjunction with rituximab, has shown promise for the management of relapsed/refractory CLL, with a 66% overall response rate [123], and may be capable of inducing complete molecular remission [124]; however, its use in the post-allograft setting may be associated with induction or worsening of GVHD [125,126]. Small molecular inhibitors of B-cell receptor signaling also promise to be of potential use for patients with post-HCT CLL relapse. Ibrutinib, an irreversible inhibitor of Bruton's tyrosine kinase available as an oral medication, is well tolerated and highly active in relapsed/refractory CLL [127,128], and if preliminary data are confirmed, this agent may play a critical role in the management of CLL post-HCT relapse. Indeed, these and other novel agents will likely become increasingly prominent in the treatment of CLL prior to consideration for allogeneic HCT, especially those, such as ibrutinib, that appear to be active in 17p-deleted CLL [34]. Only long-term follow-up and appropriate comparative trials will be able to determine whether novel agents will obviate the utility of allogeneic HCT in high-risk CLL.

Cell therapy

As discussed above, CLL is susceptible to GVL alloimmunity, but conventional allogeneic HCT also conveys the risk of GVHD. The ability to redirect autologous or allogeneic T-cell activation in response to specific antigens has recently been demonstrated by the introduction of chimeric antigen receptors (CARs). This is discussed in Chapter 69.

Conclusion

CLL is a heterogeneous disease at the molecular and clinical levels. Strategies for identifying high-risk patients now permit early consideration of transplant therapies for young and appropriate elderly patients. Although autologous HCT is feasible and associated with good outcomes, this approach does not appear to cure patients of their CLL, and the procedure is associated with a high rate of secondary malignancies. Myeloablative allogeneic HCT may cure some patients, but several studies have demonstrated that this benefit comes at an unacceptable cost in the form of very high non-relapse mortality. RIC regimens appear to offer benefits of engraftment with less toxicity and opportunities to achieve disease control via GVL activity with or without post-HCT immunomodulation. A wide array of novel treatments for CLL have recently shown early-phase efficacy, and we expect the next many years to be a period of dynamic change in common treatment algorithms for patients with CLL. Increasingly standardized molecular and/or MPFC-based MRD quantification will enable depth of response to be more precisely quantified and early progression to be detected, and it is likely that these methods will eventually facilitate the identification of patients likely to require GVL alloimmunity for adequate disease control. In the meantime, existing patient selection criteria should be used for identifying those who may benefit from RIC allogeneic HCT so that preparations for this therapy may be made prior to the development of highly treatment-refractory disease.

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CHAPTER 60

Autologous Hematopoietic Cell Transplantation for Systemic Light Chain (AL-) Amyloidosis

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Introduction

The amyloidoses are diseases in which abnormal proteins self-assemble and form extracellular deposits of insoluble non-branching linear fibrils that are 7–10nm in width, vary in length, and resist proteolysis [1]. Medical investigators at autopsy recognized such deposits over three centuries ago and thought that the material was a fatty substance, hence the term “lardaceous disease” [2]. In 1854, the pathologist Virchow described the deposits in postmortem liver tissue with the botanical term “amyloid,” meaning starch, because of the starch-like affinity that the deposits had for iodine. When viewed in visible light after hematoxylin–eosin staining, the deposits appear amorphous, hyalinized, and eosinophilic, but when viewed in polarized light after Congo Red staining, the deposits exhibit a characteristic color change from hyaline pink to flaky yellow–green, a dichromic or birefringent feature first reported in 1927 (Figure 60.1) [3]. A century after Virchow, the fibrillar nature and characteristic β-pleated sheet configuration of amyloid were identified by electron microscopy (Figure 60.1) [4,5]. In the 1970s, researchers established that all amyloid variants have fibril-precursor proteins which self-assemble into filaments and then fibrils [6,7]. Directly determining the type of amyloid in a patient’s tissue biopsy, however, remained a challenge until decades later when laser microdissection (LMD), trypsin digestion, and mass spectrometry (MS) of protein fragments with bioinformatic analysis permitted the definitive determination of type, provided that a patient’s diagnostic biopsy showed amyloidosis by Congo Red staining and contained sufficient deposits amenable to microdissection [8].

The type of amyloid is a function of the fibril-precursor protein that causes the deposits. Currently, the classification system designates the amyloid-forming protein by the first letter A for amyloid and subsequent letter(s) for the fibril-precursor protein; for example, L for light chain (AL) and TTR for transthyretin (ATTRm or ATTRwt; m = mutant or hereditary and wt = wild-type) [9]. The type of amyloidosis also designates the disease. In patients with amyloidosis associated with chronic inflammatory conditions (so-called “secondary” or AA), the fibril-precursor protein is the acute-phase apolipoprotein serum amyloid A (SAA). The gene encoding SAA has three variants, only one of which is associated with AA. In patients with hereditary amyloidosis, the fibril-precursor protein is often a mutant form of the homotetrameric protein TTR, a transporter of thyroxine and retinol. Point mutations cause amino acid substitutions that facilitate dissociation of the tetramer into monomers, conformational instability of the non-native monomers, and monomer misfolding with subsequent self-assembly. Other mutant proteins can also cause hereditary amyloidosis, including fibrinogen Aα, apolipoproteins I and II, and lysozyme. More than 100 mutant forms of TTR have been identified as causes of ATTRm hereditary amyloidosis [10]. In patients with age-related amyloidosis (also called senile systemic or senile cardiac amyloidosis), who are almost exclusively elderly men, the fibril-precursor protein is wild-type TTR (ATTRwt) [11]. Dissociation likely occurs as a consequence of abnormal glycosylation. The etiology of this age-related form of amyloidosis is poorly understood but, in contrast to the rapidly progressive clinical course of cardiac amyloidosis due to AL, ATTRwt with cardiac involvement typically has a more indolent course with atrial arrhythmias and heart failure that worsen gradually over years. Therapies currently available for both mutant and wild-type forms of TTR-related amyloid disease stabilize the tetramer and impair the accumulation of misfolded monomers [12].

In patients with light chain (AL-) amyloidosis, the fibril precursors are usually free light chains (FLCs) possessing an abnormal tertiary structure. These FLCs both aggregate into oligomers that may be toxic to cellular metabolism and assemble as filaments of β-pleated sheets that coil together to form relatively insoluble fibrils. Both AL-amyloidosis and multiple myeloma are clonal plasma cell disorders, but what distinguishes symptomatic AL-amyloidosis from symptomatic multiple myeloma is the pattern of organ damage. AL-amyloidosis typically involves the heart, causing a restrictive cardiomyopathy, the kidneys in the form of albuminuria due to glomerular amyloid deposits, the liver and gastrointestinal tract, and the peripheral nervous system. Myeloma typically involves bones in the form of lytic disease and hypercalcemia, kidneys in the form of Bence Jones proteinuria and cast nephropathy resulting in acute kidney injury, and bone marrow hematopoiesis, causing anemia and other cytopenias. It is not typical for the clinical course of a patient with AL-amyloidosis to be significantly impacted by associated myeloma organ damage. Patients with AL-amyloidosis who do not have myeloma at presentation have a small risk of subsequent evolution to frank multiple myeloma and 10–15% of

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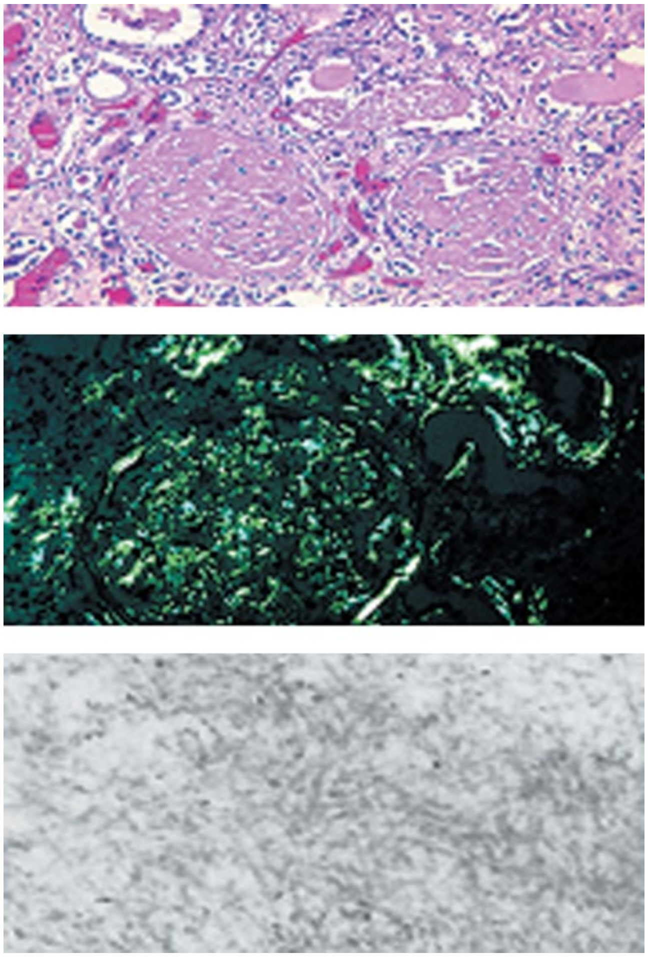

Figure 60.1 Renal biopsy showing amyloidosis. These images show three views of a renal biopsy: with periodic acid–Schiff (PAS) staining showing amorphous material in glomeruli (top), Congo Red staining showing apple-green birefringence in polarized light (middle), and electron microscopy showing non-branched linear fibrils about 10nm in length. (For a color version of this figure, please see the color plate section.)

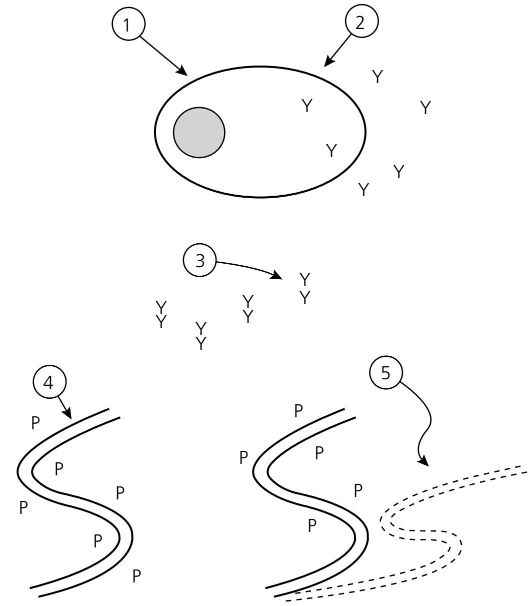

Figure 60.2 Theoretical remedies for AL-amyloidosis. There are a number of points at which intervention could, in theory, control AL-amyloidosis: (1) reducing the number of plasma cells with cytoreductive therapy; (2) reducing the secretion of clonal free light chains with drugs; (3) impairing the self-assembly and aggregation of light chains; (4) inhibiting fibril formation in tissues; and (5) dissolving or enhancing the proteolysis of amyloid fibrils. Remedies that reduce the number of plasma cells include standard- and high-dose chemotherapy. Although it is currently not available, 4-iodo-4-deoxydoxorubicin (IDOX) may enhance the proteolysis of fibrils. The other possible remedies remain theoretical.

myeloma patients may develop amyloidosis during the course of their disease. Furthermore, at diagnosis high-risk AL-amyloidosis is defined based on the extent of cardiac involvement, whereas high-risk myeloma is defined based on the genetics of the clonal disease. The distribution of heavy and light chain isotypes, and also the distribution of clonal cytogenetics and fluorescent in situ hybridization (FISH) abnormalities, differ in AL-amyloidosis and myeloma; however, the agents and approaches used to treat them are similar. In AL-amyloidosis, 75% of the pathologic clones are λ isotype whereas in myeloma only 40% are, a difference that reflects an intrinsic amyloid-forming propensity of λ light chains.

AL-amyloidosis is a disorder both of protein conformation and of clonal plasma cells [13]. In theory, the treatment of AL-amyloidosis could be directed at various aspects of its pathogenesis (Figure 60.2). Most frequently, as in myeloma, therapies have aimed to reduce or eliminate clonal plasma cells, the source of the aberrant FLCs. By the mid-1990s, no significant progress had been made with standard chemotherapy in reversing the inexorable progression of systemic AL-amyloidosis, not surprisingly in view of the visceral dysfunction it causes. At that time, because the efficacy of autologous hematopoietic cell transplantation (HCT) had been demonstrated in myeloma, HCT was tested in systemic AL-amyloidosis [14,15]. When HCT was successful, the organ damage could be reversed, the production of deposits halted, and prior amyloid deposits slowly resorbed. Organ function, performance status, and quality of life improved [16,17]. The achievement of a hematologic complete response (CR), as defined for that era, led to clinical and functional improvement and prolonged survival; however, in the early clinical trials, transplant-related mortality was high because the organ reserve of patients was compromised by cardiac, gastrointestinal, renal, and neuropathic amyloid involvement. This made patients with AL-amyloidosis different from other stem-cell transplant candidates. The evolution of HCT for patients with AL-amyloidosis has been characterized by refinement of patient selection, improvement of peri-transplant clinical management, and recently by application of post-HCT adjuvant therapy with novel agents and the development and application of validated consensus response criteria [18–21]. In this chapter, we describe the epidemiology, pathogenesis, and distinctive clinical aspects of this disease, offer an approach to the evaluation of patients with AL-amyloidosis, and summarize recent clinical research in HCT for AL-amyloidosis.

Epidemiology

The epidemiology of AL-amyloidosis is difficult to define because the disease often goes undiagnosed. Data from tertiary referral centers are not necessarily representative. AL-amyloidosis is a rare disorder with an age-adjusted incidence estimated to be 5.1–12.8

per million person-years, resulting in approximately 1275–3200 new cases annually in the United States and 255–640 cases in the United Kingdom, an incidence similar to that of chronic myelogenous leukemia or Hodgkin disease. AL-amyloidosis appears to be more common in men, but no links with race, occupation, or environmental exposures have been reported. Summary data on over 800 patients from centers in six countries are shown in Table 60.1 [21].

Pathogenesis

The final pathway in the pathologic process of AL-amyloidosis is the formation and deposition of fibrils [22]. When the AL amyloid subunit proteins are solubilized and deciphered by amino acid sequencing, the dominant constituents of deposits are the light chain variable and constant regions and less frequently the whole immunoglobulin [8,23,24]. Although the reason why some FLCs form amyloid remains unknown, comparisons of the primary structures of normal and amyloid-forming FLCs have provided some insight based on the aberrant properties of the pathologic FLCs [25]. Analyses of primary structure have emphasized the critical contribution of uncommon amino acid substitutions to the stability and interactive properties of misfolded or partially folded light chains [26]. Such partially folded intermediate forms may be prone to self-assemble. The identification of potentially destabilizing uncommon amino acid substitutions at unique positions in amyloid-forming FLCs has supported this hypothesis. Purified urinary light chains from patients with AL-amyloidosis reproduce fibrillar disease when injected into mice, whereas those from myeloma patients without AL-amyloidosis do not [27].

If the pathogenesis of AL-amyloidosis were a function of fibrillar amyloid burden, however, patients with minimal tissue deposits would be relatively asymptomatic. However, this is not always the case. A patient's clinical status is not determined simply by amyloid burden. Patients with peripheral nervous system or cardiac involvement can have minimal tissue amyloid but experience symptomatic organ dysfunction. Conversely, a significant fraction of patients who respond to treatment with elimination of the monoclonal FLCs do not show a reduction in amyloid burden despite clinical improvement and abatement of symptoms, when burden is evaluated with serial iodine-123-labeled serum amyloid P protein (SAP) total body scintiscans [28]. Such observations have led to the view that amyloid fibrils per se may not be the sole pathologic basis of the disease [29,30]. Moreover, experiments in vitro with several fibril-precursor proteins have shown that intermediate oligomers or oligomeric aggregates may be toxic to cells [31,32]. This has led to the view that fibrils may represent a more benign form of the precursor protein and may provide relative protection from cellular toxicity until the burden of fibrils overwhelms the involved organ [1]. The relationship between oligomers and fibrils in the various types of amyloidosis remains undefined, and the mechanistic role of intermediates in the disease is unclear. Therefore, we currently live with the paradox that while pathologic FLCs cause symptoms in patients with AL-amyloidosis, we cannot make the diagnosis of AL-amyloidosis without evidence of tissue deposits.

Table 60.1 Patient characteristics (*N* = 816) (data pooled from six countries) [21]

Characteristic	*n* (%) or median (25th–75th centile)
Evaluating center	
Pavia, Italy	208 (25.5)
Rochester, MN, USA	151 (18.5)
London, UK	150 (18.4)
Heidelberg, Germany	139 (17.0)
Boston, MA, USA	86 (10.5)
Athens, Greece	43 (5.3)
Limoges, France	39 (4.8)
Age (years)	63 (55–71)
Gender	
Male	489 (59.9)
Female	327 (40.1)
Organ involvement (number)	2 (1–2)
Kidney	556 (68.1)
Heart	529 (64.8)
Peripheral nervous system	153 (18.7)
Liver	131 (16.0)
eGFR (792 patients)	
≥90 mL/min/1.73 m^2	175 (22.1)
60–89 mL/min/1.73 m^2	296 (37.4)
30–59 mL/min/1.73 m^2	199 (25.1)
15–29 mL/min/1.73 m^2	67 (8.5)
<15 mL/min/1.73 m^2 or dialysis	55 (6.9)
Urinary protein loss (g/24 h)	2.9 (0.4–7.7) (800 patients)
NYHA class 3 or 4	156 (26.8) (582 patients)
Cardiac staging [129]	(517 patients)
Stage I	160 (30.9)
Stage II	226 (43.7)
Stage III	131 (25.4)
NT-proBNP (ng/L)	1587 (351–4670)
NT-proBNP ≥650 ng/L	452 (65.7) (688 patients)
cTnI (ng/mL)	0.05 (0.02–0.09) (276 patients)
cTnT (ng/mL)	0.01 (0.01–0.05) (303 patients)
Positive serum and/or urine immunofixation	725 (94.2) (770 patients)
Heavy chain type	(770 patients)
G	299 (38.8)
A	77 (10.0)
M	23 (3.0)
D	5 (0.6)
Light chain only	366 (47.6)
Quantifiable (i.e. ≥10 g/L) complete MC	249 (61.6)
Monoclonal protein concentration (g/L)	10.8 (5.0–25.5) (404 patients)
Involved light chain type	
κ	201 (24.6)
λ	615 (75.4)
Abnormal κ/λ ratio	677 (84.8) (798 patients)
iFLC (mg/L)	178 (90–477) (792 patients)
dFLC (mg/L)	157 (70–460) (792 patients)
Bone marrow plasma cell (%)	10 (5–16) (452 patients)
Treatment type	
Melphalan + dexamethasone	364 (44.6)
Autologous stem-cell transplant	129 (15.9)
Thalidomide based	119 (14.6)
Lenalidomide based	43 (5.3)
Bortezomib based	26 (3.2)
Dexamethasone alone	24 (2.9)
Melphalan + prednisone	20 (2.4)
Other	91 (11.1)

How amyloid fibrils cause organ dysfunction is not known, and how fibrils are metabolized and organs improve is no less a mystery. The liver, for example, can tolerate a large burden of amyloid without failing and, once treatment eliminates the supply of FLCs, the liver can respond with restoration of normal synthetic function and dramatic regression to normal size. These changes can happen over months. This is attributed to the regenerative capacity of the liver but begs the question as to how the fibrils are removed. Removal, when it occurs, may be related to the presence of other components of the fibrillar deposits. The interactions between amyloid-forming FLCs and the passenger constituents of amyloid deposits, such as glycosaminoglycans and apolipoproteins, may be related to the amino acid variations noted earlier, and may influence the course of disease by stabilizing filament and fibril formation and by modifying the sensitivity of fibrils to proteolysis [33]. These passenger constituents are reliably identified in the amyloid deposits evaluated by LMD/MS and provide an internal validation or control with respect to the accuracy of the technique. A major component of amyloid deposits is serum amyloid P-component (SAP), a member of the pentraxin protein family that is highly resistant to proteolysis and likely acts as a pathologic chaperone for fibrils [34]. SAP employs a calcium-dependent binding mechanism in its interactions with amyloid fibrils and may contribute to the occurrence of deficiencies in calcium-binding vitamin K-dependent coagulation factors, most notably Factor X, a clinical feature associated with hepatosplenic amyloidosis.

Monoclonal FLCs and minimal bone marrow plasmacytosis are detected in the majority of patients with AL-amyloidosis, often in association with suppression of non-involved immunoglobulin production [21,35]. About 60% of patients have marrow biopsies showing 10% or fewer clonal plasma cells [36]; 11% of patients with amyloid have over 20% plasma cells with no evidence of myeloma-defining organ damage. When a clonal immunoglobulin heavy chain is detectable in the serum, it is IgM in 7% of cases. The presence of IgM amyloidosis is not well recognized; these patients usually do not have Waldenström macroglobulinemia [37]. The clones that cause AL-amyloidosis are distinct from myeloma clones with respect to their repertoire of immunoglobulin light chain variable germline (Ig *VL*) genes, but similar in that their immunoglobulin genes are highly mutated (i.e., they are antigen-driven or post-germinal center clones) [38–40]. The repertoire of Ig *VL* genes in AL-amyloidosis is skewed, unlike that in myeloma, which is similar to the normal expressed repertoire. Half of AL clones employ one of three immunoglobulin λ light chain variable region germline genes: *IGLV2-14* [*2a2*; Z73664], *3r* (Z73647) or *IGLV6-57* [*6a*; Z73673]. AL clones using genes derived from the *3r* germline donor have a significantly higher divergence from the germline sequence than those using the *IGLV6-57* donor, a difference possibly related to both antigen selection and the inherent amyloid-forming propensity of IGLV6-57 light chains [38,41].

A puzzling feature of AL-amyloidosis is the variety of organs that can be affected. Although the basis for this tropism is unknown, evaluations of the immunoglobulin light chain genetics underlying AL-amyloidosis suggest that some light chain variable region germline donor genes are more likely to give one pattern of organ involvement than another [38,42]. Using the reverse transcriptase polymerase chain reaction to clone amyloid-forming FLC genes, and available databases for germline gene identification, several investigators have sought to test the hypothesis that the light chain variable region genes used by AL-amyloidosis plasma cell clones influenced organ tropism. In one series, light chain variable region germline genes were identified from 60 patients with AL-amyloidosis. Dominant renal involvement was linked to clones whose FLCs were derived from the *IGLV6-57* germline gene, while dominant cardiac and multisystem disease was linked to those with FLCs derived from the *IGLV2-14* and *3r* genes [38]. Despite these suggestive data, we do not know how the assembly of abnormal FLCs causes progressive organ dysfunction and clinical disease, often at a rapid tempo, nor do we know how partially folded light chain intermediates may be more toxic to cellular metabolism than fibrillar deposits [43].

Clonal AL plasma cells harbor recurring cytogenetic abnormalities, including t(11;14), del 13q and gain 1q, while t(4;14) and del 17p are not typically seen [44–46]. Interestingly, the t(11:14) was associated with worse overall survival (OS) in a series of AL-amyloidosis patients, in contrast to its association in myeloma with a relatively favorable prognosis [46]. Moreover, overexpression of *cyclin D1* in purified AL plasma cells occurs in half of patients and is associated with a high frequency of light chain M-proteins and kappa light chain-producing clones [47]. Trisomies of chromosomes 7, 9, 11, 15, and 18 have also been reported in 33–52% of patients, and trisomy X in 13% of women and 54% of men. Immunoglobulin heavy chain translocations have been reported in 55% of patients, and abnormalities of chromosome 13 are frequent [48].

Diagnosing AL-amyloidosis

The most common symptoms of amyloidosis are fatigue, weight loss, dyspnea, edema, and paresthesias. The fatigue, generally caused by cardiac involvement, may be misdiagnosed as functional or stress related since the usual features of congestive heart failure are absent. Light-headedness is common. It can occur in patients with nephrotic syndrome with hypoalbuminemia and contraction of plasma volume, and in patients early in the course of cardiac amyloidosis due to poor diastolic filling, a reduced stroke volume, and autonomic dysfunction. Often such patients have orthostatic hypotension but a normal echocardiographic ejection fraction. Amyloidosis patients with autonomic neuropathy also often have orthostatic hypotension, frequently associated with syncope. Amyloidosis should be considered when patients with a monoclonal gammopathy complain of fatigue, weight loss, and vague constitutional symptoms.

The physical findings of amyloidosis are specific but not sensitive. Purpura are seen in only one patient in six, most commonly on the eyelids, upper face, and webbing of the neck. A palpable liver is present in one-quarter of patients and may be due to infiltration of the liver or congestion related to elevated right-sided cardiac pressures. Enlargement of the tongue is the most specific finding, but is seen in only one patient in 10. It is easily recognized because it results in dental indentations on the tongue laterally and inferiorly, and enlargement of the submandibular salivary glands, often misdiagnosed as enlarged lymph nodes. An occasional patient will present because of vascular claudication due to small vessel occlusion with amyloid deposits.

Because the symptoms and physical findings are not diagnostic of amyloidosis, it is important to recognize the clinical syndromes associated with AL-amyloidosis. The seven most common presentations are infiltrative cardiomyopathy, nephrotic-range proteinuria, hepatomegaly, demyelinating peripheral neuropathy, carpal tunnel syndrome, macroglossia, and intestinal pseudo-obstruction. When one of these clinical syndromes is seen, the next step is to perform serum FLC and immunofixation studies of serum and urine. A screening serum protein electrophoresis is inadequate because of the high prevalence of light chain-only disease. Since many patients have significant degrees of albuminuria, an M-protein peak may be obscured in the urine protein electrophoresis, necessitating immunofixation. Serum FLC and/or immunofixation studies will be abnormal in over 95% of patients, and the serum FLC assay has demonstrated abnormal circulating FLCs even when the serum and urine immunofixation studies are negative [49].

Since AL-amyloidosis is usually a plasma cell disease, the finding of a monoclonal light chain in a patient with a consistent clinical syndrome is an indication to obtain a tissue biopsy for diagnosis. Rarely, AL-amyloidosis can be associated with mature B-cell non-Hodgkin lymphomas that produce clonal FLCs [50].

FLCs unassociated with heavy chains are produced in slight excess by normal plasma cells and in significant excess at times by clonal plasma cells. FLCs circulate in normal individuals and can be measured with the FLC assay. This assay is quantitative, unlike immunofixation, and is a more sensitive measure of the presence of clonal disease than immunofixation in many cases. FLC levels and ratios are abnormal in more than 95% of patients with AL-amyloidosis, and the elevated FLCs usually are the precursor protein [51,52]. When the diagnostic evaluation includes serum FLCs, serum and urine immunofixation, and clonal analysis of marrow biopsy for plasma cells, all patients with AL-amyloidosis should be recognized. If evidence for clonal plasma cell disease cannot be detected, the type of amyloidosis remains in question, and one should consider forms of non-AL-amyloidosis such as localized, age-related, or hereditary [53].

The diagnosis of amyloidosis is made by biopsy, as is standard for patients with malignancy. Patients with visceral involvement of the kidneys, heart, liver, or soft tissues may have the diagnosis confirmed by involved organ biopsy. The nature of amyloid, however, is that there is widespread deposition in blood vessels at diagnosis, and less invasive biopsies will readily establish the diagnosis. The abdominal fat is a validated surrogate site and will contain Congophilic amyloid deposits in 80% of patients, although false positives due to overstaining can sometimes be seen [54]. A bone marrow biopsy will demonstrate Congophilic deposits in 56% of patients, although marrow is not considered a valid surrogate site [55]. Other sites that can be safely biopsied include the skin, minor salivary glands, and rectum. Visualizing amyloid in visceral biopsies with both Congo Red staining and electron microscopy enhances diagnostic confidence.

The evaluation at diagnosis includes testing for organ involvement and, if indicated, for type of amyloid. Concerns about whether a patient has AL-amyloidosis or another type are warranted because myelotoxic chemotherapy has no place in the treatment of non-AL-amyloidosis, and also because failure to diagnose a hereditary disease has consequences [56,57]. Family history is not a useful guide for hereditary disease and, with the exception of renal biopsies, tissue staining for amyloid typing is unreliable. Investigators at two centers have reported patient series (n = 178, n = 350) in which 2–10% of patients presumed to have AL-amyloidosis had both a monoclonal gammopathy and a hereditary mutant protein, either transthyretin or fibrinogen Aα. Of note, a hereditary mutant transthyretin (the valine-122-isoleucine variant) occurs in 4% of African-Americans, a racial group that also has a higher incidence of monoclonal gammopathy [58]. Therefore, African-Americans with amyloid and a monoclonal gammopathy need to have their amyloid typed. We also recommend amyloid typing in patients presenting with a monoclonal gammopathy and either peripheral neuropathy or a concurrent immune disorder such as Sjogren syndrome or rheumatoid arthritis. Patients with two possible amyloid-forming proteins should be referred to centers specializing in amyloidosis for typing and treatment. Amyloid typing is also indicated in patients with amyloid by tissue biopsy with no apparent amyloid-forming protein.

The gold standard for typing amyloid is based on modern proteomic techniques. In the United States, LMD/MS, a Clinical Laboratory Approved Amendments (CLIA) approved test, employs customized bioinformatic assessment of the protein fragments from digested and fractionated Congophilic tissue deposits to identify type reliably in over 98% of cases [8]. LMD/MS is specifically indicated in cases where two potential amyloid precursor proteins are present or where none is apparent. Importantly, LMD/MS identifies the chaperone and fellow-traveler elements in amyloid deposits and also the amyloid-forming protein, providing an internal validation unavailable for any other tissue or protein-based technique. DNA sequencing of genes related to hereditary variants is also a useful tool, particularly for confirming proteomic findings and screening family members. It is important to note that LMD/MS and other proteomic techniques provide probability-based read-outs of protein constituents of Congophilic deposits, hence the use of multiple samples from one specimen or of tissue controls from normals [59]. Occasionally, serum proteins, particularly very low levels of kappa free light chains, can be identified in the read-outs, likely representing serum protein contamination; in our experience, however, these low-level contaminants can be discounted with respect to typing and treatment, although we remain curious as to their significance.

The practitioner who rarely sees patients with amyloidosis must also be sensitive to the occurrence of localized AL- or systemic age-related amyloidosis (ATTRwt). The skin, larynx, tracheobronchial tree, lungs, duodenum, and bladder are the most common sites of localized AL-amyloidosis. Localized rarely progresses to systemic disease. Age-related systemic amyloidosis usually presents with less aggressive cardiac involvement in men over 70 years of age, although the lungs may be symptomatically involved and progression can be rapid in rare cases [60]. Patients who may have localized or age-related systemic amyloidosis should also be referred to centers specializing in amyloidosis for diagnostic confirmation and recommendations regarding therapy and supportive care.

Clinical presentations

Heart

Heart involvement carries the most serious prognosis because its extent directly impacts survival [61]. The pathophysiology is infiltration of the myocardial wall leading to restriction of ventricular filling and extensive vascular involvement causing ischemia and myocardial cell drop-out. Vascular deposits in small coronary arterioles can be demonstrated on endomyocardial biopsy and can produce angina.

Patients may present with disabling fatigue and unexplained weight loss as the only symptoms. The electrocardiogram can show low voltage with no prior history of an ischemic event. The echocardiogram shows thickening of the wall, commonly misinterpreted as concentric left ventricular hypertrophy rather than infiltration. The ejection fraction is usually preserved. Thickening of the mitral and tricuspid valves due to amyloid infiltration is often an important clinical clue. Exercise-induced syncope has been associated with a median survival of 2 months [62]. Any patient with intractable fatigue or an echocardiogram showing a thickened wall needs to have studies for the presence of a monoclonal protein in the serum or urine.

A role for cardiac biomarkers has been established in AL-amyloidosis [63,64]. A staging system using both N-terminal fragment of pro-brain natriuretic peptide (NT-proBNP) and troponin has been developed [65]. Cardiac involvement is prevalent; therefore, these biomarkers are useful in screening. Patients with pronounced elevations of both NT-proBNP and troponin have a poor prognosis [66]. These biomarkers are useful in the management of patients and can decrease in cardiac patients when the clonal FLCs are reduced with therapy. Because cardiac involvement is the major determinant of survival and changes in cardiac function following therapy can be reliably assessed using NT-proBNP levels, changes in FLCs and NT-proBNP predict survival as early as 3 months after treatment initiation [21].

The mainstay of management of cardiac amyloidosis is diuretics. Many patients will have associated orthostatic hypotension and intravascular volume contraction secondary to nephrotic syndrome, making diuretic therapy difficult. The use of diuretics may precipitate syncope and can reduce renal blood flow, with a resultant rise in the serum creatinine level. The high frequency of hypotension in AL-amyloidosis can make the use of angiotensin-converting enzyme inhibitors and receptor blockers difficult. Also, stasis frequently leads to thrombi in the right and left ventricles, potential sources of embolism that may complicate the course of HCT. These thrombi can occur even in patients in normal sinus rhythm.

Kidneys

Over 60% of patients with AL-amyloidosis will have renal involvement [21], and the most common presentation of renal amyloidosis is albuminuria. About 30% of all patients will have proteinuria above 3g/day [36]. Amyloidosis is demonstrated in 3% of renal biopsies [67]. The major clinical consequences of renal amyloid are severe serum hypoalbuminemia and eventual renal failure. The loss of albumin results in reduced intravascular oncotic pressure and edema of the lower extremities and presacral area and, in severe cases, can lead to anasarca, ascites, and pleural effusions. The edema generally requires diuretics for control, but diuresis may aggravate intravascular volume contraction and exacerbate hypotension. The principal long-term complication of continuous urinary protein loss in renal amyloid is tubular damage that results in end-stage renal failure. One of the goals of HCT is to eliminate the high urinary protein losses and therefore delay or prevent the need for dialysis. The presenting 24-hour urine protein and serum creatinine predict which patients will ultimately develop end-stage renal disease. In historical cohorts, the median time from diagnosis of AL-amyloidosis to dialysis was 15 months and the median survival from the start of dialysis was 8 months. This poor outlook justified the use of HCT in patients with single-organ renal involvement. The extent of amyloid deposits seen on a renal biopsy does not correlate with the severity of proteinuria and hypoalbuminemia. Limited amyloid deposits can be associated with advanced nephrotic syndrome. The kidneys are normal in size in amyloid. If amyloid does not involve the kidneys at presentation, it rarely occurs during follow-up.

Patients with higher levels of proteinuria have greater morbidity associated with pre-transplant conditioning, including increased fluid retention and transient acute renal failure [68]. Post-HCT in renal responders, median time to response was 10 months (range 1–40 months) [69]. Renal response was associated with a longer survival than hematologic response alone. Severe proteinuria and high troponin negatively affected renal response after autologous stem-cell transplantation. Achievement of renal response was associated with improved survival [69]. Renal transplantation has been performed in patients with AL-amyloidosis and HCT for AL-amyloidosis has been performed successfully both before and after renal transplantation [70,71].

Liver and gastrointestinal tract

Hepatomegaly is found by physical examination in one-quarter of patients with AL-amyloidosis, usually associated with an unexplained elevation of the serum alkaline phosphatase level and early satiety. The majority of patients are symptomatic [72]. Hepatomegaly may be due to amyloid-induced heart failure or direct infiltration of the hepatic parenchyma. Half of the patients with hepatic amyloid have more than 1g of daily proteinuria [72]. The clues that allow the recognition of hepatic amyloid are hepatomegaly out of proportion to the degree of abnormal liver function tests, elevated alkaline phosphatase, a monoclonal protein in the serum or urine, Howell–Jolly bodies in the peripheral blood film (due to splenic infiltration), and proteinuria. Most patients will have only alkaline phosphatase elevation with normal transaminases. The median elevation of the serum alkaline phosphatase value in AL-amyloidosis patients with hepatic involvement is 2.3 times the upper limit of normal. Elevation of the serum bilirubin is generally a preterminal finding. The presence of hyposplenism on the peripheral blood film is specific for splenic amyloid. Rarely, a patient can have splenic or hepatic rupture, or portal hypertension with varices and bleeding. Ascites is usually due to associated nephrotic syndrome, hypoalbuminemia and congestive heart failure, and is not associated with portal hypertension.

Fewer than 5% of patients with AL-amyloidosis will present with symptoms referable to the gastrointestinal tract [73]. The presence of anorexia and weight loss does not correlate with the presence of gastrointestinal amyloid deposits. Malabsorption with steatorrhea is seen in less than 5% of patients. When symptoms are present, they most often include weight loss and bleeding. Patients with advanced gastrointestinal tract involvement can require long-term total parenteral nutrition for management. These patients have intractable nausea and vomiting, and do not respond to enteral feeding or pharmacologic interventions. The most common presenting symptoms are diarrhea, anorexia, dizziness, and abdominal pain. They will frequently have vitamin K deficiency due to malabsorption and low Factor X levels. Computed tomography scanning demonstrates thickening of the bowel wall. There is usually a significant delay from the onset of symptoms to the recognition of amyloid. In our experience, the median time from symptoms to histologic diagnosis is 7 months; a laparotomy may be required to establish the diagnosis. Nutritional failure is the cause of death in over half of the patients; an additional one-quarter die of cardiac amyloid. The diarrhea of amyloid is difficult to treat [74]. Loperamide, diphenoxylate, octreotide, and tincture of opium have all been tried but with limited success. Amyloidosis can present as ischemic colitis with deposits obstructing the vessels of the lamina propria and muscularis mucosa, and lead to chronic mucosal sloughing and hemorrhage. There is a risk for gastrointestinal bleeding in patients who undergo HCT. Gastrointestinal bleeding has been associated with an inferior outcome in HCT [75].

Peripheral nervous system

Peripheral neuropathy is present in one patient in five [36]. The most common symptoms are paresthesias, muscle weakness, numbness, pain, orthostasis, urinary retention, and impotence. In one-quarter of patients, dysesthesias and distal burning accompany the peripheral neuropathy. The lower extremities are involved before the upper extremities in 90% of patients, and two-thirds of patients have autonomic symptoms. Cranial nerve involvement is rare. Carpal tunnel syndrome is seen in half of patients with amyloid peripheral neuropathy, and one-third show significant weight loss. When patients present with amyloidosis, dominant neuropathy, and a monoclonal gammopathy, consideration still needs to be given to the possibility of hereditary amyloidosis and, therefore, amyloid typing with LMD/MS is necessary.

If untreated, the neuropathy is progressive over time. Marked restriction of mobility ultimately develops in three-quarters of patients, and one-third become bedridden. There is often a significant diagnostic delay with amyloid neuropathy. Patients with a peripheral neuropathy should have serum FLC and serum and urine immunofixation studies performed. Finding a monoclonal light chain expands the differential diagnosis to include gammopathy-related neuropathy, POEMS syndrome (*p*olyneuropathy, *o*rganomegaly, *e*ndocrinopathy, *M* protein, *s*kin changes), and cryoglobulinemia. Since amyloidosis preferentially causes the loss of small unmyelinated fibers, an electromyogram can be normal early in the disease course. Because amyloid is often deposited at the level of the nerve root, causing distal demyelination, the sural nerve biopsy is often negative.

Respiratory tract

Involvement of the respiratory tract is usually asymptomatic. Almost 40% of patients with amyloid deposits in the lungs have localized forms and do not have systemic amyloidosis [76]. In patients who have histologic evidence of systemic amyloidosis and lung involvement, the symptoms may be overshadowed by concomitant cardiac involvement. Even when deposits are present, gas exchange in the lungs is preserved until late in the disease. Pulmonary amyloidosis presents radiographically as an interstitial or reticulonodular infiltrate with or without pleural effusion. Bronchoscopic lung biopsy is safe and is not associated with an increased risk for bleeding. The chest X-ray is not specific, demonstrating an interstitial process that can be misinterpreted as fibrosis. In patients who have dyspnea due to interstitial lung disease, low doses of prednisone may produce symptomatic benefit. Pleural infiltration with amyloid can result in pleural effusions. Rarely, pulmonary hypertension develops in association with right-sided heart failure [77].

Hemostasis

Bleeding can be a serious complication of amyloidosis. The most common manifestation of bleeding is skin purpura. Deficiency of Factor X is seen in less than 5% of patients and is associated with hepatic involvement [78]. Normalization of Factor X levels following transplantation for hepatic amyloidosis has been reported. Bleeding associated with Factor X deficiency is generally seen only when the level falls below 25% [79]. Severe Factor X deficiency is associated with increased mortality with HCT [79]. The management of factor X deficiency prior to HCT has included splenectomy and the use of recombinant human Factor VIIa [80]. Rupture of the liver or spleen can also occur in a small fraction of patients with hepatosplenic AL-amyloidosis.

Rarely, thromboembolic disease (TED) is observed in patients with AL-amyloidosis [81]. In a single-center series of 40 patients with AL-amyloidosis and TED, thromboembolism was seen more than 1 month following the diagnosis of AL-amyloidosis in half of the patients. Twenty-nine of the 40 events were venous and 11 arterial. Risk factors for thrombosis included nephrotic syndrome, immobilization, tobacco use, heart failure, and disseminated intravascular coagulation. Five of the 40 patients had activated protein C resistance. The mortality associated with thromboembolism was 20%; 45% of the patients died within 1 year of the thrombotic event.

Prognosis and eligibility for HCT at diagnosis

Unlike multiple myeloma, the organ damage, morbidity, and mortality in AL-amyloidosis are due to the direct effects of the toxic monoclonal light chain protein, not to the proliferation of clonal plasma cells. Nonetheless, therapies that eliminate clonal plasma cells, namely those employed in myeloma, ameliorate AL-amyloidosis because they can remove the source of the toxic proteins. Survival in AL-amyloidosis depends upon rapid suppression of the synthesis of the monoclonal proteins and stabilization or recovery of heart function because the majority of patients die of complications of cardiac dysfunction. Most commonly, cardiac deaths in patients with AL-amyloidosis are the result of congestive cardiomyopathy or sudden death caused by arrhythmias or pulseless electrical activity [82]. The most important prognostic factors therefore are the presence and extent of cardiac involvement at baseline and the hematologic response to therapy, now defined by the serum FLCs [21]. Cardiac biomarker staging and standard echocardiography are useful in assessing patients with AL-amyloidosis with regard to their suitability for HCT. In the early stages of cardiac amyloid, relaxation is abnormal, and NT-proBNP and troponin levels are minimally abnormal, whereas in advanced cardiac amyloid, there is restricted filling with a shortened deceleration time, and both NT-proBNP and troponin levels can be markedly elevated.

Approximately one-quarter to one-third of newly diagnosed AL-amyloidosis patients are eligible for risk-adapted melphalan with HCT; only about one-sixth are eligible for HCT with 200mg/m^2 of melphalan [19]. Eligibility criteria for HCT usually include adequate performance status and adequate cardiac, pulmonary, and hepatic function. Patients with stage I or II cardiac involvement can do well with HCT but stage III patients tend to have a higher risk of death in the first year post-HCT. At some centers, the presence of orthostatic hypotension is an exclusion criterion for HCT, but standing systolic blood pressures greater than 90mmHg at baseline are usually deemed adequate for HCT despite orthostatic changes [18]. An elevated creatinine level has an important impact on the morbidity of HCT, and consideration of melphalan dose reduction to 140 or 100mg/m^2 must be given to patients with renal insufficiency or on dialysis [17]. Age-related criteria may play a role in specific clinical trials but, as with other diagnoses for which HCT is performed, biologic and not chronologic age may be a consideration.

We recommend that at diagnosis patients be stratified according to the extent of cardiac damage and prioritized for appropriate investigational approaches. Stratification is most widely achieved, particularly in clinical trials, by employing the cardiac biomarkers NT-proBNP and troponin and the tripartite cardiac staging system defined in the last decade [65]. Patients with stage III cardiac amyloid represent up to 25% of those seen at tertiary centers and, provided that patient age, clonal cytogenetics, and comorbidities allow, patients with advanced cardiac disease should be considered for orthotopic heart transplantation [21]. The degree of infiltration of the marrow with plasma cells should not determine eligibility provided that the patient has AL-amyloidosis and the cytogenetics and FISH show standard risk plasma cell disease [83]. Although clinical trial data supporting our view are limited, we believe that the novel agents currently available to treat plasma cell diseases can be effective in such patients after listing for heart transplant while awaiting a donor organ, and also after orthotopic heart transplant when HCT may be useful as well [84,85].

Therapy

Efficacy and end-points

We developed and validated the current categories of hematologic response based on changes in the free light chain measurements after therapy (Figure 60.3); patients achieving complete hematologic

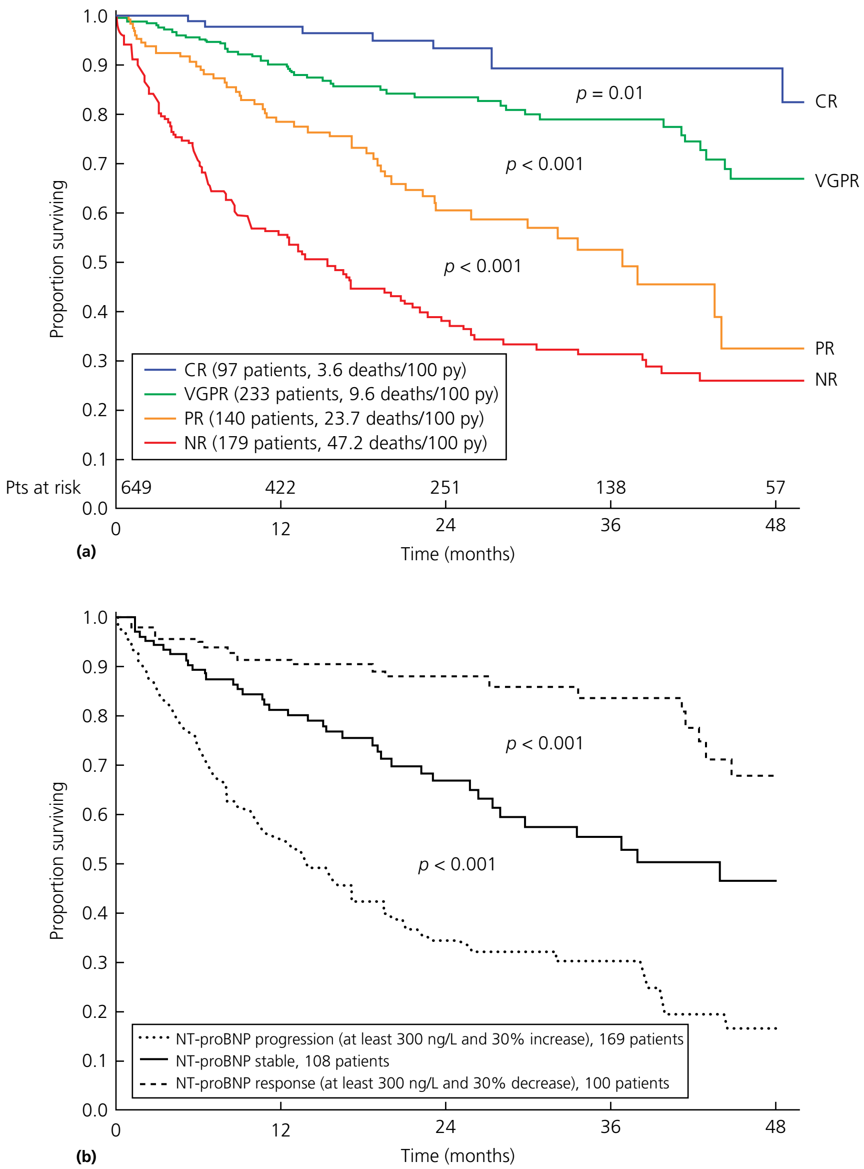

Figure 60.3 Landmark analyses of overall survival in AL patients [21]. (a) Patients in this international case series from seven centers were analyzed for survival at 3 months after beginning therapy based on metrics for hematologic response assessed at 6 months. In this series, 43.6% received oral melphalan and dexamethasone, 11.4% stem-cell transplant, 22% IMID-based therapy, and 3% bortezomib and dexamethasone. Response criteria are CR (immunofixation negative and FLC normal); VGPR [very good partial reduction; reduction in the difference between iFLC and uninvolved FLC (dFLC) to less than 40mg/L); PR (partial reduction; reduction in the dFLC by >50%); and NR (no response; less than PR). (b) Staging cardiac biomarkers were available for 53% (432/816) and 24% were stage 1 (both NT-proBNP ≤332ng/L and cTnT ≤0.035ng/mL or cTnI ≤0.1ng/mL), 52% stage 2 (either NT-proBNP >332ng/L or cTnT >0.035ng/mL or cTnI >0.1ng/mL), and 24% stage 3 (both NT-proBNP >332ng/L and cTnT >0.035ng/mL or cTnI >0.1ng/mL). In these survival curves, results for 377 patients with baseline NT-proBNP ≥650ng/L are shown according to NT-proBNP response or progression at 6 months.

response have the best survival [21]. However, although hematologic response is a key factor in prolonging survival, patients who have advanced cardiac damage at diagnosis are at high risk of sudden cardiac death and often cannot be rescued even by effective anti-plasma cell therapy [82]. Furthermore, in AL-amyloidosis, unlike myeloma, patients achieving a partial hematologic response often require additional therapy (or disease control?) to derive significant clinical benefit because ongoing light chain production may result in further organ damage. Similarly, and importantly unlike myeloma, stable clonal disease is not a benign category of response in AL-amyloidosis because of the risk of progression of organ damage. When evaluating overall response to therapy then, a partial hematologic response needs to be viewed in conjunction with organ response, and such patients must be followed closely for evidence of progression of organ damage. Patients with stable hematologic disease, that is, no response after initial therapy or after HCT, usually have short survivals.

The only effective therapeutic approach to systemic AL-amyloidosis remains reduction of amyloid-forming FLCs while providing best supportive care. Treatments that gradually reduce FLCs are likely to be ineffective because progression of amyloid organ disease continues. The FLC assay provides a direct quantitative measure of the fibril-precursor protein and of response when used serially during the course of therapy. In addition, the cardiac biomarkers, particularly the NT-proBNP, provide practitioners with an objective measure of cardiac stress. The FLC assay has significantly changed the way in which patients with AL-amyloidosis are monitored during therapy. In a prospective phase II trial using HCT and post-HCT consolidation, patients achieving a normal serum FLC κ-to-λ ratio at 3 months post-HCT had significantly better overall and progression-free survival (PFS) [19]. Furthermore, the recently updated hematologic response categories are based on FLC responses (Figure 60.3) [21]. For the 15% of patients with AL-amyloidosis and normal FLCs or dFLCs ≤40mg/L at baseline, standard criteria of response used in myeloma are available. M-proteins >500mg/dL by electrophoresis are generally considered as assessable for response, and serum and urine immunofixation identify the monoclonal component in a high proportion of patients; in such cases, the full range of newly defined FLC response categories would not be available. The use of the new FLC response criteria on clinical trials can limit accrual but the criteria have been validated and, therefore, may prove useful in registration trials of new agents.

In AL-amyloidosis, hematologic response to treatment predicts overall survival [21]. A complete hematologic response requires that, at evaluation after therapy, evidence of the monoclonal protein be absent. The serum and urine immunofixation studies must not show the baseline light chain protein, the levels and ratio of the serum free light chains should normalize, and bone marrow plasma cells should be normal in number without evidence of light chain restriction. Criteria for assessing organ responses have been defined and are in widespread use, and recently biomarker-based criteria were validated for cardiac responses (Table 60.2, Figure 60.3) [21]. Prior to the use of the FLC assay, it was difficult to gauge hematologic response and titrate therapy, although we knew that organ responses occurred frequently in patients with hematologic responses (Figure 60.4). The serologic markers of clonal disease were often difficult to interpret, particularly the urine immunofixation in the setting of evolving renal insufficiency. Also, because the improvement of amyloid organ damage lags the reduction in fibril-precursor protein, clinical correlation was not useful for assessing the need for continued therapy. Integration of the quantitative FLC assay into clinical practice allows the titration of therapy in anticipation of response before evidence of organ improvement may be available. However, and despite this advance, we do not yet have a test that can tell us the threshold below which the pathologic FLCs must be reduced (short of a hematologic complete response) in order to maximize the likelihood of stopping the disease process, and evolving renal insufficiency can also confound interpretation of the FLC assay, leading to a need for marrow biopsies to confirm or assess presumed responses or hematologic status. In the current roster of response categories, the very good partial response (VGPR) provides some help in this regard since it has been validated with respect to survival. Nevertheless, in clinical practice, we continue to follow markers of organ disease such as NT-proBNP and 24-hour proteinuria to make the determination of organ response, but the lag time must be appreciated. The major end-points of clinical research in systemic AL-amyloidosis remain hematologic response, particularly the complete hematologic response rate, organ response, and progression-free and overall survival [86].

Table 60.2 Organ response and progression criteria [21,117]

Organ	Response	Progression
Heart	NT-proBNP response (>30% and >300 ng/L decrease in patients with baseline NT-proBNP ≥650 ng/L) *or* NYHA class response (≥2 class decrease in subjects with baseline NYHA class 3 or 4)	NT-proBNP progression (>30% and >300 ng/L increase)[a] OR cTn progression (≥33% increase) *or* Ejection fraction progression (≥10% decrease)
Kidney	50% decrease (at least 0.5 g/day) of 24-h urine protein (urine protein must be >0.5 g/day pretreatment). Creatinine and creatinine clearance must not worsen by 25% over baseline.	50% increase (at least 1 g/day) of 24-h urine protein to >1 g/day or 25% worsening of serum creatinine or creatinine clearance[a]
Liver	50% decrease in abnormal alkaline phosphatase value Decrease in liver size radiographically at least 2 cm	50% increase of alkaline phosphatase above the lowest value
Peripheral nervous system	Improvement in electromyogram nerve conduction velocity (rare)	Progressive neuropathy by electromyography or nerve conduction velocity

[a] Patients with progressively worsening renal function cannot be scored for NT-proBNP progression.

Overview

Therapy for systemic AL-amyloidosis has undergone a remarkable transformation over the past four decades. Because colchicine was useful in treating patients with familial Mediterranean fever (about one-third of whom acquire "secondary" amyloidosis due to SAA protein), it was used in the 1970s to treat patients with AL-amyloidosis for whom no standard effective therapy existed [87]. Its use was not debunked until the 1990s when treatment arms containing colchicine were shown to be ineffective in phase III trials, the same trials in which oral melphalan and prednisone (MP) was shown to prolong median survival from 8 to 18 months [88,89]. The most significant side-effects of melphalan were and remain myelotoxicity and myelodysplasia. In patients who survived for more than 3.5 years, there was a 20% risk of myelodysplasia, often leading to secondary leukemia [82,90]. Approximately 5% of patients treated with alkylating agents survived for 10 years or more [91]. These long-term survivors were predominantly patients without symptomatic cardiac involvement or peripheral neuropathy who had relatively normal renal function.

By the late 1990s, oral MP had become the standard treatment for patients with AL-amyloidosis. At that time, and not without controversy, high-dose melphalan with HCT was shown in phase III trials to provide a survival benefit for patients with multiple myeloma [92]. With the high hematologic response rate noted in myeloma, phase II trials for HCT in AL-amyloidosis began. Early trials were characterized by high hematologic and organ response rates, and regression of amyloid in patients achieving hematologic complete responses (Figure 60.4). The complete response rates in these early phase II trials of HCT in AL-amyloidosis were 5–10 times higher than those seen with oral MP [15]. However, these trials also had excessive treatment-related mortality (TRM) due to sudden cardiac death, intractable hypotension, and gastrointestinal bleeding [70]. The 100-day mortality ranged from 20 to 40%, the latter in a multicenter setting, and deaths were noted during stem-cell mobilization with granulocyte colony-stimulating factor (G-CSF) alone, an unusual happenstance [93]. In the largest series ($n = 421$, covering the period 1994–2008), the overall 100-day mortality with HCT was 11.4% but for those undergoing HCT during 2003–2008 it had been lowered to 5.6% [94].

The first decade of the second millennium ushered in the era of novel agents such as thalidomide, lenalidomide, and bortezomib, all

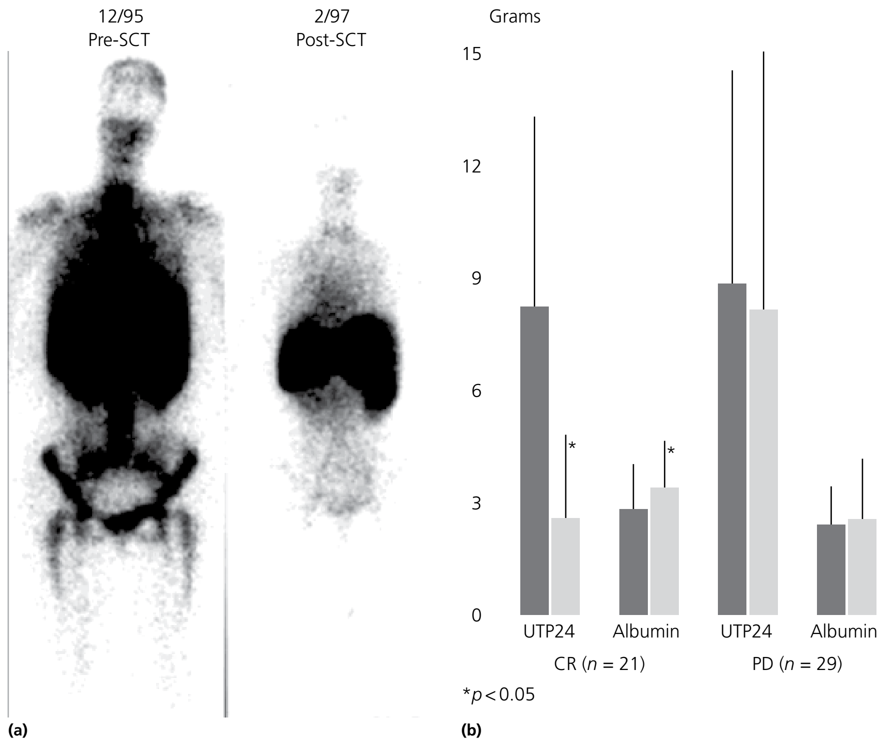

Figure 60.4 Responses of amyloid organ involvement to HCT. (a) Scintiscans employing iodine-131-labeled serum amyloid P (SAP). The black areas show uptake consistent with amyloid deposits that are extensive pre-HCT and markedly improved 14 months later. Source: reproduced with permission of Dr. Philip Hawkins. (b) Responses in 50 consecutive AL patients spilling more than 1g/day of albumin in the urine. Patients who achieved a complete response (CR, left panels) had significantly diminished daily albuminuria (UTP24) and increased serum albumin levels 1 year post-HCT ($p < 0.05$ by paired *t*-test). Those with persistent disease (PD; right panels) were basically unchanged. Mean values are shown and the whisker lines represent the standard deviations. Source: adapted from Dember et al. 2001 Ann Intern Med, 134: 746–753 [130].

of which are now approved for use in myeloma. A combination of the first of these agents, thalidomide, with MP (MPT) was compared at the phase III level with both MP and intermediate-dose melphalan (MEL) with HCT in the treatment of myeloma (MPT vs. MP vs. MEL 100), and MPT was shown to be superior [95]. Subsequently, bortezomib (Velcade) with MP (MPV) and MP (MPV vs. MP) were compared at the phase III level and MPV was shown to confer both response and overall survival advantages [96]. At last the threshold had been passed: a combination of agents had superseded MP for the treatment of myeloma patients not undergoing HCT. Notably, in AL-amyloidosis, phase II trials of dexamethasone combined in one instance with interferon-alpha and in another with oral melphalan, also showed high partial and complete response rates [97,98]. Indeed, oral melphalan and dexamethasone (MDex) became a standard therapy in AL-amyloidosis for non-transplant candidates because of its effectiveness and convenience (one 4-day course per month), despite the risk of myelodysplasia and secondary leukemia [82].

The first novel agent to be investigated in relapsed AL-amyloidosis, thalidomide, was poorly tolerated at high doses but in combination with dexamethasone in more modest doses showed efficacy [99]. The combination of oral cyclophosphamide, thalidomide and dexamethasone (CTD) showed promise in the relapsed setting [100]. In phase II trials, full-dose lenalidomide had significant toxicity, requiring dose reductions or discontinuation, and was better tolerated at 15mg/day in combination with weekly dexamethasone (LenDex), with hematologic response rates of 40–50% [101]. In recent reports on over 100 patients with relapsed AL-amyloidosis treated with LenDex, there was a 52% hematologic response rate with 16% CR, median OS of about 2 years, and, in those achieving CR, PFS of over 3 years [102]. Phase I/II studies combining LenDex with oral melphalan or cyclophosphamide had response rates not substantially different from those seen with MDex [103]. In a phase II study of pomalidomide with weekly dexamethasone (PomDex), over one-third of heavily pretreated relapsed AL-amyloidosis patients achieved a hematologic response by 6 months of treatment, highlighting the promise of this potent immunomodulatory drug (IMID) [104]. A notable confounding aspect of IMID therapy in AL-amyloidosis, however, is a rise in cardiac biomarkers that does not correlate with hematologic progression, making the use of IMIDs in AL-amyloidosis challenging [105]. Despite that complication, the IMIDs remain an active class of drugs in AL-amyloidosis.

Bortezomib is the most active and most useful of the novel agents in the treatment of AL-amyloidosis. Its safety and efficacy as a single agent in relapsed AL-amyloidosis were evaluated in a phase I/II study of 70 patients [106]. The maximum tolerated dose was not reached and evaluation of intravenous dosing at both 1.3mg/m^2 on the standard schedule (twice weekly, BIW) and 1.6mg/m^2 when given once weekly (QW) for 4 of 5 weeks was completed on the phase II level. The most common side effects were gastrointestinal with ≥grade 3 vomiting and diarrhea in five patients (prophylaxis with loperamide and granisetron was routinely used). In the QW cohort there were no instances of ≥grade 3 peripheral neuropathy (PN) and no discontinuations due to PN. Hematologic response and CR rates were the highest ever reported for a single agent: 69% with 38% CR QW, 67% with 24% CR BIW ($n = 34$), and 39% with

11% CR at lower doses. PFS at 1 year exceeded 75% and OS 90%. Renal responses were seen in 14/49 (29%), and objective cardiac responses in 5/39 patients (13%). Similar efficacy was seen in a retrospective series of 94 patients (81% previously treated) receiving bortezomib BIW and dexamethasone. There was a 71% hematologic response rate with 25% CR, a median time to progression of 25 months and 1-year survival of 75% [107]. Recently, use of the combination of oral cyclophosphamide, bortezomib and dexamethasone (CyBorD) for newly diagnosed patients with AL-amyloidosis has spread rapidly; it is particularly effective at achieving prompt reductions in the pathologic FLCs, although the durability of responses remains under study [108].

We are in a new era in the treatment of clonal plasma cell diseases. The integration of traditional therapies and novel agents continues to be a complex process. Phase II trials in AL-amyloidosis using a variety of combinations of traditional and novel agents have produced response rates similar to those seen with MDex or with HCT alone. Currently, there is no standard therapy for newly diagnosed patients who are not transplant candidates. In this new era, major differences between myeloma and AL-amyloidosis, as suggested above with respect to response categories, continue to be delineated. For instance, high-risk myeloma is defined based on the genetics of the clonal disease whereas high-risk AL-amyloidosis is defined based on the extent of cardiac involvement. Also, with respect to the role of HCT, in myeloma HCT is being challenged by the high response rates and prolonged disease control associated with maintenance or continuous therapy, despite the numerous phase III trials showing an overall survival advantage associated with HCT, whereas in AL-amyloidosis the uses of HCT are expanding as a platform for initial treatment combining risk-adapted melphalan dosing with post-HCT consolidation, after a response to initial treatment with regimens such as CyBorD, and as second-line therapy after a sub-optimal response to initial treatment. Although we now can distinguish to some extent front-line and second-line options for AL-amyloidosis patients, there is as yet no consensus on how to combine, sequence, or customize traditional and novel approaches. Also, just as there is no standard therapy for newly diagnosed patients who are not HCT candidates, there is no standard therapy for patients who do not respond to, or relapse after, initial therapy. The numerous phase II trials that are ongoing will inform us on the activity of new agents as single agents and in combination. However, phase III trials such as the one phase III trial opened for relapsed AL-amyloidosis patients (a global registration trial for the novel oral proteasome inhibitor MLN9708, ixazomib) are needed to define new standards of care. It is clear that MP is no longer standard therapy and, although the clinical options are in flux, there are clearly plenty of them. Also, just as MP is no longer a standard therapy for AL-amyloidosis, HCT for AL-amyloidosis is not investigational. The latter is a particularly important point. A case–cohort analysis has shown a survival advantage in AL-amyloidosis for good-performance status patients treated with HCT, and a second study has shown that the quality of life improves significantly in patients who respond to HCT [109,110].

Hematopoietic stem-cell transplantation for AL-amyloidosis

The dose-intensive regimens used to treat patients with AL-amyloidosis have been melphalan based, and the experience parallels that in myeloma. Melphalan alone is preferred to melphalan plus total body irradiation. In the mid-1990s, phase II trials were performed giving patients with AL-amyloidosis dose-intensive intravenous melphalan and autologous HCT [15,70]. Investigators at numerous centers documented the effectiveness of HCT in two-thirds of surviving patients, and SAP scintiscans demonstrated resorption or stabilization of deposits after the clonal plasma cells were reduced or eliminated [111]. The experience with HCT was initially controversial because of TRM. Therefore, refinement of patient selection became a priority. The extent of amyloid organ involvement clearly accounted for much of the TRM. The causes of TRM included cardiac arrhythmias, sepsis, intractable hypotension, gastrointestinal bleeding, and multiorgan failure. Adverse events during HCT occurred more frequently in patients with AL-amyloidosis than in those who received HCT for other indications. Although it is difficult to distinguish amyloid-related from treatment-related toxicities, the frequency and grade of strictly defined treatment-related toxicities appear to be a function of the dose of intravenous melphalan, as indicated by the lower-grade toxicities experienced by HCT patients with AL-amyloidosis treated with 100 as opposed to 200mg/m^2 of melphalan [15,70]. Of particular note, the gastrointestinal toxicity with 200mg/m^2 of melphalan is striking, as are the higher rates of edema and bleeding. It is reasonable to conclude that amyloidosis confers on HCT patients an increased risk; however, the toxicities per se are regimen related, and are amenable to clinical management by patient selection and dose reduction (risk-adapted melphalan dosing).

In a series of 270 patients transplanted at the Mayo Clinic for AL-amyloidosis, melphalan dosing was risk adapted [112]. Two-thirds of the patients received melphalan at 200mg/m^2, over 80% had one or two organ systems involved, and the overall TRM was 11%. In a proportional hazards model, predictors of outcome included number of organ systems affected and the baseline NT-proBNP level (Figure 60.5). Patients achieving a complete or partial response had significantly better survival than those with no response (median survival <12 months). The hematologic complete response rate with risk-adapted melphalan dosing was 33%. In a series of 421 patients transplanted at Boston Medical Center between July 1994 and December 2008, melphalan dosing was risk adapted, and the TRM was 11.4% overall and 5.6% in the last 5 years [94]. The median OS and event-free survival (EFS) post-HCT were 6.3 and 2.6 years, respectively (Figure 60.5). On an intent-to-treat basis, the hematologic CR rate at 1 year post-HCT was 34% [145 of 421; 19% of patients (n = 81) did not survive for 1 year to be evaluated]. CR was associated with median OS and EFS of 13.2 and 8.3 years, respectively. Organ responses occurred in almost 80% of patients achieving CR and 50% of those surviving to 1 year without achieving CR.

In a report from the Center for International Blood and Marrow Transplant Research, a series of 107 patients from 48 transplant centers was described [113]. The 30-day TRM was 18% and median PFS 4 years. The key predictor of survival was the experience of physicians and caregivers at the transplant center. In a multicenter phase II trial conducted by the Eastern Cooperative Oncology Group, the TRM was 10%, suggesting that treatment-related complications need not occur at a higher rate in the multicenter setting [114]. In a series of 92 patients transplanted in the United Kingdom over a 10-year period, the overall TRM was 23%, but it was only 13% over the second half of that period [111]. On multivariate analysis, TRM was associated with the number of major organ systems involved, cardiac involvement, age, performance status, and albumin level. More than 80% of patients had a greater than 50% reduction of the pathologic FLC, and at 1 year post HCT, the hematologic complete response rate was 21% by intent-to-treat (19 of 92)

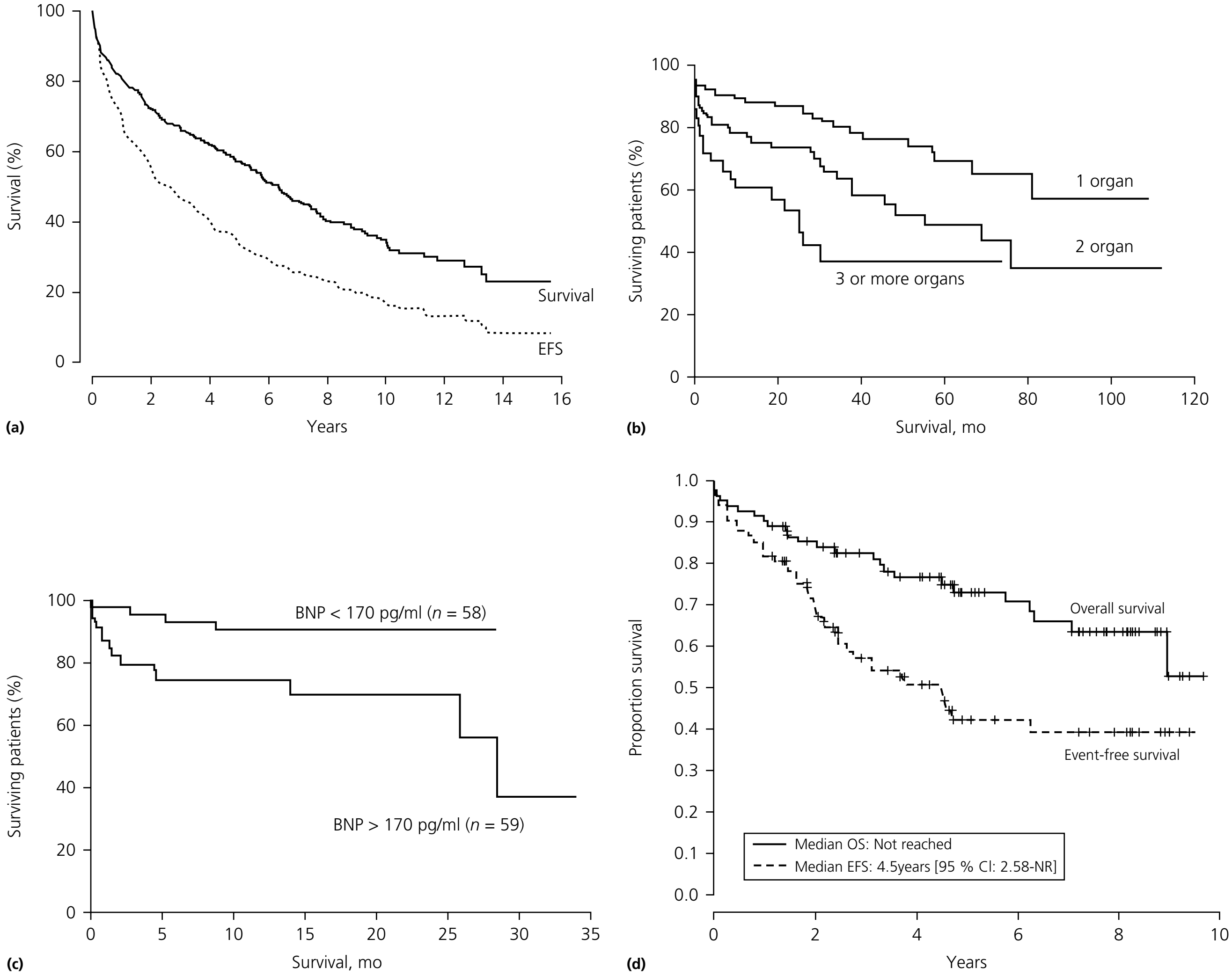

Figure 60.5 Survival after HCT [94,112,128]. These survival curves describe outcomes in (a) 421 patients transplanted at Boston Medical Center between July 1994 and December 2008 with median OS and PFS of 6.3 and 2.6 years; (b and c) 270 patients transplanted at the Mayo Clinic showing the impact of number of organs involved and NT-proBNP on survival; and (d) 83 patients transplanted at the Memorial Sloan-Kettering Cancer Center between 2002 and 2011 in phase II trials with risk-adapted melphalan dosing, HCT and adjuvant therapy for those not achieving CR with HCT; with median follow up of 5.1 years, median OS is not reached and median EFS is 4.5 years.

and 36% in evaluable patients. At 18 months post-HCT, the organ response rate was 48%. Whole-body amyloid load by SAP scintigraphy was reduced in 65% of patients evaluated pre- and post-HCT. The median survival was 5.3 years.

In a large single-center randomized prospective phase II clinical trial, 100 patients were stratified based on organ involvement and time from diagnosis, and then randomized to initial HCT or HCT after two cycles of MP [115]. The 100-day TRM was 20%, including 12% of patients who died in association with stem-cell mobilization. There were no significant differences between the two arms with respect to OS at a median of 4 years follow-up. At 5 years post-HCT, the OS was 50% for immediate and 39% for delayed HCT. The CR rates were 21 and 17%, respectively, and 42% of patients achieved an organ response at 1 year. In this trial, fewer patients randomized to initial oral therapy received HCT because progression of disease rendered them ineligible for HCT. This affected patients with cardiac involvement disproportionately. Newly diagnosed untreated patients with AL-amyloidosis eligible for HCT did not benefit from two cycles of MP. The number of organ systems affected and the presence of cardiac involvement were predictors of outcome.. For patients with one or two major organ systems affected (heart, kidneys, liver/gastrointestinal tract, and peripheral nervous system), and for those without cardiac involvement, median survival had not been reached at a median of 4 years' follow-up. For the others, median survivals were 9.3 and 5.2 months for patients with more than two organs involved, and 9.6 and 4.8 months for cardiac patients. This trial demonstrated that HCT could be safely applied to patients with AL-amyloidosis who do not have more than two symptomatic organ systems involved or advanced cardiac amyloid characterized by recurrent pleural effusions, cardiac syncope, or symptomatic arrhythmias, and made clear that the other patients are at a high risk of dying in the peri-transplant period. French investigators conducted a randomized prospective multicenter phase III trial in which stem-cell transplant with high-dose melphalan was compared with MDex given continuously for up to 18 months [116]. In the HCT group, there was a 44% early failure rate due largely to treatment-related deaths and progression of disease. TRM was 24%. Analysis of outcomes between the groups demonstrated a center effect in that patients treated at the majority of centers (27 of 29) had far better survival if they received oral therapy ($p < 0.01$), whereas patients in the two centers in which the largest fraction of HCT was performed had a trend to better survival with HCT. Median survival overall was 48 months for HCT and 58 months for MDex. Comparisons of response rates and survival between those alive at 6 months after receiving their assigned treatment showed no difference between groups. For both groups, the hematologic response rates were 65%. The organ response rate was higher in the HCT group (52% vs. 40%). Surprisingly, no cases of myelodysplasia were reported in the MDex group. Despite the apparent center effect and the need to be cautious in interpreting results with such a high TRM, this phase III trial did not define a standard initial therapy for systemic AL-amyloidosis.

Viewing HCT as a treatment platform, we conducted two phase II trials for newly diagnosed AL-amyloidosis patients combining risk-adapted melphalan dosing and HCT and incorporated consolidation for patients who had not achieved CR following HCT. In the first trial, we used post-HCT thalidomide and dexamethasone (TD), and in the second, bortezomib and dexamethasone (BD) [19,20]. In these trials, we showed that HCT for AL-amyloidosis can be performed with a low TRM using risk-adapted melphalan at 100, 140 or 200mg/m^2 based on age, renal function, and cardiac involvement, and that post-HCT consolidation significantly and safely improved hematologic response rates [19,20]. In the first trial, TRM was 4.4% (2 of 45), with both deaths in patients with cardiac involvement; there were no deaths during consolidation. At 3 months post-HCT, the hematologic response rate was 63% with 21% CR and no difference in OS or CR rates based on the dose of melphalan. Thirty-one patients received post-HCT TD, but only 52% ($n = 16$) completed all 9 months as planned, with 16 and 32% discontinuing for progression of disease and toxicity, respectively. The median nightly dose of thalidomide was 150mg (range 50–200mg), and the majority of patients received one or two dexamethasone pulses per month. Despite the high discontinuation rate, 42% of patients receiving consolidation had an improved hematologic response at 1 year post-HCT. The overall response rate at 1 year was 78% [95% confidence interval (CI) 65–90%], with 39% achieving a complete hematologic response (95% CI 24–53%). By intent-to-treat, these rates were 71% (95% CI 57–84%) and 36% (95% CI 21–50%), respectively, again with no significant difference seen based on melphalan dosing. For patients with cardiac involvement, estimates of 1- and 2-year OS were 63% at both times (95% CI 34–91%). Median PFS was 40 months with 74% (95% CI 61–87%) of patients remaining progression free at 2 years. In univariate analysis, the baseline characteristics associated with OS were the number of organ systems involved and the baseline troponin level. In multivariate analysis, having a normal serum FLC kappa-to-lambda ratio at 3 months post-HCT and achieving a hematologic response at 12 months post-HCT were associated with improved OS and PFS.

In the second trial, we used BD as consolidation after risk-adapted melphalan HCT in 40 untreated AL-amyloidosis patients with renal (70%), cardiac (65%), liver/gastrointestinal (15%), or nervous system (13%) involvement.. Four patients with advanced cardiac AL-amyloidosis died within 100 days of SCT (10% TRM). Survival at 12 and 24 months was 88 and 82%, respectively, overall and 81 and 72%, respectively, in patients with cardiac AL-amyloidosis. At 3 months post-HCT, 45% had a hematologic response (≥PR), including 27% CR. Twenty-three patients received consolidation and in 86% the response improved, all within one cycle. At 12 and 24 months, 79 and 60%, respectively, had ≥PR and 58 and 40%, respectively, had CR. Organ responses occurred in 55 and 70% at 12 and 24 months, respectively. Eight patients relapsed but only one patient with serologic relapse had worsening organ function. In both of these trials, unlike the studies reported above, hematologic response criteria were strictly FLC based [21,86,117]. The overall TRM was 7%, and the CR rate following SCT was 24%, increasing to 48% with post-SCT adjuvant therapy. The CR rates increased at 1 year compared with 3 months post-SCT to 36% with TD and to 60% with BD. With a median follow-up of 5.1 years, EFS is 4.5 years and median OS has not been reached (Figure 60.5). These results indicate that risk-adapted melphalan dosing with HCT and consolidation is an effective initial treatment strategy for patients with AL-amyloidosis and results in low TRM, high CR rates, maintained organ improvement, and long survival.

Mobilization and immune reconstitution

Mobilized hematopoietic stem cells collected by apheresis of blood have provided the marrow rescue products for patients with AL-amyloidosis receiving HCT. In an early trial, two-thirds of patients had amyloid identified in the bone marrow, but this did not impair mobilization [15]. Contamination with clonotypic plasma cells has been demonstrated in these stem-cell products collected after growth factor priming, and CD34$^+$ cell selection is possible with adequate yields, but the effect on the disease course of having a positively selected apheresis product remains unknown [118]. Currently, G-CSF mobilization is used at the majority of centers transplanting AL-amyloidosis patients regularly, although G-CSF combined with plerixafor has been shown to enhance CD34$^+$ cell mobilization and collection in myeloma and even has single-agent activity in that regard [119,120]. The mechanism of action of G-CSF mobilization in the marrow stem-cell niche has been the subject of intensive study over the past decade and the data support a role for a "stress response" in which mediators of the sympathetic nervous system cause alterations in the CXCR4–CXCL12 axis, resulting in the release and egress of CD34$^+$ cells into the marrow sinusoids and peripheral blood [121,122]. In order to evaluate this association clinically, we collected 24-hour urine samples prior to G-CSF mobilization from 40 AL-amyloidosis patients enrolled in a phase II clinical trial using risk-adapted melphalan dosing and analyzed them for epinephrine, norepinephrine, and dopamine excretion. Baseline urinary excretion of epinephrine and dopamine correlated with the total CD34$^+$ cells per kilogram collected (0.33, $p = 0.005$; and 0.47, $p = 0.05$, respectively). An optimal collection defined as $\geq 5 \times 10^6$ CD34$^+$ cells in two collections was associated with baseline epinephrine ($p = 0.02$) and dopamine ($p = 0.05$) levels [123]. These preliminary data may help guide the addition of plerixafor to G-CSF for mobilization in AL-amyloidosis patients.

Given the impaired visceral reserve, vasculopathy, and coagulopathies associated with AL-amyloidosis, it was predictable that treatment-related toxicity would be more prominent in these patients. It was not expected, however, that there would be significant toxicity associated with the mobilization and collection of blood stem cells. At Boston Medical Center, of 312 patients who underwent stem-cell mobilization with cytokines from July 1994 to June 2002, 13 died and 22 had complications of mobilization that prevented them from undergoing HCT [124]. Deaths have been reported during the mobilization of patients with symptomatic cardiac amyloid and multisystem disease [115]. Excessive fluid accumulation during mobilization has been shown to be an independent predictor of first-year mortality post-HCT [68]. In a retrospective study of 126 patients who underwent HCT at the Mayo Clinic between July 1997 and June 2003, body weight increased more than 2.0% during mobilization in over half of patients. Those with >2.0% weight gain had more proteinuria, more organ systems involved, lower serum albumin, and greater diuretic dependence. They also had significantly higher first-year mortality (33.9% vs. 9.8%, $p = 0.002$). In multivariate analysis, independent predictors of first-year mortality included weight gain, glomerular filtration rate, and septal thickness. The increase in mortality was noted despite diuresis of the excess weight prior to HCT.

Immune recovery after autologous HCT for AL-amyloidosis has been studied prospectively in a cohort of patients with AL-amyloidosis [125]. At 3 months following HCT, $CD4^+$ cells were significantly decreased and T-cell function was depressed. In contrast, $CD8^+$ cells, monocytes, natural killer cells, B-cell number, and functional activity (measured by both quantitative immunoglobulin levels and the proliferative response to staphylococcal antigen) had returned to the normal range. These outcomes are similar to those reported for patients with other hematologic diseases after autologous HCT, although the prompt recovery of humoral immunity is somewhat atypical. Opportunistic infections, including *Pneumocystis jiiroveci* pneumonia and herpes zoster, were seen within the first year post-HCT, and several deaths due to sepsis occurred between 6 and 12 months post-HCT. This experience in the year post-HCT clearly supports the use of pre-HCT trimethoprim/sulfamethoxazole or aerosolized pentamidine and post-HCT acyclovir prophylaxis.

Peri-transplant management

Cardiac complications, gastrointestinal bleeding, and multiorgan failure with hypotension have been significant causes of early mortality with HCT in AL-amyloidosis [126]. These complications usually occurred within the first 14 days of HCT; the degree to which sepsis, autonomic dysfunction, occult chronic infections, immunologic impairments, and amyloid vasculopathy contribute to these complications is unknown.

Gastrointestinal bleeding is unusual after autologous HCT in all patients, and in frequency and severity is unique to patients with AL-amyloidosis. If amyloid extensively infiltrates the submucosa of the stomach or lower gastrointestinal tract, the potential for severe mucositis with hemorrhage must be anticipated, while neuropathic compromise of the enteric plexus often results in atony, persistent post-transplant nausea, and failure to thrive. For these reasons, pre-transplant planning and peri-transplant supportive care become critical elements of the care plan. Recommendations with respect to pre- and peri-transplant management have been described in detail, and include stool guaiac testing for occult blood [17]. Pre-transplant patient evaluation should also include a detailed review of gastrointestinal signs and symptoms, endoscopic studies to define pathology when indicated by history or the presence of occult blood in stool, and a complete assessment of coagulation status. It is important to note that major gastrointestinal bleeding can present atypically; for example, as new-onset atrial fibrillation or supraventricular tachycardia. In HCT patients with known gastrointestinal amyloid, if stool guaiac results are positive, the hemoglobin should be kept above 9g/dL and platelets maintained over 50,000/μL. It is also important to keep in mind that visceral rupture (e.g., splenic rupture) can occur acutely in patients with AL-amyloidosis. Vague or atypical left-sided abdominal or shoulder pain should raise a concern about splenic hemorrhage and lead to imaging the abdomen. Splenic rupture occurring during this period has been successfully managed surgically. Other viscera, such as the esophagus or small bowel, can also perforate and present life-threatening challenges. Peri-transplant, proton pump inhibitors should be used for gastrointestinal prophylaxis and, because dose-intensive intravenous melphalan can cause delayed emesis, an antiemetic regimen combining low-dose dexamethasone, lorazepam, and ondansetron may be useful beginning the day after the infusion of the graft.

In our experience, the peri-HCT mortality rate in patients with cardiac amyloid and congestive heart failure, or with a history of cardiac arrhythmias, syncope, or recurrent pleural effusions, approaches 100%, while patients with uncomplicated or well-compensated cardiac amyloid and no other symptomatic organ involvement have a low peri-HCT mortality and 65–85% 1-year survival. In patients with dominant cardiac amyloid with minimal symptoms, preserved left ventricular function usually assures diuretic responsiveness. Maintenance of normal electrolyte levels in cardiac patients receiving diuretic therapy is an obvious requirement. In a detailed study of arrhythmia events in 24 consecutive AL-amyloidosis cardiac patients undergoing risk-adapted melphalan and SCT with continuous telemetric monitoring, the relation between number and severity of ventricular arrhythmias [ventricular tachycardia/ventricular fibrillation (VT/VF)] and baseline clinical, laboratory, and echocardiographic data were determined. All patients displayed atrial and ventricular arrhythmias and non-sustained VT was the most frequent event. In 20/24 patients therapeutic intervention for arrhythmias was required usually with low-dose β-blockers; in three patients, life-threatening arrhythmias were detected and treated. There was an inverse relation between VT/VF and cardiac output ($r = -0.72$, $p < 0.0001$), cardiac index ($r = -0.71$, $p = 0.0001$), and stroke volume ($r = -0.59$, $p = 0.0029$). There was also a relation between VT/VF and brain natriuretic peptide (BNP) before SCT ($r = 0.47$, $p = 0.019$), average BNP levels during SCT ($r = 0.62$, $p = 0.0012$), troponin I levels at diagnosis ($r = 0.47$, $p = 0.022$), and serum creatinine levels before SCT ($r = 0.62$, $p = 0.001$). It was concluded that decreased cardiac output was strongly associated with risk of significant ventricular arrhythmias and that continuous telemetric monitoring contributed to patient safety during HCT.

The mortality associated with cardiac amyloid in HCT recipients is due to sudden cardiac death and to cardiopulmonary failure, resulting in hypotension and hypoxia. Patients rarely, if ever, survive hypotensive bradycardic episodes despite the addition of appropriate medications and the use of advanced life support measures. Hemodynamically stable tachycardias, on the other hand, are frequent and tolerated. Use of β-blockade simply for control of sinus tachycardia should not be routine. Whether prophylactic pacemakers or implanted automated defibrillators may impact peri-HCT mortality in these patients remain matters for further clinical investigation.

The management of intravascular volume and hypotension is a critical aspect of care. Nephrotic syndrome causes salt avidity and hypoalbuminemia, and therefore often leads to significant edema. The risk of overdiuresis, however, may be greater than the risk of allowing some peripheral edema in the setting of clinical euvolemia. Even mild intravascular volume depletion may exacerbate nausea and emesis; therefore, twice daily orthostatic vital signs and normal saline support when indicated are recommended. Nevertheless, maintaining a diuresis concurrently with melphalan administration and graft infusion is reasonable. Since a major factor causing pulmonary and peripheral edema is hypoalbuminemia, albumin infusions should be used throughout the treatment period to maintain a serum level above 2.0g/dL. In these patients, albumin infusions can help mobilize fluid in the tissues and therefore administering albumin along with diuretics can be effective. Other supportive measures such as compression garments and/or lymphedema therapy should also be utilized in patients with peripheral edema and low blood pressure who require diuresis.

The multiorgan failure syndrome and intractable hypotension seen as a toxicity of HCT in patients with AL-amyloidosis likely reflect the limitation on visceral reserve imposed by fibril deposition. Volume depletion, bleeding, sepsis, and hypoadrenalism, followed by worsening autonomic neuropathy, are the most likely causes of hypotension post-HCT. The cellular and hormonal aspects of the syndrome remain poorly characterized. Use of morphine or fentanyl to treat mucositis can affect both blood pressure and urine output, and can complicate acyclovir prophylaxis. When renal perfusion and urine output are reduced, risk of acyclovir toxicity increases. Midodrine and fludrocortisone are useful treatments for orthostasis but do not work reliably in the transplant setting. At the time of neutrophil recovery or myeloid engraftment, it is not uncommon for patients to experience orthostasis requiring more aggressive hydration.

At the Mayo Clinic, post-HCT G-CSF is not used to support hematopoietic recovery because of the morbidity, particularly the fluid retention, associated with its use. In the series of 270 patients from that institution, median time to recovery of an absolute neutrophil count over 500/μL was 14 days, whereas in contrast the median time at other major centers was 10 days [112]. A detailed cost–benefit comparison has not been performed, but now with the availability of pegfilgrastim (Neulasta), a long-acting preparation requiring a single administration and hence minimal nursing and pharmacy costs, such a comparison would be welcome and useful [127]. Longer periods of neutropenia may result in an increased frequency of fevers requiring laboratory evaluation, empiric antibiotic use, and inadvertent side effects.

Relapse

The population of AL-amyloidosis patients living with hematologic responses and stable or improved organ function or with hematologic relapse after response to initial therapy is growing. Recently, we developed consensus definitions of relapsed and refractory disease in AL-amyloidosis. We defined relapsed disease as progression of hematologic or organ disease after a response to initial therapy, refractory hematologic disease as no response to initial therapy or stable disease despite therapy, and refractory organ disease as persistent significant organ disease causing symptoms despite prior therapy and best supportive care [86].

Data on relapse and outcomes with second-line therapy are just being reported. There are no patient series describing second HCT as salvage therapy in AL-amyloidosis. In the largest series of HCT patients (*n* = 421), at a median of 6.9 years follow-up (range 1–15.6 years), among those who achieved CR, 28% (40/145) had hematologic relapse and 40% (16/40) of relapsed patients died. In contrast, among those with maintained CR, 24% (25/105) had died. In the two phase II trials for newly diagnosed untreated AL-amyloidosis patients using risk-adapted melphalan dosing with HCT and post-HCT consolidation, 16 patients died prior to relapse and 26 relapsed by FLC criteria with a cumulative incidence of 8, 18, and 29% at 1, 2, and 3 years, respectively, while the remainder remained progression free. About 31% (8/26) of relapsed patients did not require second-line therapy, likely due to the very sensitive serum free light chain assay that detects low-level hematologic progression in the absence of organ progression. Among those who required second-line therapy, 78% (14/18) responded, including 44% (8/18) with CR. The median OS following relapse was 5 years (95% CI 2.6–5.8 years). In a retrospective study of 193 patients undergoing HCT at the Mayo Clinic between 2005 and 2009, 27 died prior to relapse, 41% (*n* = 79) relapsed and the remainder remained progression free. Among the relapsed patients, the median time to relapse was 14.4 months and hematologic progression (68%) was more common than progression of organ damage (37%). About 90% (*n* = 70) received second-line therapy with response rates of 40–55%. With a median 26 months of follow-up of survivors, the 1- and 3-year OS were 87 and 59%, respectively.

Conclusion

AL-amyloidosis remains a disease for which our tests, treatments, and knowledge continue to evolve. Survival remains the primary end-point for both patients and clinical investigators. LMD/MS is the newest tool available, allowing unequivocal typing of tissue amyloid. The FLC assay is the most important development for baseline assessment and for monitoring the course of therapy. HCT for AL-amyloidosis remains most effective in patients who are younger or have limited disease and, by combining risk-adapted melphalan dosing and HCT with novel agents such as bortezomib, the response rates, PFS and OS of patients improve over the short term. As we come to understand more fully the basis of the organ disease that amyloid-forming light chains cause, novel cytoprotective pathways may be identified and simple and effective drug treatments developed. The ideal treatment for AL-amyloidosis in the future will involve a combination of approaches aimed at eliminating the fibril-precursor light chains, inhibiting their self-assembly, and enhancing resorption of fibrillar deposits. In the future, such therapeutic combinations will continue to include HCT because of the extent to which it enhances recovery and extends the survival of patients with AL-amyloidosis.

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CHAPTER 61

Autologous Hematopoietic Cell Transplantation for Breast Cancer and Germ-cell Tumors

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Introduction

Autologous hematopoietic progenitor-cell support (AHPCS), derived from bone marrow or from peripheral blood progenitor cells (PBPCs), allows for the dose escalation of chemotherapy up to 10-fold, exploiting the dose–response effect of alkylators and other drugs. While AHPCS circumvents myelosuppression, extramedullary organ toxicities become dose limiting. These substantial dose increments clearly increase the antitumor activity of high-dose chemotherapy (HDC) compared with standard-dose chemotherapy (SDC), with the goal of improving patient outcome. Widespread substitution of PBPCs for bone marrow support and other improvements in patient care have increased the safety of HDC to current treatment-related mortality (TRM) rates below 5%.

Hematopoietic cell transplantation for breast cancer

Despite the advances in standard therapy, more than 50% of patients with high-risk primary breast cancer (HRPBC) and virtually all patients with metastatic breast cancer (MBC) receiving SDC experience treatment failure and ultimately die from their disease. The initial prospective phase II trials of HDC in breast cancer, dating back to the late 1980s, were followed by randomized studies in both settings. Some argued that the impressive results of the phase II trials could be explained by patient selection, staging biases, or short follow-up. Thus, controversy has surrounded HDC for breast cancer almost since its inception [1,2].

High-dose chemotherapy trials in metastatic breast cancer

Prospective HDC trials in MBC targeted in a sequential fashion patients with refractory [3,4], untreated [5], and responding disease [6–8]. It soon became clear that not only did HDC produce the highest response rates (RRs) and complete response (CR) rates ever reported in MBC, but also a consistent long-term event-free survival (EFS) rate of 10–25% in patients transplanted after response to first-line chemotherapy, which appeared unprecedented (Figure 61.1). These results triggered phase III evaluation of HDC, employing a variety of regimens, for MBC (Table 61.1).

In the Philadelphia study, Stadtmauer et al. randomized 184 patients responding to first-line therapy to receive HDC with STAMP-V [cyclophosphamide/thioTEPA (*N*-triethylenethiophosphoramide)/carboplatin] or maintenance CMF (cyclophosphamide/methotrexate/fluorouracil) for 18 months or until progression [9]. At its final update at a median follow-up of 5 years there were no differences in EFS (4% vs. 3%) or overall survival (OS) (14% vs. 13%). A significant interaction was detected between age and treatment, with young and older patients apparently benefiting from HDC and CMF, respectively. With only 45 patients in CR treated, this study was grossly underpowered to detect any meaningful difference in this subset. Strikingly, the PR to CR conversion rate was higher after CMF than after STAMP-V (9% and 6%, respectively). The low CR conversion rate in the transplant arm seemed much lower than in most HDC trials, where CR conversion rates of 20–50% were typically reported.

In the National Cancer Institute of Canada (NCIC) trial, Crump et al. randomized 224 responsive MBC patients to additional SDC or HDC with cyclophosphamide/mitoxantrone/etoposide [10]. The dropout rate in the transplant arm was 20%. Transplant-related mortality was 6%. At a median follow-up of 4 years, intent-to-treat comparisons showed significant differences in favor of transplant in EFS (median 11 vs. 8 months, $p = 0.006$), but not OS (median 24 vs. 28 months, $p = 0.4$).

In the French PÉGASE 03 study, Biron et al. randomized 180 patients with responsive tumors to receive HDC with cyclophosphamide/thioTEPA or observation [11]. The CR rate increased from 11 to 24% after HDC ($p = 0.0002$). At a median follow-up of 48 months, a significant EFS advantage was observed in favor of HDC (27% vs. 10%, $p = 0.0005$), without significant OS differences (38% vs. 30%, $p = 0.7$).

Crown et al. from the International Breast Cancer Dose Intensity Study (IBDIS) group randomized 110 patients to receive doxorubicin/docetaxel × 4, followed by either CMF × 6 or sequential high-dose cycles of ifosfamide/carboplatin/etoposide (ICE) and cyclophosphamide/thioTEPA with AHPCS [12]. The HDC arm produced higher RR (71% vs. 44%) and CR rates (29% vs. 6%). At a median follow-up of 42 months, the study was positive for its primary end-point, EFS (HDC 16% vs. SDC 9%, $p = 0.01$), with a trend for an OS advantage in the HDC arm in an intent-to-treat analysis (45% vs. 37%, $p = 0.1$).

In the PÉGASE 04 trial, Lotz et al. randomized 61 responding MBC patients to additional SDC or HDC with cyclophosphamide/mitoxantrone/melphalan [13]. In its final analysis at a median follow-up of 7.5 years, significant benefits from HDC were shown

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in PFS (18.7% vs. 0%, median 12 vs. 6 months, $p < 0.005$) and OS (36.8% vs. 13.8%, median 44 vs. 19 months $p = 0.02$).

In the German Breast Cancer Dose Intensity Study (GEBDIS) group trial, Schmid et al. randomized 92 untreated MBC patients to receive adriamycin/paclitaxel × 6–9 or tandem cycles of high-dose cyclophosphamide/mitoxantrone/etoposide, 6 weeks apart with AHPCS [14]. At a median follow-up of 52 months, there were no significant differences between the high-dose and control arms in PFS (27% vs. 20%, $p = 0.5$) or OS (18% vs. 21%, $p = 0.75$).

Investigators at Duke University conducted two randomized trials with a crossover design, comparing early versus late use of HDC, using cyclophosphamide/cisplatin/BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine)] (STAMP-I). In the first trial, 100 hormone-refractory MBC patients in first CR were randomized to immediate HDC or observation with HDC at subsequent relapse [15]. At a median follow-up of 10.6 years, EFS was superior in the immediate transplant arm (26% vs. 10%, $p = 0.03$), with a median EFS of 10 versus 4 months ($p < 0.006$). Six-year OS rates were 26.5% versus 35% ($p = 0.69$), with median OS times of 2.1 versus 3.3 years ($p = 0.2$). The combined TRM from transplant in both arms was high (8.6%).

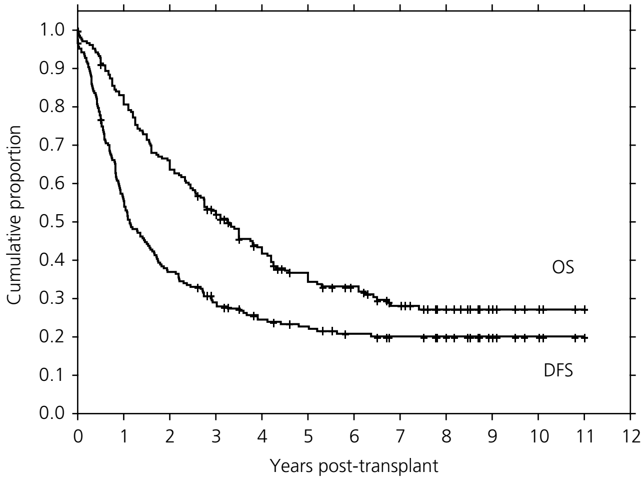

Figure 61.1 Long-term disease-free survival (DFS) and overall survival (OS) of prospective phase II trials with cyclophosphamide/cisplatin/BCNU (carmustine) (STAMP-I) as first-line therapy for MBC patients at the University of Colorado.

In the second Duke University trial, 69 patients with hormone-refractory bone-only MBC were randomized after first-line SDC to immediate HDC and radiotherapy of all bony metastases, or to radiotherapy and observation, again with HDC upon progression [16]. At a median follow-up of 8.1 years, EFS was superior in the immediate transplant arm (17% vs. 0%, $p < 0.0001$) with median EFS times of 12 versus 4 months ($p < 0.0001$). The OS rates were not significantly different between the immediate and late transplant arms (17% vs. 9%, $p = 0.1$) with median OS times of 3 versus 1.8 years. As in the other Duke trial with STAMP-I, the overall transplant TRM was high (9.7%).

In summary, a significant benefit in EFS in favor of HDC was noted in six of the eight trials, but only one study (PÉGASE 04) showed an OS advantage. The lack of a direct comparison between HDC and non-HDC arms in the two Duke crossover trials obviated OS analyses complicating their interpretation.

Berry et al. conducted a meta-analysis based on the individual patient data from six of those trials (excluding the two Duke crossover studies), involving a total of 866 patients [17]. A significant PFS benefit from HDC was detected [median 11 vs. 8 months, hazard ratio (HR) of 0.76, $p < 0.001$], with the OS differences not reaching significance (HR 0.87, $p = 0.08$). There was no detrimental effect of HDC on OS after progression (HR 1.01, $p = 0.95$).

High-dose chemotherapy in high-risk primary breast cancer (HRPBC)

The groups at Duke University [18] and the National Tumor Institute in Milan [19] pioneered the evaluation of HDC in HRPBC patients. Long-term results of Peters and colleagues at Duke University in patients with ≥10 involved (+) axillary nodes showed a 10-year EFS rate of 72% [20]. In the Milan study, Gianni et al. explored a sequential high-dose single-agent approach and reported a 57% EFS rate at median follow-up of 4 years [19].

Table 61.1 Randomized trials of high-dose chemotherapy in metastatic breast cancer

Trial	Population	*n*	HDC regimen	FU (mo)	Event-free survival rates			Overall survival rates		
					HDC	Control	*p*	HDC	Control	*p*
NCIC [10]	Responsive	224	CPA/Mito/Eto	48	NR Median 11 mo	NR Median 9 mo	0.006	37% Median 24 mo	38% Median 28 mo	0.4
Philadelphia [9,95]	Responsive	184	CPA/TT/Cb (STAMP-V)	67	4% Median 10 mo	3% Median 9 mo	0.3	14% Median 26 mo	13% Median 24 mo	0.6
PÉGASE 03 [11]	Untreated	180	CPA/TT	48	27%	10%	0.0002	38%	30%	0.7
IBDIS-1 [12]	Untreated	110	Sequential Ifos/Cb/Eto → CPA/TT	42	25% Median 14 mo	20% Median 9 mo	0.01	39% Median 32 mo	35% Median 23 mo	0.1
Duke crossover-1 (patients in CR) [15]	CR	100	CPA/CDDP/BCNU (STAMP-I)	127	25% Median 10 mo	10% Median 4 mo	<0.006	26.5% (#)	38% (#)	0.7 (#)
GEBDIS [14]	Untreated	92	Tandem CPA/Mito/Eto ×2	52	27% Median 11 mo	20% Median 11 mo	0.7	18% Median 27 mo	21% Median 23 mo	0.77
Duke crossover-2 (bone only) [16]	HR (bone only)	69	STAMP-I	97	17% Median 12 mo	0% Median 4 mo	<0.0001	17% Median 3 yr[a]	9% Median 1.8 yr[a]	0.1[a]
PÉGASE 04 [13]	Responsive	61	CPA/Mito/Mel	90	19% Median 12 mo	0% Median 6 mo	<0.005	37% Median 44 mo	14% Median 19 mo	0.02

n, No. of patients; FU, follow-up; NCIC, National Cancer Institute of Canada; NR, not reported; PÉGASE, Programme d'Étude de la Greffe Autologue dans les Cancers du Sein; IBDIS, International Breast Cancer Dose Intensity Study; GEBDIS, German Breast Cancer Dose Intensity Study; CR, complete response; HR, hormone refractory; CPA, cyclophosphamide; Mito, mitoxantrone; Eto, etoposide; TT, thioTEPA; Cb, carboplatin; Ifos, ifosfamide; CDDP, cisplatin; Mel, melphalan; mo, months; yr, years.

[a] Not evaluable for a direct OS comparison, due to crossover design.

The encouraging prospective phase II data of HDC in HRPBC (Figure 61.2) prompted subsequent randomized phase III trials, which have undergone final analyses (Table 61.2). The Dutch trial by Rodenhuis et al. randomized 885 patients with ≥4+ lymph nodes to receive fluorouracil/epirubicin/cyclophosphamide (FEC) × 4 followed by one more cycle of FEC or high-dose cyclophosphamide/thioTEPA/carboplatin (CTC) [21,22]. At a median follow-up of 7 years, a trend for an EFS advantage was noticed in favor of HDC (64.3% vs. 59%, HR 0.84, $p = 0.07$), with no detectable OS differences (73% vs. 70%, $p = 0.2$). Prospectively planned subset analyses showed a substantial EFS improvement among patients with ≥10+ nodes (68% vs. 49%, $p = 0.01$). In the 4–9+ node group, EFS rates were 72% (HDC) versus 65% (SDC) ($p = 0.5$). Other subgroup analyses, which were unplanned, suggested that young patients, those with HER2 negative, or those with lower grade tumors might benefit from HDC. Specifically, patients with

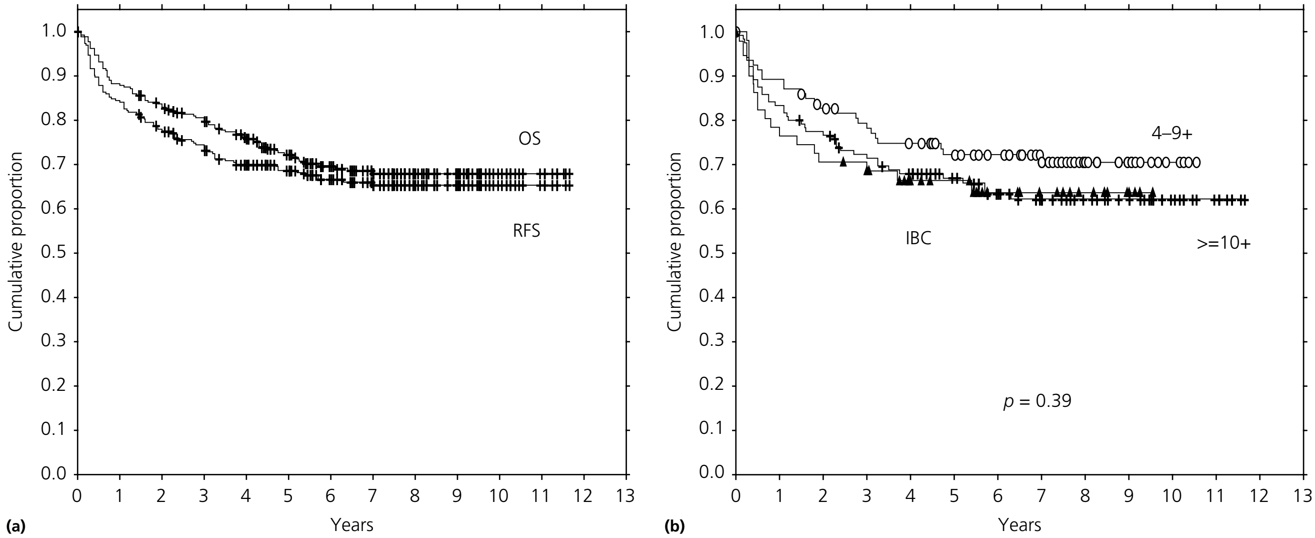

Figure 61.2 HDC for HRPBC. (a) Long-term relapse-free survival (RFS) and overall survival (OS) of 264 patients enrolled in prospective phase II trials of cyclophosphamide/cisplatin/BCNU (carmustine) (STAMP-I) at the University of Colorado, at a median follow-up of 7 years. (b) RFS in the different populations enrolled in those trials: 4–9+ nodes, ≥10+ nodes, and inflammatory breast cancer (IBC).

Table 61.2 Randomized trials of high-dose chemotherapy in high-risk primary breast cancer

Trial	Population (No. of + nodes)	*n*	HDC regimen	Median FU (yr)	Event-free survival rates			Overall survival rates		
					HDC	Control	*p*	HDC	Control	*p*
NWAST [21,22]	≥4	885	CPA/TT/Cb (CTC)	7	64	59	0.07	73	70	0.2
CALGB [23]	≥10	785	CPA/CDDP/BCNU (STAMP-I)	5.1	61	58	0.2	71	71	0.75
Anglo-Celtic [24]	≥4	605	CPA/TT (CT)	6	57	54	0.7	62	64	0.4
ECOG [25]	≥10	540	CT	6.1	55	48	0.1	58	62	0.3
SWOG [25]	≥4	523	CPA/TT/Cb (STAMP-V)	NR	75	78	NS	84	87	NS
SBCG [27,28]	>5 to 8	525	STAMP-V	5	45	51	0.07	56	60	0.3
WSG [29]	≥10	403	Epi/CPA/TT × 2	4	60	44	0.0006	75	70	0.02
Michelangelo [31]	≥4	382	Sequential Single agents	11	52	44	NS	60	51	NS
IBCSG [32]	≥10	340	Epi/CPA × 3	5.8	52	43	0.07	70	61	0.1
PÉGASE 01 [33]	>7	314	CPA/Mito/Mel	2.75	71	55	0.002	84	85	0.33
GABG [34,96]	≥10	302	CPA/TT/Mel	6.1	51	41	0.1	62	57	0.3
ICCG [35]	≥4	281	STAMP-V	5.6	57	59	0.7	66	67	0.4
JCOG [94]	≥10	95	CT	5.25	52	37	0.1	63	62	0.7
Dutch pilot [97,98]	Axillary level III involvement	81	CTC	6.9	49	47	0.37	61	62	0.85
MDACC [99,100]	≥10, or ≥4 after chemo	78	CPA/Eto/CDDP ×2 (DICEP)	12	26	40	0.1	42	47	0.1
MDACC/EBMT meta-analysis [101]	≥4	6210	Various	6	HR 0.87 in favor of HDC		0.0001	HR 0.89 (HDC)		0.16

n, No. of patients; FU, follow-up. NWAST, Netherlands Working Party on Autologous Transplantation in Solid Tumors; CALGB, Cancer And Leukemia Group B; ECOG, Eastern Collaborative Oncology Group; SWOG, Southwest Oncology Group; SBCG, Scandinavian Breast Cancer Group; WSG, West German Study Group; IBCSG, International Breast Cancer Study Group; GABG, German Autologous Bone Marrow Transplant Group; ICCG, International Collaborative Cancer Group; JCOG, Japan Clinical Oncology Group; MDACC, M.D. Anderson Cancer Center; EBMT, European Blood and Marrow Transplant Group; CPA, cyclophosphamide; TT, thioTEPA; Cb, carboplatin; Epi, epirubicin; Mito, mitoxantrone; Mel, melphalan; Eto, etoposide; HR, hazard ratio; NR, not reported; NS, not significant; mo, months; yr, years.

HER2-negative disease (n = 621) experienced a marked benefit from HDC in EFS (72% vs. 59%, $p = 0.002$), and OS (78% vs. 71%, $p = 0.02$), with an HR for relapse decreased by about one-third after HDC.

In the Cancer and Acute Leukemia Group B (CALGB) 9082 trial, Peters et al. enrolled 785 patients with ≥10+ nodes, who received four cycles of CAF and were randomized to HDC with cyclophosphamide/cisplatin/BCNU (STAMP-I) or to one additional cycle of those drugs at intermediate doses (IDs) with G-CSF support [23]. At a median follow-up of 7.3 years, there were no significant differences between the HDC and ID arms in EFS (61% vs. 58%, $p = 0.2$) or OS (71% for both groups, $p = 0.7$). There was a 31% reduction in the number of relapses in the HDC arm, particularly among women younger than 50 years of age, which was offset by a high TRM rate of 8.1% in the HDC arm (versus 0% in the ID arm).

In the Anglo-Celtic trial, Leonard et al. randomized 605 patients with ≥4+ nodes to receive four cycles of doxorubicin followed by either high-dose cyclophosphamide/thioTEPA (CT) or CMF × 8 [24]. At a median follow-up of 6 years, there were no differences in EFS (57 and 54%, respectively, $p = 0.7$) or OS (62 and 64%, respectively, $p = 0.4$).

Tallman et al. from the Eastern Cooperative Oncology Group (ECOG) randomized 540 patients with ≥10+ nodes to receive CAF × 6, with or without high-dose CT, administered with AHPCS from bone marrow or, towards the end of the study, PBPCs [25]. At a median follow-up of 6 years, there were no significant differences between HDC and SDC in EFS (55% vs. 48%, $p = 0.1$) or OS (58% vs. 62%, $p = 0.3$).

The Southwest Oncology Group (SWOG) trial randomized 539 patients with ≥4+ nodes (92% of them with 4–9+ nodes) to receive conventional treatment with sequential dose-dense adriamycin/paclitaxel/cyclophosphamide, or Adriamycin (doxorubicin)/cyclophosphamide (AC) × 4 followed by HDC (using STAMP-V in 86% and STAMP-I in 14% of patients) [26]. At a median follow-up of 70 months, there were no differences in EFS (78% vs. 73%, $p = 0.35$) or OS (87% vs. 83%, $p = 0.4$).

Bergh et al. for the Scandinavian Breast Group randomized 525 patients with ≥8+ nodes [or ≥5+ nodes plus an estrogen receptor (ER)-negative and high S-phase fraction tumor] to receive nine cycles of individually tailored dose-intensive FEC, or conventional FEC × 3 followed by HDC with STAMP-V [27]. At a median follow-up of 5 years, there was a trend in favor of tailored FEC in EFS (51% vs. 45%, $p = 0.07$), with no significant differences in OS (60% vs. 56%, $p = 0.3$) [28]. A major problem in interpreting this study is that the control arm received higher cumulative doses of chemotherapy than the transplant arm.

In the West German Study Group (WSG), Nitz et al. randomized 403 patients with ≥10+ nodes to receive a dose-dense arm with epirubicin/cyclophosphamide (EC) × 4 followed by CMF × 6 or a transplant arm with two initial cycles of EC followed by tandem cycles of high-dose epirubicin/cyclophosphamide/thioTEPA administered 4 weeks apart with AHPCS [29]. At a median follow-up of 4 years, HDC resulted in improved EFS (60% vs. 44%, $p < 10^{-3}$) and OS (75% vs. 70%, $p = 0.02$). An unplanned retrospective analysis showed a large benefit from HDC for tumors with a basal-like phenotype [ER/PR (progesterone receptor) negativity, HER2 negativity, and basal cytokeratin positivity], with HR for relapse of 3.06 (95% CI 1.41–6.06) if receiving dose-dense chemotherapy compared with tandem HDC [30].

The Michelangelo study by Gianni et al. compared the Milan high-dose sequential single-agent approach with SDC in 382 patients with ≥4+ nodes [31]. At a median follow-up of 11 years, non-significant differences in favor of the high-dose arm in EFS (52% vs. 44%) and OS (60% vs. 51%) were noted.

In the International Breast Cancer Study Group (IBCSG), 344 patients with ≥10+ nodes received AC/EC × 4 followed by CMF × 3, or a more dose-intense arm of three cycles of EC at supraconventional, but not myeloablative, doses every 3 weeks with AHPCS [32]. At the current follow-up of 6 years, the EFS rates were 52 and 43% for the dose-intense and the standard arms, respectively ($p = 0.07$), with OS rates of 70% versus 61% ($p = 0.1$).

In the PÉGASE 01 trial, Roché et al randomized 314 patients with >7+ nodes to receive SDC followed by high-dose cyclophosphamide/mitoxantrone/melphalan or observation [33]. At a short median follow-up of 33 months, there were significant differences in EFS (71% vs. 55%, $p = 0.002$), without an OS advantage (84% vs. 85%, $p = 0.3$).

Zander et al. from the German Autologous Bone Marrow Transplant Group (GABG) randomized 307 patients with ≥10+ nodes to receive EC × 4 followed by HDC with cyclophosphamide/thioTEPA/mitoxantrone, or CMF × 3 [34]. At a median follow-up of 6 years, there were non-significant differences in favor of HDC in EFS (51% vs. 41%, $p = 0.1$) and OS (62.5% vs. 57 %, HR 0.84, $p = 0.3$).

In the study by the International Collaborative Cancer Group, 281 patients with ≥4+ nodes were randomized to receive FEC × 6 or FEC × 3 followed by HDC with STAMP-V [35]. At a median follow-up of 5.7 years, there were no differences in EFS (59% vs. 57%, $p = 0.76$) or OS (67% vs. 66%, $p = 0.4$).

A meta-analysis based on individual patient data from 15 randomized included a total of 6210 patients. After a median follow-up of 6 years, a benefit in EFS was observed after HDC (HR 0.87, $p < 0.001$), but not in OS (HR 0.94, $p = 0.13$) [36].

Prognostic factors of benefit from HDC

Retrospective studies have identified the MBC populations who are most likely to achieve long-term remissions after HDC, such as those with low tumor burden, in CR after SDC, or without liver involvement [37,38]. A prospective study of HDC with STAMP-I for MBC patients early in the course of their disease with low tumor burden (oligometastatic) was conducted at the University of Colorado [39]. At a median post-transplant follow-up of 5 years, the EFS and OS rates of 60 patients were 52 and 62%, respectively, with median EFS and OS times of 4.3 and 6.7 years, respectively (Figure 61.3a). HER2-negative status and a single metastatic site were independent favorable predictors of outcome (Figure 61.3b) [40].

Likewise, retrospective analyses have identified prognostic factors for HDC for HRPBC, including hormone receptor status, tumor size, axillary nodal status (particularly when determined by the nodal ratio between number of positive nodes and number of sampled nodes) [41,42]. HER2 status (Figure 61.4a) [43], epidermal growth factor receptor (EGFR) overexpression (Figure 61.4b) (in particular when co-overexpressed with HER2, Figure 61.4c) [44], or tumor angiogenesis (Figure 61.4d) [45].

Conclusions

Twenty-five years after its first trial for MBC, the use of HDC for breast cancer has almost ceased, with no active trials currently registered in the United States. Randomized trials in the MBC and HRPBC settings have largely failed to show OS benefits. While many of those trials were underpowered to detect realistic survival differences, their meta-analyses did not show a global OS

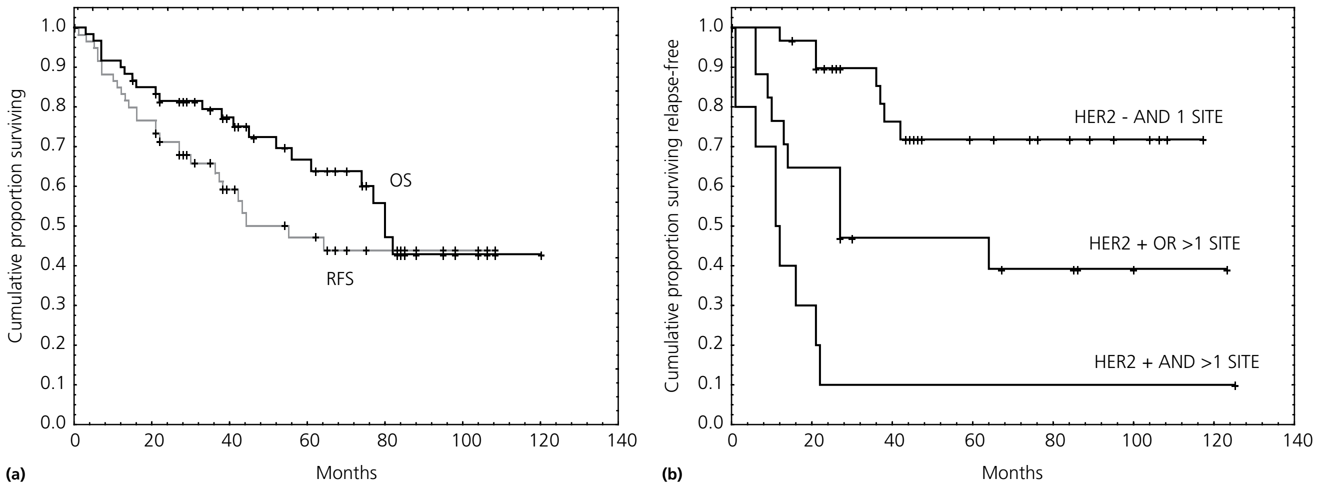

Figure 61.3 HDC for oligometastatic disease. (a) Relapse-free survival (RFS) and overall survival (OS) results of a prospective trial of STAMP-I; (b) RFS curves in this trial according to HER2 and number of metastatic sites.

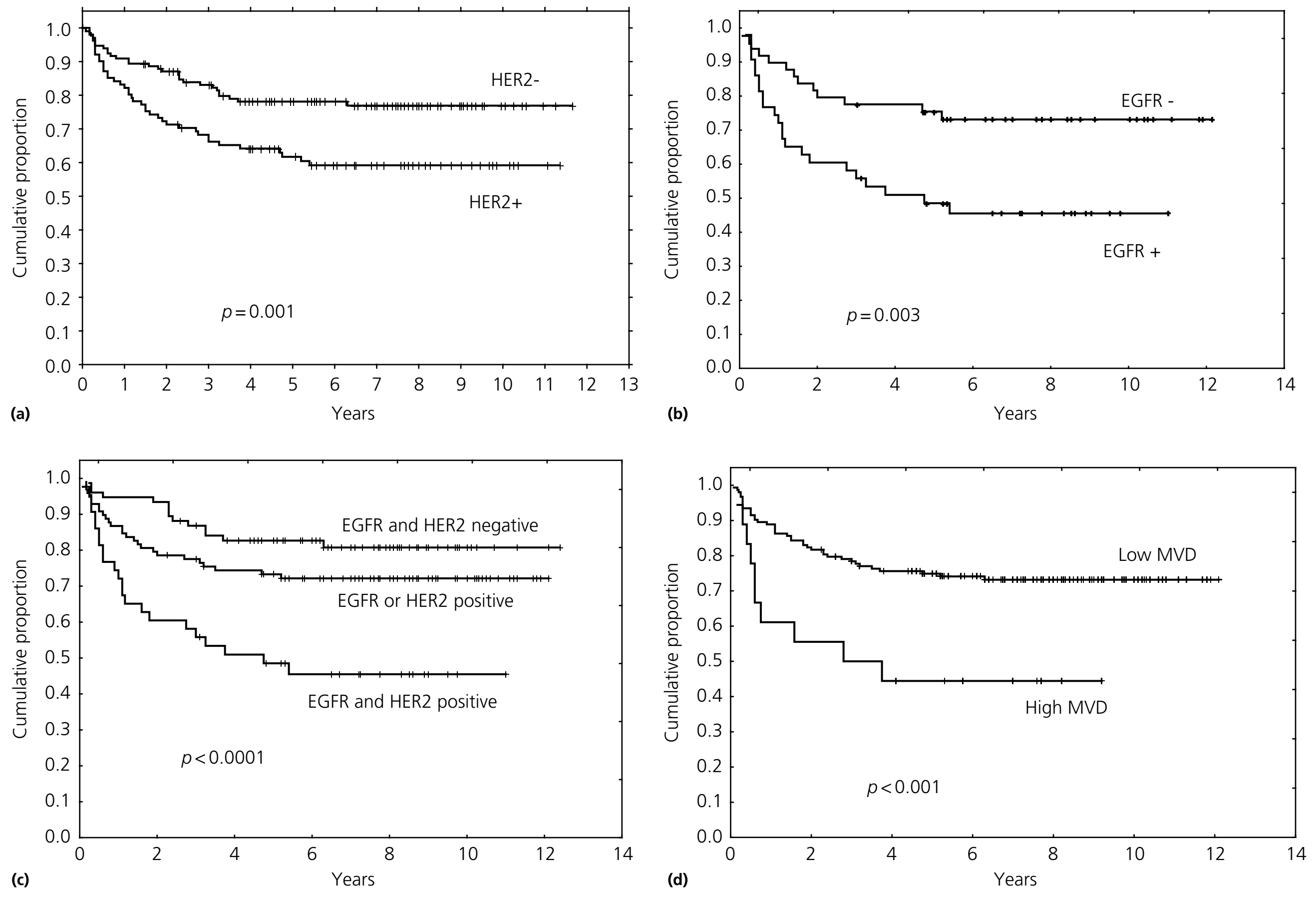

Figure 61.4 Effect of biologic prognostic factors on EFS of patients with HRPBC after HDC. (a) Effect of HER2 overexpression; (b) effect of EGFR expression; (c) combined effect of HER2 and EGFR expression; (d) effect of intratumor microvessel density (MVD).

benefit in either setting [17,36]. Those overviews, however, did detect a statistically significant 13% decrease of risk of relapse in HRPBC and a 24% decrease of risk of progression in MBC. In our view, the data do not justify the abandonment of the transplant approach to breast cancer, given the persistent need for curative treatment strategies for its advanced stages. Clearly, a fraction of patients may benefit from a high-dose approach, such as those with oligometastatic disease. In addition, and independent of whether the superior tumor debulking capacity of HDC translates into improved outcomes, post-transplant minimal residual disease would still provide an optimal scenario for application of novel therapies. In contrast to an all too common opinion, HDC and novel therapeutics targeting signal-transduction pathways are not mutually exclusive. In solid tumors, available data suggest that these promising new agents may help improve outcome when combined with chemotherapy but are unlikely to have a substantial impact alone. The anti-HER2 monoclonal antibody trastuzumab or other biological agents can be easily administered after transplant, or integrated into the high-dose regimen exploiting their synergy [46].

Personalized medicine has re-emphasized the importance of evaluating small patient populations who achieve uncommon benefit following a novel treatment [47]. For example, many trials of HDC for MBC demonstrated that 10–15% patients with advanced disease experienced EFS of more than 5-years (Table 61.3). If a sizable fraction of these patients continue in remission 15 years later, this observation may suggest the re-examination of the "incurability" of metastatic breast cancer for selected patients. These types of long-term follow-up should be reported. It would be of interest if personalized medicine concepts induce a re-examination of the importance of the "tail" of Kaplan–Meier curves using novel statistical tools [48], which might reflect a subset of cured patients.

Table 61.3 Overall results from major phase II trials of HDC with ASCT for breast cancer and germ-cell tumors

Tumor	Setting	Long-term EFS (%)	Ref.
Breast	4–9 nodes	60–70	[41]
	10+ nodes	55–65	[19,20,41,42]
	Inflammatory carcinoma	55–65	[41]
	Metastatic chemosensitive disease	10–20	[38]
	Oligometastatic disease	55	[40]
	Refractory or high-burden relapse	<5	
NSGCT	Refractory or ≥2nd relapse	15	[53–55]
	1st relapse	50	[56–67]
	First-line high-risk disease	50–70	[84–86]

NSGCT, non-seminomatous germ-cell tumor.

Germ-cell tumors

Germ-cell tumors (GCTs) represent the most common malignancy in young men between the ages of 15 and 35 years. Since the advent of modern cisplatin-based chemotherapy, cure rates for metastatic disease have reached 70–80%. The standard first-line regimen of bleomycin/etoposide/cisplatin (BEP) yields long-term EFS rates of 90% in advanced-disease good-risk patients, as defined by the International Germ Cell Consensus Classification, which decreases to 80% for intermediate-risk GCT and 50% for poor-risk patients (Table 61.4) [49]. Standard salvage regimens, based on ifosfamide and cisplatin, plus either vinblastine (VeIP) [50], etoposide (VIP) [51], or paclitaxel (TIP) [52], result in CR rates of 50–60% and long-term EFS of 20–30% of patients in first relapse. Patients in second relapse are largely incurable with standard treatment.

HDC for refractory/relapsed NSGCT

The use of tandem high-dose cycles of carboplatin/etoposide (CE) with autologous stem-cell transplantation (ASCT) as salvage therapy was pioneered by Nichols and colleagues at Indiana University in the 1980s. In sequential studies, this treatment resulted in long-term EFS rates of 15% for patients in second relapse [53–55] and 38% for those transplanted in first relapse [56,57]. The introduction of PBPCs and improvement of supportive measures allowed the re-escalation of the dose of these drugs and improvement of results [58]. Einhorn et al. subsequently reported 68% EFS at a median follow-up of 48 months in a more recent report from the Indiana group in 184 patients (135 transplanted in second-line therapy and 49 in third-line or later therapy) who received tandem cycles of carboplatin (2100mg/m^2) and etoposide (2250mg/m^2) [59]. The EFS rates in patients transplanted in first or in second or later relapse were 70 and 45%, respectively. The outcome was similar in patients with seminoma and non-seminoma histologies (74% vs. 60%). These excellent results reflect, in part, patient selection, with no patients with late relapses or primary mediastinal tumors included, and around one-third of the patients presenting features predictive of good outcomes with SDC alone.

Several groups have tested the addition of a third drug to the CE backbone. Motzer et al. treated 58 cisplatin-refractory patients with two cycles of carboplatin/etoposide/cyclophosphamide (CEC), with a 40% CR rate and 21% EFS rate at a median follow-up of 28 months [60].

Table 61.4 Risk stratification of newly diagnosed patients (International Germ Cell Consensus Classification)

	Non-seminoma		Seminoma	
Low risk	• Testis or retroperitoneal primary *and* • No non-pulmonary visceral metastases *and* • Good markers (AFP < 1000ng/mL *and* B-HCG < 5000IU/L *and* LDH < 1.5ULN)	• 56% patients • 90% EFS at 5 years	Any primary site *and* • No non-pulmonary visceral metastases *and* • Normal AFP, any B-HCG, any LDH	• 90% patients • 80% EFS at 5 years
Intermediate risk	Testis or retroperitoneal primary *and* • No non-pulmonary visceral metastases *and* • Intermediate markers (AFP 1,000–10,000ng/mL *or* B-HCG 5,000–50,000IU/L *or* LDH 1.5–10ULN)	• 28% patients • 80% EFS at 5 years	• Testis or retroperitoneal primary *and* • Non-pulmonary visceral metastases *and* • Normal AFP, any B-HCG, any LDH	• 10% patients • 67% EFS at 5 years
Poor risk	• Mediastinal primary, *or* • Non-pulmonary visceral metastases *or* • Poor markers (AFP >10,000ng/mL *or* B-HCG >50,000IU/L *or* LDH >10ULN)	• 56% patients • 50% EFS at 5 years		

ULN, upper limit of normal.

Siegert et al. treated 74 patients with recurrent disease with two SDC cycles followed by one cycle of HDC with ifosfamide/carboplatin/etoposide (ICE), with 50% 2-year EFS rates among patients with sensitive tumors, but only 4% EFS in those with refractory disease [61]. Margolin et al. reported a 45% EFS rate after tandem cycles of high-dose ICE in 20 patients with relapsed, cisplatin-sensitive tumors [62]. The same group subsequently treated 31 relapsed patients with sequential cycles of HDC with paclitaxel (425mg/m^2 over 24 hours)/carboplatin/etoposide (cycle 1) and ICE (cycle 2) [63]. At a median follow-up of 5.5 years, 12 patients (39%) remained free of disease. Rick et al. treated 80 patients, most with cisplatin-sensitive disease, with 3–4 cycles of SDC followed by one cycle of high-dose carboplatin/etoposide/thioTEPA [64]. At a median follow-up of 3 years, the EFS rate was 26%. Overall, high-dose triplets achieve 40–50% EFS rates in patients with cisplatin-sensitive tumors, and 4–20% in those with refractory disease [65,66].

Feldman et al. tested the use of three cycles of HDC, and reported encouraging results in 107 patients with relapsed disease and unfavorable prognostic features treated with two biweekly cycles of paclitaxel/ifosfamide followed by high-dose CE × 3 (TICE), with 47% DFS at 5 years [67].

A novel strategy to improve results attempts to modulate the effect of HDC with biologic agents. GCTs are highly vascularized and express vascular endothelial growth factor (VEGF) prominently. The expression of this angiogenic factor correlates closely with microvessel density within metastatic GCT lesions, which has been associated with prognosis [68,69]. Use of a single dose of the anti-VEGF monoclonal antibody bevacizumab before chemotherapy decreases the tumor interstitial fluid pressure and enhances access of the cytotoxic drugs into the solid tumor lesions [70]. Attempting to exploit their synergy, investigators at the M.D. Anderson Cancer Center are conducting a phase II trial of sequential cycles of bevacizumab–HDC in highly refractory or multiply relapsed patients. A preliminary analysis of their phase II trial of bevacizumab–gemcitabine/docetaxel/melphalan/carboplatin followed by bevacizumab–ICE showed very encouraging early results, with a 2-year EFS of 60% in a population with very poor prognosis [71].

Can we predict the outcome of patients with relapsed GCT receiving HDC?

Beyer et al. analyzed 283 relapsed/refractory patients treated with CE [72]. They identified the following independent adverse predictors: refractoriness to cisplatin (progression within 4 weeks after treatment with cisplatin), absolute refractoriness to cisplatin (no response to initial platinum chemotherapy), primary mediastinal tumor, and high tumor markers [human chorion gonadotropin (HCG) >1000U/L] at the time of HDC. A prognostic score based on these variables was developed (Table 61.5). The resulting good-, intermediate-, and poor-risk groups presented 2-year EFS of 51, 27,

Table 61.5 Prognostic models for HDC for NSGCT

Model		Factor		Points	2-year EFS (%)
Beyer	Variables	Progressive disease before HDC		1	
		Mediastinal primary tumor		1	
		Cisplatin-refractory disease(relapse within 4 weeks of completion of first-line chemotherapy)		1	
		Absolute cisplatin-refractory disease(PD as best response to prior therapy)		2	
		B-HCG > 1000IU/L before HDC		2	
	Stratification	Low risk		0	51
		Intermediate risk		1–2	27
		High risk		>2	5
Einhorn	Variables	HDC at third-line or subsequent line of treatment		3	
		Refractory disease before HDC(relapse within 4 weeks of completion of first-line chemotherapy)		2	
		High-risk IGCCCG stage		2	
	Stratification	Low risk		0	80
		Intermediate risk		2–3	60
		High risk		>3	40
International Prognostic Factors Study Group	Variables	Histology	Seminoma	–1	
			Non-seminoma	0	
		Primary tumor site	Mediastinal	3	
			Retroperitoneal	1	
			Gonadal	0	
		Response to first-line chemotherapy	CR/PRm–	0	
			PRm+/SD	1	
			PD	2	
		Progression-free interval following first-line chemotherapy	>3 months	0	
			≤3 months	1	
		AFP at salvage	Normal	0	
			≤1000	1	
			>1000	2	
		B-HCG at salvage	≤1000	0	
			>1000	1	
		Liver/brain/bone metastases	No	0	
			Yes	1	
	Stratification	Very low risk (seminoma + low risk)		–1	92
		Low risk		0	64
		Intermediate risk		1–2	53
		High risk		3–4	33
		Very high risk		>4	22

and 5%, respectively. This model was externally validated in a group of 45 patients with cisplatin-refractory relapsed disease, treated with HDC using ICE × 2, where the subsets with good, intermediate and poor risk had 62, 13, and 0% EFS rates, respectively, at a median follow-up of 32 months [73]. The Beyer model was based on patients treated between 1984 and 1993, and most of them had received a single course of HDC. Of these patients, 91% had failed at least one prior salvage regimen.

Einhorn et al. developed a different prognostic model based on their experience in 184 patients treated at Indiana University with tandem CE [59]. Patients with primary mediastinal tumors were not included for this analysis owing to their earlier findings that none of the patients that the authors had treated were alive and free of disease at 2 years [74]. Three independent adverse prognostic factor were identified: an International Germ Cell Cancer Collaborative Group (IGCCCG) poor-risk classification at initial diagnosis, cisplatin-refractory disease [defined as progressive disease (PD) within 4 weeks of most recent cisplatin-based chemotherapy] and administration of HDC as third-line or later treatment. Patients in the low-, intermediate-, and high-risk categories had EFS rates of approximately 80, 60, and 40%, respectively.

The International Prognostic Factor Study Group (IPFSG) analyzed 1594 patients treated after 1990 with HDC (n = 821) or SDC (n = 773) as first salvage therapy [75]. Among patients receiving HDC, half received a single cycle and the other half received tandem cycles. Seven factors were found to be independent predictors of outcome, after either SDC or HDC: primary tumor site (mediastinal vs. retroperitoneal vs. gonadal), response to first-line therapy, length of prior progression-free interval, α-fetoprotein (AFP) at salvage, β-HCG at salvage, and presence of non-pulmonary visceral metastases. A composite score based on these factors assigns patients to a very low (only seminoma), low, intermediate, high or very high risk categories. Within each prognostic category, PFS at 2 years was significantly superior after HDC compared with SDC, which translated into improved OS (Table 61.6). The strengths of this international analysis are its very large sample size and the requirements of a well-documented unequivocal progression or relapse, and also of prior use of modern first-line and salvage treatments. It is applicable to patients receiving either SDC or HDC at the time of first recurrence, a setting in which it seems more applicable than the other two models. In both the IPFSG and Beyer models, HDC was administered in either one (50 and 91% of the patients, respectively) or tandem (50 and 9%, respectively) cycles. In contrast, all patients in the Indiana model received tandem cycles.

Table 61.6 EFS and OS rates in patients treated with HDC or SDC (International Prognostic Factors Study Group)

Patients	Treatment	2-yr EFS (%)	*p*	5-yr OS (%)	*p*
All patients	SDC	28	<0.001	41	<0.001
(n = 1594)	HDC	50		53	
Very low risk	SDC	58	<0.001	64	<0.01
(n = 76)	HDC	92		89	
Low risk	SDC	40	<0.001	66	0.98
(n = 257)	HDC	64		64	
Intermediate risk	SDC	32	<0.001	45	<0.001
(n = 646)	HDC	53		58	
High risk	SDC	17	<0.001	23	<0.005
(n = 351)	HDC	33		35	
Very high risk	SDC	2	<0.001	3	<0.001
(n = 105)	HDC	22		27	

Both the Beyer and the Indiana models are applicable to patients in second or later relapses. Whereas the Indiana model reflects better the common US practice of using tandem HDC cycles, the Beyer score seems more capable of detecting a very poor prognosis subset of patients, for whom novel approaches are clearly needed.

Randomized trials of HDC in the salvage setting

Retrospective matched-pair comparisons of HDC with SDC for patients in first relapse have suggested a large benefit from transplant [76,77]. Pico et al. at the European Group for Blood and Marrow Transplantation (EBMT) conducted the only randomized trial to date comparing HDC with SDC in relapsed patients. They randomized 280 relapsed patients to receive three cycles of SDC followed by one more cycle (SDC arm) or a single course of high-dose CEC with ASCT (HDC arm) [78]. At a median follow-up of 45 months, no differences in EFS (35% vs. 42%, p = 0.16) or OS (53% in both arms) were apparent. This trial has been criticized because of a 30% drop-out rate in the HDC arm, the higher than expected treatment-related mortality in both study arms (3 and 7%, respectively), and the use of a single rather than sequential cycles of HDC.

Lorch et al. randomized 216 patients with relapsed or refractory disease to receive one cycle of high-dose CE or two sequential cycles of those drugs at slightly lower doses, requiring AHPCS in both arms [79]. Accrual to this study stopped after an excessive 14% TRM was detected in the single cycle arm. Ata median follow-up of 3 years, the differences in favor of the sequential HDC arm in EFS (40% vs. 37%) and OS rates (80% vs. 61%) did not reach statistical significance.

In summary, there is no consensus yet as to whether HDC should be considered for all patients at the time of first relapse. Results of tandem cycles of HDC as salvage treatment appear superior to those expected with SDC, which have prompted many to consider HDC the standard salvage treatment [80], despite the absence of evidence of benefit from randomized studies. It is possible that more than one cycle of HDC is necessary to achieve a favorable outcome, and that a single cycle, as administered in the European randomized study, may not be sufficient to improve results. Of note, in the IPFSG analysis, the 2-year DFS rates for patients receiving sequential HDC cycles appeared superior to those receiving a single high-dose cycle (55% vs. 44%, $p < 0.001$). An international randomized phase III trial comparing TICE with three cycles of HDC with to SDC with four cycles of TIP will soon address this important question [81].

Other areas of uncertainty

It is unclear whether patients with relapsed primary mediastinal GCT can achieve benefit from HDC. Patients with relapsed primary mediastinal germ-cell tumors (PMGCTs) have typically been reported to present very poor outcomes after HDC, which has led some investigators to abandon this strategy for these patients [82]. In contrast, others have reported long-term CRs within this population in their HDC studies [67,79].

Late relapses, defined as recurrence 2 years or longer following a CR to cisplatin-based first-line therapy, are a disconcerting syndrome characterized for its poor response to chemotherapy. While surgery is the preferred approach [83]. it remains unclear whether patients with unresectable late relapses might benefit from HDC as some data have suggested [79].

First-line HDC for poor-risk NSGCT

Motzer et al. at the Memorial Sloan Kettering Cancer Center developed a strategy of switching to HDC those patients showing a reduced clearance and prolonged half-life of tumor markers during

SDC [84]. This approach yielded a CR rate of 57%, EFS of 50% at a median follow-up of 30 months, and significantly longer OS than for historical controls from the same institution.

Schmoll et al. reported long-term results of HDC in 221 patients with advanced poor-prognosis disease, who received one cycle of SDC followed by three cycles of high-dose VIP (high-dose etoposide/ifosfamide + standard-dose cisplatin) [85]. At a median follow-up of 4 years, the EFS rate was 69%. Non-randomized matched-pair comparisons of patients enrolled in this trial with others enrolled in trials of SDC suggested superiority of HDC [86].

The role of HDC in first-line therapy has been evaluated in three randomized studies. A European randomized trial conducted in the 1980s failed to show a therapeutic benefit for HDC in 115 untreated poor-risk patients, who were randomized to receive 3–4 cycles of SDC or two cycles followed by one cycle of a stem-cell-supported high-dose cisplatin-containing regimen [87]. Patients randomized to the transplant arm received less total cisplatin than those on the standard-dose arm, and also an HDC regimen that can be considered substandard by present criteria. It is therefore felt that this study did not appropriately address the role of first-line HDC in poor-risk GCT.

A European study compared SDC with BEP × four cycles with one cycle of VIP followed by three cycles of high-dose VIP with ASCT in patients with poor-risk features by the IGCCCG criteria [88]. This study closed prematurely after slowly enrolling only 137 of the 222 planned patients. At its first analysis with a median follow-up of 4.4 years, the 2-year EFS (45% vs. 58%, $p = 0.06$) and 2-year OS (66% vs. 73%, $p > 0.05$) differences did not reach statistical significance.

In a US Intergroup trial, Motzer et al. randomized 219 patients with newly diagnosed disease with intermediate- or poor-risk features according to the IGCCCG criteria to receive four cycles of BEP or two cycles of BEP followed by two cycles of HDC with CEC [89]. The 1-year durable CR rates did not differ significantly between the transplant and control arms (48 vs. 52%, $p = 0.5$). There were no significant differences in EFS ($p = 0.4$) or OS ($p = 0.9$). A planned subset analysis according to early tumor marker clearance suggested a significant benefit of HDC among those patients experiencing a slow marker decline (1-year EFS 61% vs. 34%, $p = 0.03$), in contrast with similar outcomes in both arms in the group of patients with a satisfactory marker drop ($p = 0.5$). Given the small size of this subset analysis, these provocative observations are not robust enough to allow firm conclusions to be drawn.

In summary, the available data from randomized trials indicate that there is currently no standard role for HDC in first-line treatment of GCT.

Special considerations

Surgical management after HDC

A retrospective review of the German experience identified 216 patients treated in three consecutive HDC trials between 1989 and 1999 [90]. Partial remissions with negative or positive tumor markers after high-dose chemotherapy were achieved in 128 patients (59%). Of these patients, 57 (45%) proceeded to residual tumor resection; the remaining 71 (55%) could not be operated on because of PD, unresectability of the tumor masses, poor performance status, or patient refusal. With a median follow-up of 87 months, 37/57 patients (65%) were alive, 34 of whom (59%) remained continuously disease free. Viable cancer was documented in the resected specimen of 26 (46%) of the 57 patients. The 5-year EFS rates in patients with and without viable cancer were 42 and 84%, respectively ($p < 0.01$). Of note, all patients in this series had been treated with a single cycle of high-dose chemotherapy, which likely influenced the incidence of viable cancer in resected specimens. This report indicates that post-chemotherapy resection can contribute significantly to overall treatment outcome, and that residual tumor resection should be considered in patients in partial remission after HDC.

Management of relapse post-HDC

A retrospective review of the Indiana University experience identified 101 patients who relapsed after HDC after a median interval of 10 months. Forty-seven of them subsequently received salvage SDC, with an overall RR of 18.2% and a CR rate of 5%. Seven patients underwent surgery alone, with only one long-term survivor [91].

Two prospective trials have studied salvage SDC for relapse after HDC. In a phase II trial at Indiana University, 32 patients with PD after CE × 2 received paclitaxel/gemcitabine [92]. Patients who had received either agent prior to HDC were ineligible. A response was achieved by 10 patients (31%), including six CRs and four PRs (of 2–6 months' duration). Four patients in CR (12.5%) were continuously disease free without any additional treatment at 20–57 months from paclitaxel/gemcitabine. One additional CR patient was rendered disease free after two subsequent resections of tumor. A German study enrolled 32 patients with relapse after HDC and nine patients with cisplatin-refractory disease who were rescued with gemcitabine/oxaliplatin/paclitaxel [93]. Two patients (6%) achieved a CR, 34% achieved a marker-negative PR, and 12% achieved marker-positive PR, with an overall RR of 51%.

Therefore, although outcomes have been largely unfavorable for patients who relapse after HDC, these trials point to some possibility of successful chemotherapeutic salvage even in heavily pretreated and cisplatin-refractory patients.

Conclusion

There is currently no standard role for HDC in first-line treatment of GCT. Definition of its role as part of first-line salvage awaits completion and analysis of the upcoming international randomized trial. HDC is widely considered a valid treatment option for patients with refractory disease or in second relapse or later (Table 61.3).

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CHAPTER 62

Hematopoietic Cell Transplantation for Renal Cell Carcinoma and Other Solid Tumors

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Introduction

Our perception of the mechanisms through which malignant cells are eradicated following allogeneic hematopoietic cell transplantation (HCT) has evolved substantially over the past four decades. No longer merely thought of as a means of rescuing hematopoietic function following high-dose conditioning, allogeneic transplantation is now known to be a powerful type of immunotherapy capable of curing patients with otherwise fatal malignant diseases. This conceptual evolution has translated into a diversification of the indications for allogeneic HCT and led to the development of reduced-intensity transplant approaches whose beneficial antineoplastic effects can be attributed largely to the transplanted donor immune system. Over the past 10–15 years, several groups have sought to determine whether non-hematologic malignancies might likewise be susceptible to allogeneic immune attack. In this chapter, we highlight recent work that has improved our awareness and understanding of the graft-versus-tumor (GVT) effect, and discuss the current results of its application as an investigational therapeutic modality in the treatment of metastatic solid tumors.

Allogeneic transplantation as immunotherapy: the GVT effect

Acute leukemias have traditionally been viewed as “chemosensitive” malignancies. The inability to “cure” the majority of adults with leukemia following conventional chemotherapeutic regimens was attributed at least in part to a failure to deliver the sufficiently high doses of antineoplastic agents required to eradicate all malignant cells. Dose-limiting toxicities, including prolonged delays in lymphohematopoietic recovery, significantly hindered efforts to intensify such regimens. Pioneering work by E.D. Thomas and others led to the development of allogeneic HCT as a means of accelerated recovery of lymphohematopoietic function following the administration of high-dose chemotherapy [1,2].

For the next two decades, allogeneic HCT became increasingly used as a therapeutic option to treat patients with a variety of different treatment-resistant hematologic malignancies. Initially, the curative potential of the procedure was ascribed solely to the cytotoxic effects of the conditioning regimen, be it chemotherapy alone or in conjunction with total body radiation. It was not until years following its inception that investigators first documented the very important and powerful immune mechanisms that contributed to the curative potential of the approach in humans.

Throughout the 1980s, several landmark observations provided insights into this phenomenon known as the “graft-versus-tumor” effect. First, in an analysis of over 200 patients with hematologic malignancies undergoing human leukocyte antigen (HLA)-matched HCT from a sibling donor, investigators noted that patients who experienced moderate-to-severe acute graft-versus-host disease (GVHD) or chronic GVHD had a significantly lower incidence of leukemic relapse compared with patients with little or no GVHD [3]. Similar benefits in disease-free survival were observed in recipients of HLA-identical (non-twin) sibling donor allografts compared with those receiving syngeneic allografts [4], and also in recipients of T-cell-replete grafts versus those receiving T-cell-depleted transplants [4–7]. These observations suggested a very important role for donor T cells in mediating this effect, and also implicated the targets of GVT as falling outside the realm of antigens encoded by the major histocompatibility complex (MHC). Definitive evidence that GVT effects were clinically meaningful came from studies in patients with chronic myelogenous leukemia who relapsed post-transplant and were subsequently induced back into remission following a donor lymphocyte infusion (DLI) [6].

Over the last two decades, numerous investigators have successfully exploited GVT effects against a variety of malignancies of hematopoietic origin, including both acute and chronic leukemias, lymphomas, multiple myeloma, and Epstein–Barr virus (EBV)-related lymphoproliferative disorder [8–16]. The ability of GVT to induce remission varies among these malignancies, with the critical determining factors dictating susceptibility to GVT being not entirely understood.

The immune system and solid tumors

Tumors such as metastatic melanoma and renal cell carcinoma (RCC), with their ability to undergo occasional spontaneous regression or maintain prolonged periods of stable disease, have long fascinated investigators and led to the concept that the immune system might play a role in the regulation or control of malignant cells. Indeed, the first spontaneous regression of metastatic RCC after nephrectomy was described in 1928 and was attributed to an antibody-mediated immune response [17]. The propensity of nude (athymic) mice to develop lymphoreticular tumors, and the increased incidence of malignancies in patients with congenital or acquired immunodeficiency states, lent further credence to the

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notion that the immune system might play a role in the control of cancer [18,19]. The development of immune-based therapeutic strategies against metastatic solid tumors was a logical consequence of these observations.

At the National Cancer Institute (NCI), pilot immunotherapy trials in patients with advanced solid tumors – particularly metastatic RCC and metastatic melanoma – were conducted in the early 1980s by Rosenberg and colleagues [20]. The earliest trials were intended to non-specifically stimulate innate immune responses against tumors using cytokines such as interleukin-2 (IL-2). In their preliminary experience using IL-2 in patients with either metastatic RCC or melanoma, overall response rates of 20% (RCC) and 17% (melanoma) were observed. Importantly, some patients achieved complete and durable remission of disease, providing valuable proof of concept of the therapeutic potential of immune-based therapeutic strategies. Subsequent studies conducted at the NCI and also by the Cytokine Working Group employed adoptive transfer of lymphokine-activated killer cells or ex vivo-expanded tumor-infiltrating lymphocytes in conjunction with IL-2. Although response rates in early trials approached 35%, further evaluation suggested that tumor-infiltrating lymphocyte therapy provided little additional therapeutic benefit over cytokine treatment alone [21–27]. More recently, investigators have evaluated the use of immunosuppressive chemotherapy prior to infusion of IL-2 and autologous tumor infiltrating lymphocytes in an attempt to promote selective in vivo expansion of cytotoxic T-lymphocytes (CTLs). This approach has met with some success in patients with metastatic melanoma, but is yet to gain widespread acceptance in clinical practice [28,29]. The low response rates and significant morbidity that were frequently associated with such therapy (particularly high-dose IL-2) provided the impetus for the exploration and development of immune strategies that would selectively target the tumor.

The foundation for tumor-specific cellular immunotherapy was laid following the identification of a number of different tumor-associated antigens (TAAs) in melanoma and other solid tumors [30–32]. Several clinical trials have evaluated the safety and efficacy of cancer vaccines designed to enhance immune responses to TAAs. Although immune correlative studies have shown that some vaccines significantly expand TAA-reactive T-cell populations in vivo, early clinical results from such trials have been modest.

Several factors likely contribute to the poor clinical efficacy of conventional vaccine strategies designed to enhance "self" immunity against TAAs. To date, most trials have used an MHC class I-restricted peptide-based approach. As a consequence, resultant immune responses are limited to a single antigenic epitope devoid of a critical CD4$^+$ helper T-cell component [33]. Furthermore, such vaccine strategies may favor the selection of tumor cells lacking the targeted antigen, a phenomenon described as "antigen escape." Modifying this approach by simultaneously immunizing with multiple tumor antigens that are both MHC class I and II restricted, or by using the tumor itself as a vaccine (tumor lysates, tumor apoptotic bodies, etc.), may partly overcome this limitation [34]. It has also been suggested that tumors may evade CTLs by downregulating expression of accessory molecules required by CTLs to mediate lysis or by engaging inhibitory cell surface receptors such as CTLA-4 and PD-1 expressed on T-lymphocytes. Indeed, inhibitors of both CTLA-4 and PD-1 are being actively explored in patients with solid tumors as a means of circumventing tumor-mediated immune suppression and promoting innate antitumor immunity. Ipilimumab, an antibody against human CTLA-4, has been shown in randomized phase III studies to prolong survival in patients with metastatic melanoma, while several antagonists of the PD-1 pathway are currently being evaluated in early-phase clinical trials with promising activity in several solid tumors including melanoma and RCC [35].

Perhaps one of the greatest limitations of conventional immunotherapy is that the host immune system that it attempts to enhance has intrinsic functional compromise. A number of lines of evidence support this concept. First, the host immune system is often rendered incompetent by prior chemotherapy and/or by tumor-related factors. Second, longstanding immune tolerance to tumor antigens, including TAAs targeted by conventional cancer vaccines, may exist. Theoretically, these limitations could be circumvented by allogeneic HCT, a procedure that culminates in complete host immune replacement. Perhaps more importantly, unlike innate host immunity, an allogeneic immune system would have the capacity to mount immune responses to polymorphic variants of tumor-specific or broadly expressed minor histocompatibility antigens (mHAs) [36–38].

Solid tumors as a GVT target

Animal models exploring graft-versus-solid tumor effects

The notion that immune responses following allogeneic HCT can be directed against solid tumors was first tested in animal models in the early 1980s. Using a mouse model, Moscovitch and Slavin showed that the high incidence of spontaneous lymphosarcomas in NZ B/W hybrids could be significantly diminished by the transplantation of allogeneic hematopoietic stem cells from BALB/c mice [39]. Subsequently, this group demonstrated the ability of minor and major histocompatibility antigen-mismatched transplants to protect mice from developing metastatic disease following mouse mammary adenocarcinoma (4T1) tumor challenge [40,41]. These experiments showed that alloimmune responses against mHAs that were expressed on tumors could be generated following hematopoietic stem cell transplantation.

Early clinical data suggesting GVT effects against solid tumors in humans

Compelling clinical evidence supporting the therapeutic utility of the GVT effect in hematologic malignancies prompted investigators to explore allogeneic HCT as a therapeutic tool against metastatic solid tumors in the late 1990s. The first report suggesting a possible GVT effect against a tumor of epithelial origin noted the incidental regression of a metastatic breast adenocarcinoma lesion following allogeneic HCT for relapsed acute myeloid leukemia [42]. Subsequently, Eibl et al. reported tumor regression coincident with acute GVHD in a patient with chemotherapy-resistant metastatic breast cancer following a high-dose transplant from an HLA-identical sibling [43]. The ability to expand mHA-specific CTLs (obtained from the patient at the time of clinical response) that lysed breast cancer cell lines in vitro lent credence to the argument that the tumor regression was at least in part mediated by alloimmune T-cell-mediated mechanisms. Investigators at the M.D. Anderson Cancer Center reported their experience in 10 patients with metastatic breast cancer who received an allogeneic HCT from an HLA-matched sibling following high-dose conditioning [44]. One complete response (CR) and five partial responses (PRs) were noted. GVT effects were implicated in at least two patients whose tumor regression was temporally associated with the onset of acute GVHD and withdrawal of immunosuppression. Delayed regression of metastatic lesions following high-dose allogeneic HCT in a woman with ovarian cancer offered further evidence that some tumors of epithelial origin were indeed susceptible to GVT effects [45].

Use of reduced-intensity conditioning in allogeneic transplantation for solid tumors

By the late 1990s, there was sufficient interest in studying GVT effects against solid tumors based on available preclinical and clinical data. The major factor limiting the initiation of pilot trials was the significant morbidity and mortality associated with conventional allogeneic HCT. Dose-intensive conditioning used to provide both tumor cytoreduction and promote donor engraftment contributed in part to the morbidity and mortality associated with HCT. Subsequently, it was recognized that reducing the intensity of the preparative regimen might translate to a reduction in the risk of early, conditioning-related morbidity and mortality.

In the late 1990s, transplant regimens using reduced-intensity conditioning (RIC) were designed by a number of investigators, and were evaluated for their engraftment potential and toxicity profile [46–50]. Two major factors impacted on the design and development of these dose-reduced conditioning regimens. First was the recognition that GVT effects alone might be sufficient to eradicate some hematologic malignancies in the absence of dose-intensive therapy. Second was the realization that the primary role of the conditioning regimen might be limited to preparing the recipient for engraftment by inducing adequate host immunosuppression. Thus, RIC regimens were designed using agents that would induce adequate immunosuppression to facilitate donor immune engraftment while maintaining a low toxicity profile. Such "low-intensity" regimens were first evaluated in hematologic malignancies known to be responsive to allogeneic HCT. Pilot trials utilizing RIC HCT demonstrated that such regimens were generally well tolerated, having a decreased incidence of transplant-related morbidity and mortality, while achieving sufficient donor immune engraftment to induce sustained remissions of some hematologic malignancies [46–50].

One of the major contributions of RIC was that it allowed for the extension of allogeneic HCT to debilitated and older patients who, because of the high risk of treatment-related mortality (TRM) with conventional myeloablative conditioning, would ordinarily have been denied the benefit of a procedure with curative potential. Importantly, investigators anticipated that the overall risk of TRM would be lower with a reduced-intensity approach, a notion which emboldened them to explore for GVT effects against solid tumors.

Clinical results of reduced-intensity allogeneic HCT transplantation in solid tumors

RCC: the National Institutes of Health experience

The current worldwide clinical experience of RIC HCT for solid tumors is limited, and the approach continues to be experimental. Because there was no convincing evidence to support the existence of a GVT effect in solid tumors, and given the potential for considerable morbidity with HCT, early pilot trials were restricted primarily to terminally ill patients with advanced treatment-refractory metastatic disease. At present, RCC remains the solid tumor in which allogeneic antitumor responses have been best characterized.

Several factors provided an incentive to study the susceptibility of this malignancy to a GVT effect. First, metastatic RCC is a uniformly fatal cancer in which the majority of patients succumb to their disease within 2–3 years of diagnosis. Second, despite the recent introduction of targeted agents against the vascular endothelial growth factor (VEGF), therapeutic options are extremely limited, with conventional chemotherapy and radiotherapy being largely ineffective [51]; furthermore, these targeted agents are seldom, if ever, curative, with the majority of patients experiencing disease progression within 1 year of treatment initiation. Third, RCC is considered an "immunoresponsive" tumor based on its susceptibility to cytokine therapy [20,52,53], reports of occasional spontaneous regression [17,54], and the existence of tumor-infiltrating T-lymphocytes in regressing metastatic lesions [22,26]. These considerations led to the development of a clinical protocol at the National Institutes of Health (NIH) that sought to test the safety and efficacy of RIC HCT in patients with cytokine-refractory metastatic RCC [46,55].

The primary considerations in the design of the allogeneic HCT trial for patients with metastatic kidney cancer were the following:

1. devising a conditioning regimen that would ensure maximal host immunosuppression to favor rapid and complete donor immune engraftment with relative sparing of recipient myeloid progenitors;
2. devising a post-transplant immunosuppressive approach that would provide optimal prophylaxis for GVHD without precluding the ability to initiate a donor immune-mediated GVT effect;
3. judicious use of DLIs to overcome donor tolerance to host antigens for the promotion of a GVT effect;
4. appropriate patient selection: choosing patients with treatment-refractory metastatic disease who would be anticipated to survive at least as long as would be required for a delayed GVT effect to occur (i.e., 4–6 months).

Therefore, the presence of evaluable metastatic disease, a good performance status (Eastern Cooperative Oncology Group grade 0–1), adequate organ function, and a life expectancy of at least 3 months were prerequisites for patient enrolment.

Cyclophosphamide and fludarabine have profound immunosuppressive effects and are well tolerated when given in combination to treat patients with low-grade lymphoproliferative disorders [56,57]. Accordingly, we devised an RIC HCT approach in which patients received a conditioning regimen consisting of cyclophosphamide [60mg/kg × 2 days) and fludarabine [25mg/m^2 × 5 days), followed by infusion of an unmanipulated, granulocyte colony-stimulating factor-mobilized peripheral blood hematopoietic cell graft from a 6/6 or 5/6 HLA antigen-matched sibling donor (Figure 62.1). Lineage-specific engraftment of donor myeloid ($CD14^+$/$CD15^+$) and T cells ($CD3^+$) was quantitated from post-transplant peripheral blood lymphocyte samples using a polymerase chain reaction-based analysis of either variable number tandem repeats or short tandem repeats polymorphic between patient and donor (Figure 62.2).

Based primarily on animal data showing a decreased risk of GVHD following RIC HCT, we chose to use single-agent cyclosporine (CSP) as GVHD prophylaxis in our initial patient cohort. Much to our surprise, a high probability of grade II–IV acute GVHD (actuarial probability 56%) was observed in the first 25 patients with RCC who were treated (which was lethal in three cases). Consequently, mycophenolate mofetil (MMF) and, more recently, methotrexate, were added to CSP as GVHD prophylaxis in the second cohort of RCC patients. Gradual withdrawal of CSP/MMF was initiated on day 30 or 60 after transplantation, with the timing and rapidity of immunosuppression tapering being dictated by the rate of tumor progression, degree of donor T-lymphoid chimerism, and presence or absence of GVHD. One or more dose-escalated DLIs were administered to all patients with mixed T-cell chimerism, progressive disease, or partial remissions in the absence of acute or chronic GVHD. Since interferon (IFN) has been shown

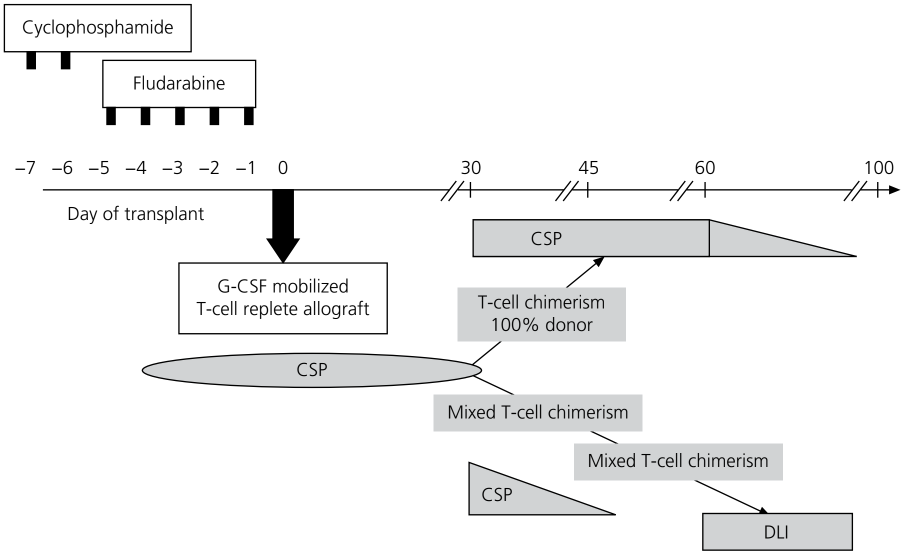


Figure 62.1 Cyclophosphamide- and fludarabine-based reduced-intensity conditioning hematopoietic cell transplantation protocol for renal cell carcinoma. CSP, cyclosporine; DLI, donor lymphocyte infusion; G-CSF, granulocyte colony-stimulating factor.

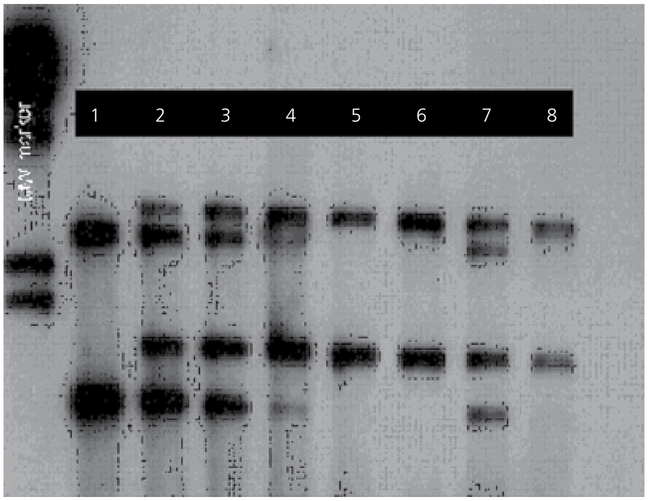


Figure 62.2 Example of polymerase chain reaction-based analysis of two different minisatellites polymorphic between patient and donor. Patient (lane 1) and donor (lane 5) lanes show two patient-specific and two donor-specific bands. Also shown are control mixtures of donor-into-patient DNA representing 30% (lane 2), 50% (lane 3), and 90% donor chimerism (lane 4), and post-transplant blood sample sorted into lineages using magnetic beads showing 100, 60, and 99% donor chimerism in $CD2^+/CD3^-$ natural killer cell (lane 6), $CD14^+/15^+$ myeloid cell (lane 7), and $CD3^+$ T-cell fractions (lane 8), respectively.

to upregulate MHC expression on RCC tumor cells in vitro (Figure 62.3), we hypothesized that using this cytokine post-transplant might make the tumor a better target for a GVT effect. Therefore, patients without GVHD who failed to respond to DLI were eligible to receive post-transplant cytokine therapy with IFN-±, either alone or in combination with IL-2.

The initial experience of RIC HCT in metastatic RCC was published in 2000 [46]. Ten of the first 19 patients treated with this transplant approach had tumor shrinkage, including three who had a CR and seven who had a PR. At present, 74 patients have undergone RIC HCT for RCC at the NIH. Of these, 73 demonstrated durable engraftment, achieving 100% donor T-cell chimerism by day 100 post-transplant. Twenty-nine of the 74 patients (39%) have had a disease response, including seven CRs and 22 PRs [58]. Five patients who were deemed to be “non-responders” had radiographic evidence for a mixed response. Disease regression was temporally associated with acute and chronic GVHD in many patients, was typically delayed in onset, and did not occur until CSP was tapered, consistent with an alloimmune-mediated GVT effect. Several patients have had a durable disease response, including the first complete responder, who remains without evidence of metastatic disease now over 14 years post-transplant (Figure 62.4). Regression of disease in multiple metastatic foci has been observed, although pulmonary responses appear to occur most frequently. On occasion, disease responses have been dramatic and have included complete resolution of large pulmonary metastases and bulky adenopathy (Figure 62.5).

Preliminary data suggest that disease response following RIC HCT is a clinically meaningful phenomenon since regression of metastatic RCC appears to be associated with a trend towards improved survival. Median survival in non-responders has been less than 6 months, in contrast to those achieving a PR, who survived a median 2.5 years post-transplant. In general, patients with metastatic kidney cancer have tolerated this conditioning regimen well. Although virtually all patients developed febrile neutropenia, we observed no sinusoidal obstructive syndrome of the liver or chemotherapy-associated mucositis. About 28% (21 of 74) of patients developed cytomegalovirus antigenemia, with only one case of cytomegalovirus disease (esophagitis) that was responsive to gancyclovir therapy. Mortality associated with transplant-related complications occurred in eight of 74 patients (11%), with either infection or acute or chronic GVHD being the major cause of TRM. Since kidney cancer frequently metastasizes to the lungs, some patients with RCC may be at high risk for the development of postobstructive pneumonia following transplantation. Such a complication occurring in the profoundly immunocompromised can lead to disastrous infectious sequelae, as was observed in two of our RCC patients who died from bacterial sepsis related to obstructive bronchial pneumonia. Most deaths related to acute GVHD occurred

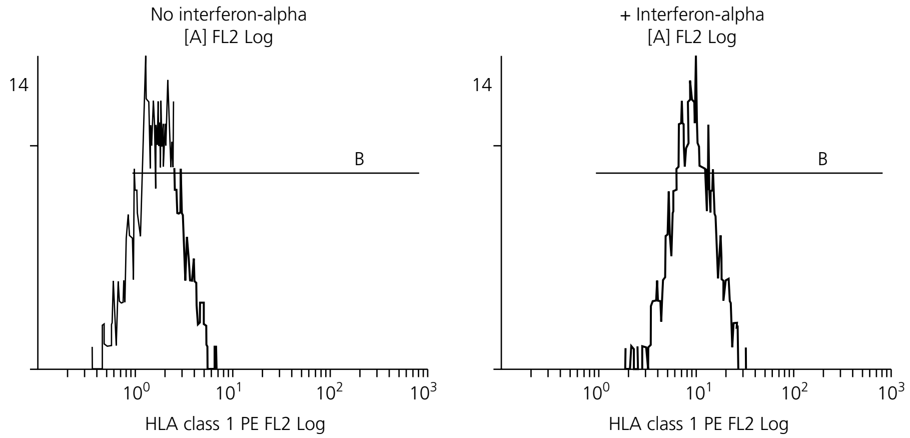
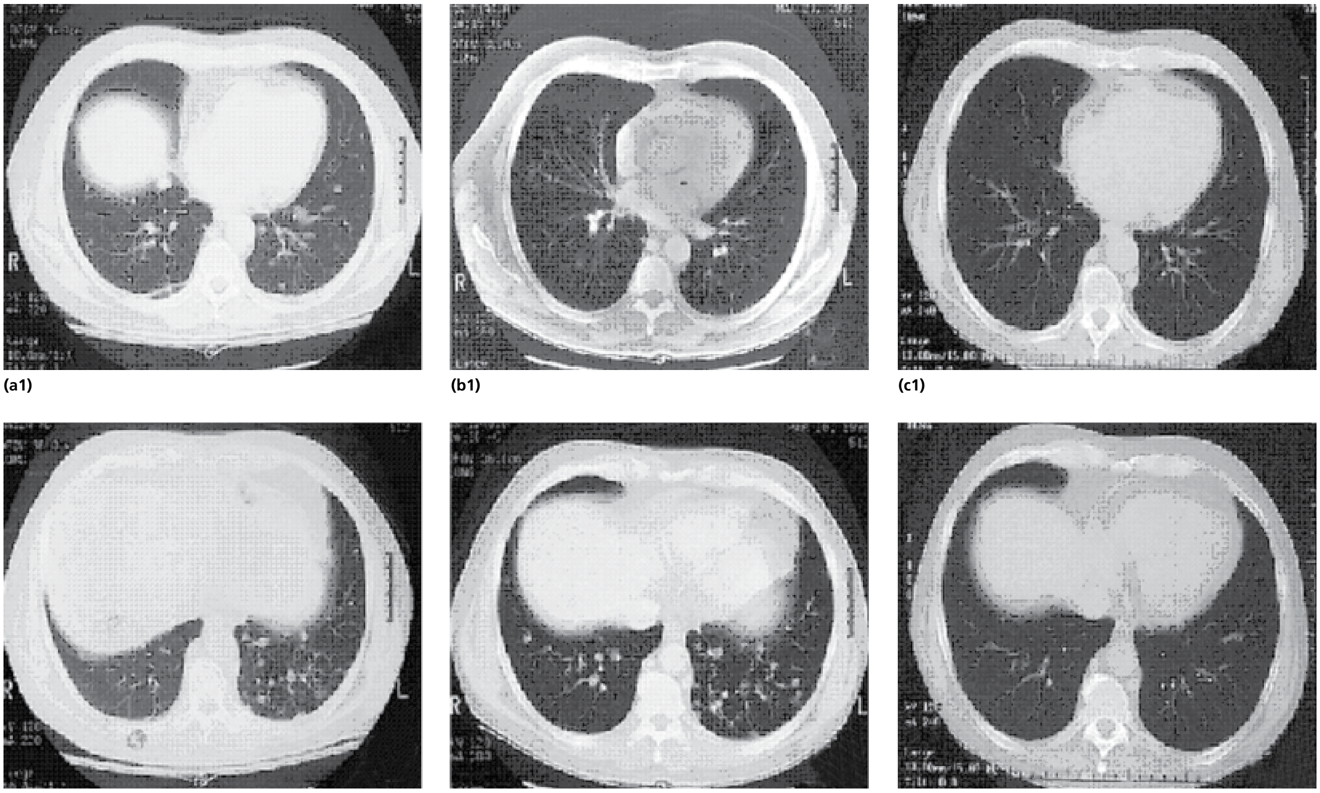


Figure 62.3 Upregulation of human leukocyte antigen (HLA) class I expression (both percent positive and mean channel fluorescence) on renal cell carcinoma cells 24 hours after exposure to interferon-alpha (10,000U/mL).



(a1) (b1) (c1)

(a2) (b2) (c2)

Figure 62.4 Renal cell carcinoma metastatic to the lungs (pre-transplant images a1 and a2). There was no change in metastatic disease 30 days following conditioning (b1 and b2). Following cyclosporine withdrawal, metastasis regressed completely by 110 days post-transplant (c1 and c2). The patient remains without evidence of disease over 9 years after transplantation.

during the first 2–3 years following inception of the trial; more recently, advances in our ability to manage severe or steroid-refractory GVHD have translated into a greatly reduced risk of mortality from this complication [59].

Acute GVHD has been the greatest contributor to morbidity and mortality in our RCC patients undergoing RIC HCT. Because MMF is a potent inhibitor of inosine monophosphate and has been shown in vitro to block proliferative responses of cytotoxic T cells, we added MMF to CSP as prophylaxis for GVHD in our second cohort of patients with RCC. Unfortunately, an interim analysis of the first 30 patients treated with both drugs revealed no difference in the incidence of grade II–IV or grade III–IV acute GVHD between the CSP alone and the CSP + MMF cohorts (actuarial probability of acute grade II–IV GVHD 56 and 61%, respectively). Based on these observations, we have withdrawn MMF from the transplant regimen and are currently investigating the effect of adding low-dose methotrexate (MTX) to CSP as GVHD prophylaxis.

A recent analysis of 230 patients with a variety of malignancies and non-malignant hematologic disorders undergoing RIC HCT

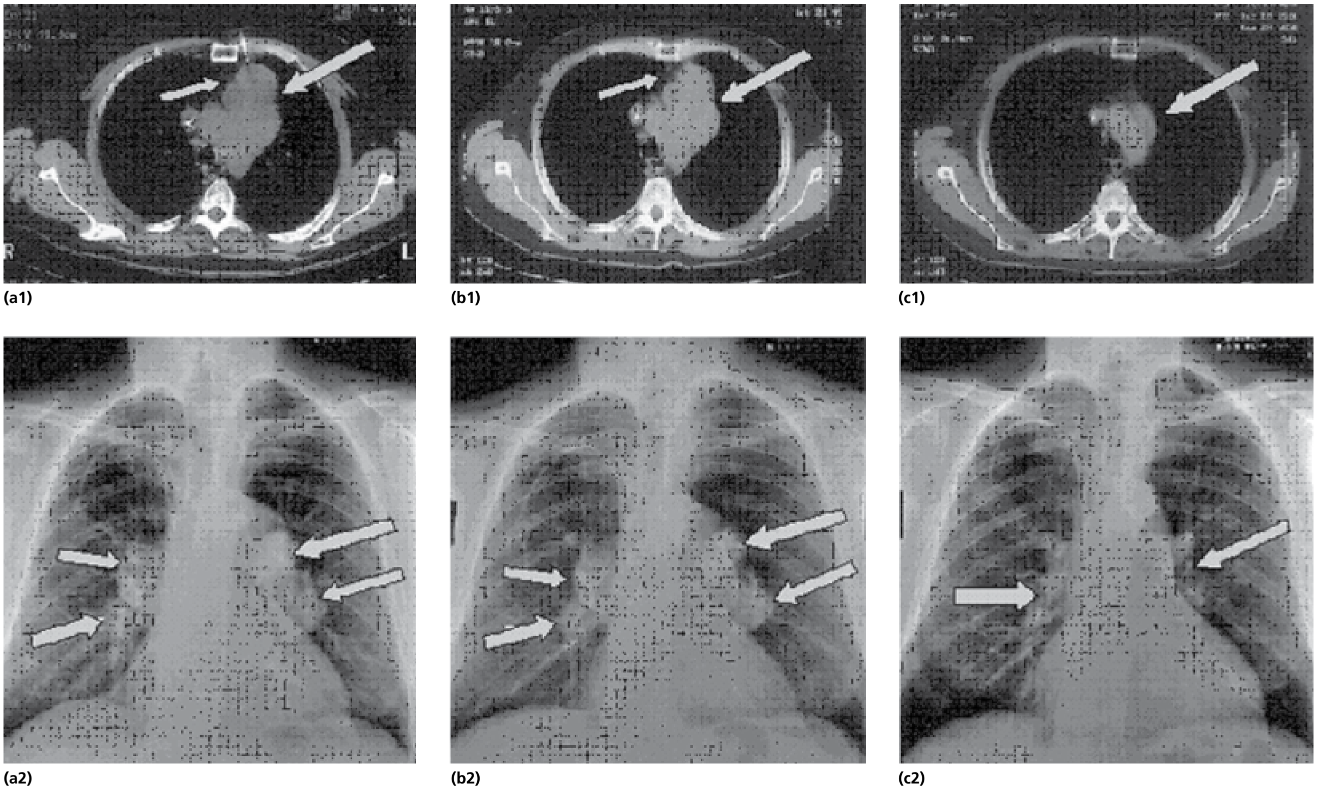


Figure 62.5 Delayed regression of renal cell carcinoma. Pre-transplant images showing bulky anterior mediastinal (a1) and hilar (a2) adenopathy. Stable disease seen 6 months post-transplant (b1 and b2). Regression of bulky adenopathy was observed 9 months post-transplant (c1 and c2).

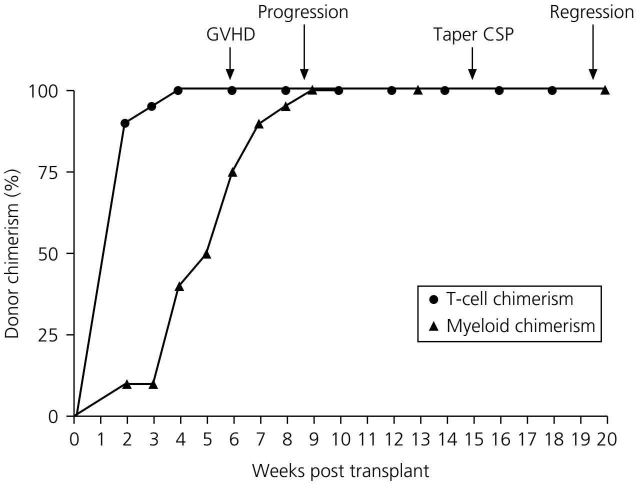


Figure 62.6 Representative lineage-specific engraftment profile following cyclophosphamide- and fludarabine-based conditioning. The percentage donor chimerism in T cells (●) and myeloid cells (▲) is shown in a patient who had a disease response 17 weeks post-transplant. CSP, cyclosporine; GVHD, graft-versus-host disease.

with cyclophosphamide and fludarabine conditioning at our institution revealed that the incidence of grade II–IV and III–IV acute GVHD was significantly less in those patients who received CSP and MTX as GVHD prophylaxis than in those who received either CSP alone or CSP + MMF. Importantly, the addition of MTX to CSP did not appear to abrogate the ability to induce GVT effects against RCC, with four of 18 patients who received this regimen demonstrating an objective response (including one patient with a CR).

Assessment of donor chimerism following RIC HCT is important not only to document the establishment of donor engraftment, but also to allow for post-transplant manipulation of the donor immune system to optimize the chances of inducing a GVT effect. In our experience, GVT effects following RIC HCT typically do not occur until the immune system has converted from mixed to predominantly donor T-cell chimerism. As a consequence, serial measurements of donor engraftment in both lymphoid and myeloid lineages are performed on all patients undergoing this approach. Representative lineage-specific engraftment profiles and their relationship to clinical outcome are shown in Figure 62.6.

Although donor T-cell engraftment typically precedes myeloid engraftment in patients receiving cyclophosphamide- and fludarabine-based conditioning, engraftment patterns may vary considerably among individual patients [60]. Several factors influence the degree and rapidity of donor engraftment, including the agents used in the conditioning regimen, the prevailing state of host immunity, and allograft cell doses. In a multivariate analysis of 36 patients with metastatic solid tumors who underwent RIC HCT at the NIH, pre-transplant exposure to chemotherapy and increased allograft CD34$^+$ cell dose were found to facilitate significantly the engraftment of donor myeloid and T cells [61]. Therefore, tailoring the intensity of RIC in solid tumor patients based on prior chemotherapy exposure seems worthy of exploration.

Although the exact mechanisms underlying the regression of metastatic RCC following allogeneic HCT are yet to be unraveled, several observations (both laboratory and clinical) suggest that an alloimmune effect mediated at least in part by donor T cells is at work (Table 62.1) [46]. The majority of patients who ultimately achieved a disease response showed early tumor growth in the first few months after transplantation, a period when the newly engrafted donor immune system was kept in check by immunosuppressive therapy or when mixed T-cell chimerism prevailed, leading to "tolerance" of host tissues (including the tumor). Tumor regression was typically delayed (4–8 months) and followed conversion to predominantly donor T-cell chimerism after immunosuppression had been withdrawn or was being tapered.

These observations highlight the importance of utilizing a transplant approach that favors rapid donor T-cell engraftment and incorporates judicious and timely withdrawal of GVHD prophylaxis. As had previously been described in patients with hematologic malignancies, a prior history of acute GVHD was associated with an increased probability of having a tumor response. One might speculate that disease regression in this setting could be the consequence of alloreactive T cells targeting mHAs that are broadly expressed on both normal tissues and tumor cells. Importantly, however, tumor shrinkage has also been observed to occur in the absence of or temporally distant from GVHD, an observation which might imply that tumor cells are particularly sensitive to allogeneic immune attack or that tumor-specific immune effectors might be involved in mediating RCC regression in some patients.

Finally, regression of RCC following a DLI or after treatment with low-dose subcutaneous IFN-α has also been observed. Disease regression after DLI suggests that the mediators of the GVT effect may be analogous to those mediating leukemia regression. Interestingly, some patients who had failed to respond to IFN-α before transplantation had disease regression when the drug was given post-transplant. This observation suggests that the mechanism by which IFN-α promotes tumor regression in the post-transplant setting relates to the ability of the drug to make the tumor a better target or enhance the allogeneic immune system rather than to a direct antineoplastic effect.

Table 62.1 Tumor response patterns consistent with a graft-versus-tumor effect after reduced-intensity conditioning hematopoietic cell transplantation

- Delayed onset of response (>100 days post-transplant)
- Response following withdrawal of immunosuppression
- Response following or concomitant with graft-versus-host disease
- Response following donor lymphocyte infusions
- Prolonged duration of response in tumors previously treatment refractory

Emerging role of RIC HCT for RCC

Although allogeneic HCT is clearly an investigational approach for the treatment of metastatic RCC, several other investigators have begun to report promising results in this disease (Table 62.2). Investigators at the University of Chicago initially reported on 15 patients undergoing HCT following a fludarabine- and cyclophosphamide-based conditioning regimen [62]. The first four patients treated were conditioned with an extremely low-intensity regimen consisting of fludarabine (90mg/m^2) and cyclophosphamide (2g/m^2) given at doses that failed to achieve adequate levels of host immunosuppression. As a consequence, three of the four patients (75%) failed to achieve stable donor T-cell engraftment and ultimately rejected their transplant. Subsequent patients received higher doses of the same conditioning agents (fludarabine 150mg/m^2 and cyclophosphamide 4g/m^2), and achieved successful and sustained donor engraftment. Of the 12 patients who engrafted and were evaluable for a disease response, four (33%) had radiographically documented disease regression consistent with a PR, including one patient who had tumor regression in his primary kidney tumor. These data have since been updated to include the first 19 patients transplanted, four of whom achieved a PR. Although all patients have relapsed at a median of 609 days following HCT, all responders were alive with a median follow-up of 41 months [compared with a median overall survival (OS) of 14 months for the entire cohort] [63].

As with other trials of RIC HCT for solid tumors, a dominant donor immune system (determined by T-cell chimerism studies) was a prerequisite for the generation of a meaningful GVT effect. Only those patients who had stable and predominantly donor immune engraftment demonstrated a disease response. In fact, one patient with graft rejection (a non-responder) underwent a second transplant with the intensified conditioning regimen, achieved complete donor engraftment, and subsequently had a PR. Only two of 12 patients (17%) experienced grade II or greater acute GVHD, perhaps the consequence of a more gradual termination of GVHD prophylaxis. Whether delaying the withdrawal of post-transplant immunosuppression will decrease the incidence of acute GVHD without negating beneficial GVT effects will need to be determined from larger studies.

A Cancer and Leukemia Group B (CALGB) intergroup trial evaluated the feasibility of performing HCT for metastatic RCC in a multi-institutional setting in the United States [64]. Twenty-two patients underwent HCT from an HLA-matched sibling donor following cyclophosphamide plus fludarabine-based conditioning.

Table 62.2 Summary of clinical trials of reduced-intensity conditioning hematopoietic cell transplantation in metastatic renal cell carcinoma

Study	Conditioning agents	GVHD prophylaxis	Acute GVHD (II–IV) (%)	Chronic GVHD (%)	TRM (%)	Response (partial or complete) (%)
Childs et al. [46] and unpublished data	CY + FLU	CSP (first 25 patients); CSP + MMF (subsequent patients)	53	21	11	53
Rini et al. [62], Artz et al. [63]	CY + FLU	Tacro + MMF	22	39	14	22
Bregni et al. [73]	CY + FLU + thioTEPA	CSP + MTX	86	71	0	57
Pedrazzoli et al. [96]	CY + FLU	CSP + MTX	0	N/A	29	0
Blaise et al. [97]	FLU + BU + ATG	CSP	42	60	9	8
Nakagawa et al. [98]	FLU/CLA + BU + ATG	CSP	44	44	0	11
Ueno et al. [99]	FLU + MEL	Tacro + MTX	47	27	33	27
Hentschke et al. [100]	FLU + TBI ± ATG	CSP + MMF	50	30	40	0
Massenkeil et al. [101]	FLU + CY + ATG	CSP ± MMF	29	57	14	29
Tykodi et al. [102]	FLU + TBI	CSP + MMF	50	50	13	13
Barkholt et al. [65]	Multiple FLU-based regimens	CSP ± MMF or MTX	40	33	16	32
Rini et al. [64]	FLU + CY	Tacro + MTX	32	23	9	0
Peres et al. [103]	FLU + CY or FLU + TBI	CSP + MMF	44	38	12	32

ATG, antithymocyte globulin; BU, busulfan; CLA, cladribine; CSP, cyclosporine; CY, cyclophosphamide; FLU, fludarabine; GVHD, graft-versus-host disease; MEL, melphalan; MMF, mycophenolate mofetil; MTX, methotrexate; N/A, not available; Tacro, tacrolimus; TBI, total body irradiation; TRM, treatment-related mortality.

Seventeen of nineteen evaluable patients achieved >90% donor chimerism by day 120 after transplantation. Acute (grade II–IV) GVHD was seen in 50% of patients, and 23% of patients experienced chronic GVHD. No objective responses were seen, and median OS was only 5.5 months, with most patients dying from disease progression (with a median time to progression of 3 months). Inclusion of a number of patients with multiple adverse prognostic factors, sparing use of DLI (only two of 22 patients received DLIs despite disease progression in the majority), and inclusion of patients with non-clear cell histology are some of the factors that may account for the poor outcome observed in this trial. This trial clearly highlights the importance of appropriate patient selection and the need for identifying prognostic factors likely to predict for a favorable outcome.

The European Group for Blood and Marrow Transplantation (EBMT) reported on their experience in 124 patients with metastatic RCC undergoing HCT at multiple centers from an HLA-identical (n = 106) or partially matched (n = 5) family donor, or from an HLA-matched unrelated donor (n = 13) (Table 62.2) [65]. A variety of fludarabine-based RIC regimens were utilized. CSP alone or in combination with MMF or MTX was used for GVHD prophylaxis in the majority of patients. Durable engraftment was achieved in 121 patients. The incidences of acute grade II–IV and chronic GVHD were 40 and 33%, respectively. Twenty-eight of 98 evaluable patients had an objective tumor response, including four patients with a CR. Responses were typically delayed, with PRs and CRs occurring a median of 135 and 265 days, respectively. A short interval from diagnosis of RCC to HCT, the presence of acute GVHD, and the use of an HLA-mismatched donor were each associated with a higher probability of response. Transplant-related mortality was 16% at 1 year, and OS at 2 years was 30%. In a multivariate analysis, chronic GVHD, good performance status (Karnofsky score ≥80), DLI administration, and fewer than three sites of metastatic disease were identified as factors favorably impacting survival. The EBMT Solid Tumor Working Group also attempted to identify key prognostic factors in a study of 70 patients undergoing HCT from matched sibling donors following fludarabine-based conditioning. Performance status, LDH and C-reactive protein were identified as factors of prognostic value in a multivariate analysis; the authors were able to incorporate these variables in a Cox regression model to identify patients with poor (median OS 3.5 months) and good (OS 23 months) prognosis [66,67].

Toxicities and limitations of RIC HCT in metastatic RCC

Although there is ample evidence now to support the susceptibility of metastatic RCC to an allogeneic GVT effect, there are a number of potentially life-threatening toxicities of RIC HCT that currently limit the broader application of this approach (Table 62.3). Although the incidence of early TRM appears to be less with RIC, overall 5–20% of patients will ultimately die from a transplant-related cause.

Table 62.3 Limitations of reduced-intensity conditioning hematopoietic cell transplantation in solid tumors

	Incidence (%)
HLA-matched sibling donor available	25–30
Acute GVHD	30–60
Chronic GVHD	40–70
Cytomegalovirus reactivation	20–40
Graft rejection	5–10
Treatment-related mortality	10–20

GVHD, graft-versus-host disease; HLA, human leukocyte antigen.

Table 62.4 Patient characteristics likely to predict a favorable outcome after reduced-intensity conditioning hematopoietic cell transplantation for solid tumors

- Good performance status (Eastern Cooperative Oncology Group grade 0–1)
- Younger patient age (<65 years)
- Small tumor volume
- Slow tumor growth kinetics
- Absence of central nervous system metastatic disease
- Evidence (clinical or in vitro) that tumor is susceptible to immune-mediated attack

Infection and complications related to acute GVHD are the greatest risks associated with the procedure. In fact, three of the first 25 patients with RCC transplanted at the NIH died from GVHD-related causes [46]. Unfortunately, the addition of MMF to CSP as GVHD prophylaxis did not reduce the incidence of grade II–IV or III–IV acute GVHD. However, new second-line therapies for severe steroid-refractory GVHD such as daclizumab (monoclonal antibody directed against the alpha chain of the IL-2 receptor) and infliximab (monoclonal antibody directed against the tumor necrosis factor-alpha receptor) have shown early promise [68,69]. Our preliminary experience suggests that these monoclonal antibodies are effective in reducing mortality from steroid-refractory GVHD, particularly when given with lipid-complexed amphotericin B or voriconazole as *Aspergillus* prophylaxis, concomitant with a rapid reduction in corticosteroid dose once steroid resistance is apparent [59]. Larger studies will be required to determine whether these agents will ultimately result in a reduction in the risk of GVHD-related mortality without compromising the ability to generate a GVT effect.

Careful patient selection, with particular attention to performance status, medical comorbidities, and tumor growth kinetics may further improve transplant-related outcome. Although the use of RIC HCT for solid tumors is currently limited, preliminary clinical experience provides insight into patient characteristics that would be expected to impact favorably on transplant outcome (Table 62.4).

RIC HCT in melanoma

Despite the recent availability of novel, clinically effective immunotherapeutic and targeted approaches, the prognosis for patients with metastatic melanoma remains poor [70,71]. Nevertheless, along with kidney cancer, malignant melanoma has long shared the reputation of being an “immunoresponsive” tumor. In fact, more immunotherapy trials have been targeted at melanoma than any other solid tumor. It would seem logical, therefore, to investigate whether this tumor might be a target for a GVT effect following allogeneic HCT. Unfortunately, and rather surprisingly, the results of RIC HCT for melanoma at the NIH and at other centers worldwide have been far from inspiring.

We reviewed the outcome of 25 patients with metastatic melanoma treated at four different institutions using one of three different RIC HCT conditioning regimens: cyclophosphamide (120mg/kg) + fludarabine (125mg/m^2) (n = 18), busulfan (8mg/kg) + fludarabine (150mg/m^2) + antithymocyte globulin (ATG) (n = 5), or 200cGy total body irradiation + fludarabine (90mg/m^2) (n = 2) [72]. Engraftment was documented in 24 of 25 patients. Twelve patients (48%) developed grade II–IV acute GVHD, including 11 who had GVHD involving the skin. In 11 patients (92%), GVHD responded to immunosuppressive therapy, and one died from grade IV steroid-refractory liver GVHD. Five patients (20%) had radiographically documented disease regression consistent with a disease response (all PRs); four responses occurred in the immediate post-transplant period, likely the consequence of a chemotherapy effect, and one response was delayed in onset and occurred in association with chronic skin GVHD consistent with a GVT effect. None of the disease responses were durable, with melanoma progression occurring within 1–3 months in all responders. Six patients were treated with a DLI for tumor progression without evidence for a response. None of the 25 patients transplanted survive. Two (8%) died from transplant-related complications (acute GVHD and idiopathic encephalitis), and 23 (92%) died from progression of metastatic disease. Median survival was only 100 days (range 7–660 days), with no meaningful survival difference seen between the five responding patients (median survival 114 days) and the 20 non-responding patients (median survival 93 days).

The disappointing outcome of RIC HCT in this tumor is perplexing, particularly when clinically meaningful GVT effects in RCC have been demonstrated. Rapid tumor kinetics and bulky metastatic disease may explain at least in part why some patients failed to manifest a GVT effect. Of more concern, however, was the observation that some patients appeared to have acceleration in the pace of tumor growth in the immediate post-transplant period following RIC HCT (D. Niederweiser, personal communication). While the description of this phenomenon is admittedly subjective and could simply reflect the natural evolution of tumor growth kinetics, it is tempting to speculate that transplant-associated immunosuppression could promote tumor progression as a consequence of the destruction of innate host immune surveillance. These preliminary data demonstrate that transient conditioning-related regression of metastatic melanoma may occur following RIC HCT, although the likelihood of a clinically meaningful GVT effect appears low. The high risk of death from rapid disease progression has discouraged investigators from pursing similar RIC HCT studies in melanoma patients. Preclinical studies of “tumor-targeted” allogeneic transplant approaches are needed and, if successful, could lead to future “second-generation” RIC HCT trials in metastatic melanoma.

RIC HCT in other solid tumors

The collective global experience with RIC HCT in solid tumors other than RCC and melanoma is limited to a handful of publications, all with small patient numbers. Early enthusiasm for this approach, based on the important observation of GVT effects in patients with RCC, led to a rapid rise in the number of experimental transplants conducted for other types of treatment-refractory solid tumor. Indeed, as discussed previously, GVT effects against metastatic breast carcinoma following high-dose conditioning and allogeneic HCT have been described in some patients in association with acute GVHD [43,44]. The high prevalence of breast carcinoma in the general population, together with the disappointing results of autologous transplantation trials, inspired a number of investigators to explore RIC HCT in patients with this malignancy. Bregni et al. treated six patients with metastatic breast cancer with an RIC HCT following conditioning with cyclophosphamide, fludarabine, and thioTEPA (*N*-triethylenethiophosphoramide) [73]. Two patients had a PR that was delayed and did not occur until several months following transplantation. In both cases, responses were preceded by DLIs and GVHD, consistent with a GVT effect.

At the NCI, 16 patients with treatment-refractory metastatic breast cancer received a T-cell-depleted peripheral blood hematopoietic cell graft from an HLA-matched sibling following fludarabine and cyclophosphamide conditioning; escalating doses of DLIs were then administered on days 42, 70, and 98 post-transplant. All 15 evaluable patients achieved complete donor T-cell chimerism. Delayed responses (beyond day 28 after transplantation) consistent with a GVT effect were seen in six of 16 patients, including two patients who had experienced early disease progression following transplantation [74].

A joint International Bone Marrow Transplant Registry–EBMT registry review identified 76 patients with metastatic breast cancer undergoing allogeneic HCT between 1992 and 2000 [75]. As would be expected from the time frame of the study, a variety of both high-dose and RIC regimens were employed. Although no engraftment data were provided, approximately 51% of patients developed acute GVHD, and 25% of patients had chronic GVHD. With a median follow-up of approximately 25 months, progression-free survival in this cohort was 9%, and OS at 2 years was only 22%. The median time to progression and median OS were 8 months.

Carella et al. described a novel approach of evaluating allogeneic immunotherapy in 17 patients with breast cancer whose tumors were first maximally debulked with intensive chemotherapy followed by autologous HCT [76]. Four patients (24%) had delayed regression of metastatic breast cancer as the consequence of a GVT effect. However, in contrast to studies of allogeneic HCT alone, where only partial regression of metastatic breast cancer was typically observed, 18% of patients in this study undergoing tandem autologous/allogeneic HCT achieved long-term disease-free survival. Remarkably, three patients had delayed and complete tumor regression associated with the development of acute GVHD, and remained in remission 3.6, 4.2, and 5.9 years after transplantation. Of note, three of five patients who had partial disease regression after autologous HCT subsequently experienced a sustained CR as a result of a GVT effect, in contrast to no CRs after allogeneic HCT in the 11 patients who failed to respond to high-dose chemotherapy.

The results presented here suggest that a tandem transplant approach can induce long-term disease-free survival in a minority of patients with metastatic breast cancer. More importantly, they highlight the importance of reducing pre-transplant tumor burden to facilitate and enhance allogeneic graft-versus-solid tumor effects.

A small number of RIC HCTs have also been performed in patients with advanced ovarian cancer. One group reported delayed tumor regression in a patient with metastatic ovarian carcinoma who received an HLA-matched transplant from her sibling donor [45]. A small series of patients (n = 5) with treatment-refractory ovarian carcinoma who underwent HCT following either high-dose (one of five) or reduced-intensity (four of five) conditioning was reported by Bay et al. [77]. Three patients had regression of metastasis and/or a decrease in disease-related serum markers (i.e. CA125) early in the post-transplant period. The proximity of tumor shrinkage to chemotherapeutic conditioning makes it unclear whether these responses were truly related to an alloimmune effect. Longer follow-up and additional patients should clarify whether this tumor is indeed responsive to a GVT effect.

Another group reported the results of RIC HCT that used either cladribine or fludarabine in combination with busulfan and antithymocyte globulin in seven patients with a variety of different solid tumors [78]. Partial disease regression ascribed to a GVT effect was reported in a patient with osteosarcoma and two patients with RCC.

Since effective T-cell-mediated alloimmune responses can be generated against EBV-mediated lymphoproliferative disorders, it is tempting to speculate that EBV-mediated solid tumors may also be susceptible to GVT. Recently, Toh et al. reported their experience with RIC HCT in patients with EBV associated advanced nasopharyngeal carcinoma [79,80]. Twenty-one patients received a peripheral blood transplant from a 6/6 or 5/6 HLA-matched sibling donor following a conditioning regimen comprising cyclophosphamide, thymic irradiation, and rabbit ATG. Seven patients (33%) were reported to have a partial response, including three who had delayed and durable responses in the context of increasing donor T-cell chimerism and/or DLI administration. Patients with delayed responses also demonstrated a concomitant decrease in blood EBV DNA levels. Similar to the experience of allogeneic transplantation for RCC, the presence of GVHD was associated with a higher probability of response.

In summary, insufficient data are available at present to comment on the efficacy of RIC HCT in most solid tumors. Interpreting these limited data is made even more difficult as it is often not clear whether disease regression is related to chemotherapeutic agents in the conditioning regimen or is truly the sequela of a donor immune-mediated GVT effect. Strict adherence to criteria such as those outlined in Table 62.1 may help substantiate that a GVT effect has indeed occurred and should minimize the risk of inadvertent over-interpretation of clinical data. The efficacy of GVT in terms of its ability to induce remission varies among these malignancies, with the critical determining factors dictating neoplastic susceptibility to GVT not entirely understood.

Mechanisms underlying GVT effects in solid tumors

Disease regression following allogeneic HCT in some patients with treatment-refractory RCC has stimulated interest in exploring the mechanisms through which GVT effects occur. A better understanding of both the targets and effectors of the GVT response could lead to the development of safer and more efficacious transplant regimens. Based on clinical data and our knowledge of the mediators of the GVT effect, T-lymphocytes (both $CD8^+$ and $CD4^+$) and natural killer (NK) cells probably play a role in mediating immune responses against solid tumors [81–85]. The observation that disease regression (1) is associated with an increase in the percentage of circulating $CD8^+$ T cells with an activated phenotype ($DR^+CD38^+CD57^+$), (2) does not occur until T-cell chimerism is predominantly donor, and (3) sometimes follows a DLI provides strong evidence to support this hypothesis [46,86].

What, if any, role allogeneic NK cells play in mediating GVT effects in solid tumors is currently unknown. Researchers have shown that NK cells may play a critical role in mediating GVT effects in patients with acute and chronic myelogenous leukemia undergoing transplants from haploidentical donors with killer immunoglobulin-like receptor (KIR) incompatibility in the GVHD direction (i.e., patient Cw allele mismatched to donor and therefore unable to function as a ligand for donor NK cell KIR) [87,88]. We have conducted in vitro experiments showing that NK cell clones generated from KIR-mismatched allogeneic donors can kill both melanoma and RCC tumor cells preferentially through KIR incompatibility [89]. These preliminary data suggest that the antitumor effects seen against acute myelogenous leukemia following KIR-incompatible allogeneic HCT might also occur against select solid tumors in the setting of haplotransplantation.

A retrospective analysis in a subset of patients with metastatic RCC who underwent a T-cell-replete RIC HCT from an HLA-matched sibling donor at the National Heart, Lung, and Blood Institute provides preliminary clinical evidence in support of this hypothesis [90]. In this study, KIR incompatibility (defined as the presence of one or more KIR genotypes in donor cells for which the corresponding inactivating HLA-B or -C ligand is missing from the recipient) appeared to predict higher objective response rates and also improved overall survival, an effect most pronounced in patients lacking HLA-Bw4 who received a transplant from donors with genotypic evidence of KIR3DL1.

The identification of target antigens involved in GVT effects is an area of intense investigation, and recent findings suggest that both lineage-restricted and broadly expressed mHAs are potential targets. In an attempt to clarify the role of mHAs in alloimmune T-cell responses against RCC, previously characterized mHA-specific CTL clones were tested for cytotoxicity against EBV-transformed B cells (EBV-LCLs) (HLA matched for the appropriate mHA-restricting allele) first to identify RCC patients expressing the relevant antigen. Subsequently, CTLs recognizing patients' EBV-LCLs were tested for their ability to recognize mHAs present on patient RCC cells. In the majority of cases, CTLs recognizing patient EBV-LCLs also recognized patients' RCC cells, suggesting that broadly expressed mHAs could serve as targets for immune-mediated tumor regression in the setting of acute GVHD [91].

Preliminary data obtained from patients with RCC regressing after allogeneic transplantation suggest that the target antigens may differ in those who experience tumor regression in the context of GVHD as opposed to those without. T-cell clones that specifically lysed patient RCC cells or both RCC and patient hematopoietic cells were isolated and expanded from one responder where a GVT effect occurred in the absence of GVHD. In contrast, we could only expand T-cell clones that reacted against both tumor and hematopoietic cells in a patient who had disease regression that occurred in the setting of acute GVHD [86]. Hence it is conceivable that cellular immune responses directed against antigens shared by tumor and normal tissues would result in GVHD in addition to GVT, whereas GVT without GVHD would be expected to occur in those having a cellular immune response against tumor-restricted antigens (Figure 62.7).

The identity of antigens that serve as targets for a GVT effect, specifically those that appear to be restricted to the tumor, could provide clues to the intricate mechanisms of GVT and provide valuable insight that might aid in the development of future tumor-targeted immunotherapeutic approaches. Using T cells isolated from a patient who had regression of metastatic RCC following allogeneic transplantation, we recently identified a new solid tumor antigen that appears to be overexpressed in the majority of kidney tumors. $CD8^+$ T-cell clones isolated from lymphocytes obtained from this patient after transplantation and following tumor regression lysed the patient's RCC cells in vitro. Using complementary DNA expression cloning, the target antigen of these allogeneic T cells (donor in origin) was found to be a 10-mer peptide tumor antigen that was recognized in the context of HLA-A11. The genes encoding this antigen (HERV-E) were found to be derived from a human endogenous retrovirus that was found to be overexpressed in more than 50% of clear cell carcinomas but not in any normal tissues [58]. Remarkably, HERV-E expression was found to be restricted to the clear cell subtype of RCC (ccRCC), which at present is the only histological subtype of kidney cancer where GVT effects have been observed. Clear cell RCC is characterized by an inactivation of the von Hippel–Lindau (*VHL*) tumor suppressor gene with subsequent stabilization of hypoxia-inducible transcription factors HIF-1α and -2α. Recent data show that expression of HERV-E is associated with *VHL* inactivation and appears to be regulated by hypoxia-inducible factor 2α (HIF2α); HERV-E expression in ccRCC was found to

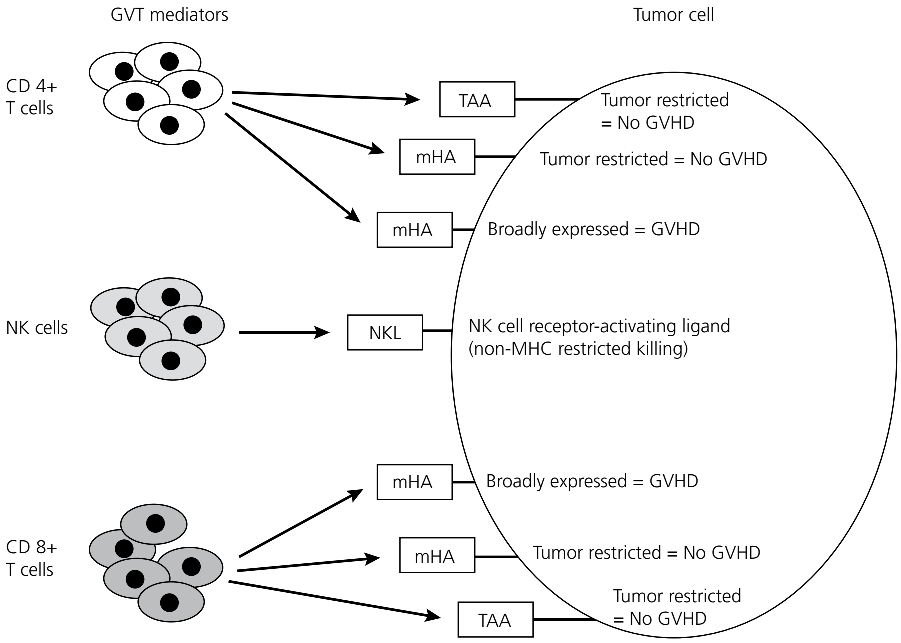

GVT mediators
Tumor cell
CD 4+ T cells
NK cells
CD 8+ T cells
TAA
Tumor restricted = No GVHD
mHA
Tumor restricted = No GVHD
mHA
Broadly expressed = GVHD
NKL
NK cell receptor-activating ligand (non-MHC restricted killing)
mHA
Broadly expressed = GVHD
mHA
Tumor restricted = No GVHD
TAA
Tumor restricted = No GVHD

Figure 62.7 Immune populations hypothesized to contribute to graft-versus-tumor (GVT) effects following reduced-intensity conditioning hematopoietic cell transplantation. Minor histocompatibility antigens (mHAs) are either restricted to the tumor or broadly expressed on normal and malignant tissues. Tumor cells need to express a dominant natural killer (NK) cell receptor-activating ligand (i.e., MICA) to initiate NK cell-mediated killing. GVHD, graft-versus-host disease; MHC, major histocompatibility complex; NKL, natural killer ligand; TAA, tumor-associated antigen.

correlate linearly with HIF-2α levels and could be silenced in tumor cells by transfection of normal *VHL* or by siRNA inhibition of HIF-2α [92]. Using chromatin immunoprecipitation, HIF-2α was found to serve as transcriptional factor for HERV-E by binding with HIF response elements (HREs) localized in the HERV-E 5′LTR. These data provide the first evidence that inactivation of a tumor suppressor gene can result in aberrant proviral expression in a human tumor that may have the net effect of enhancing the immunogenicity of clear RCC.

These studies provide the first insights into the mechanisms accounting for the selective expression of a HERV in a solid tumor, demonstrating that inactivation of a tumor suppressor gene can result in aberrant expression of a highly immunogenic provirus in ccRCC. The antigen encoded by this HERV-E is the first solid tumor antigen identified using allogeneic T cells from a patient undergoing HCT, and could provide a potential target for cellular immunity against RCC both within and outside the context of allogeneic HCT.

Future directions

The lack of efficacy of chemotherapeutics, radiotherapy, and cytokine-based immunotherapy for many patients with metastatic cancer has catalyzed enthusiasm for exploring allogeneic immunotherapy against solid tumors. While there is little doubt about the potential for its application in oncology, extensive use of RIC HCT in the treatment of solid tumors will likely remain limited until both the safety and efficacy of the approach are improved. Further progress in the fields of tumor vaccination and adoptive “tumor-targeted” T-cell infusion are needed to address these problems. Recently, murine models have demonstrated that tumor-specific immunity can be boosted following post-transplant tumor immunization. In an MHC-matched but mHA-disparate allotransplant model, immunization of the recipient post-transplant against either leukemia or fibrosarcoma resulted in enhanced antitumor activity without exacerbating GVHD [93]. A similar strategy could be used in humans for those patients who fail to achieve a CR after RIC HCT. Likewise, adoptive transfer of ex vivo-expanded tumor-specific CTLs that are generated from the donor could be used to enhance a GVT effect. Recent developments in our understanding of the role played by NK cells in both enhancing GVT effects and minimizing GVHD offer the prospect of incorporating “KIR-incompatible” or “KIR-mismatched” NK cell therapy into allogeneic immunotherapy strategies.

In vitro, allogeneic KIR-incompatible NK cells have enhanced cytotoxicity against RCC cells compared with autologous or KIR-matched NK cells [89]. The adoptive infusion of alloreactive donor NK cells has been found to reduce significantly the risk of acute GVHD and potentiate graft-versus-RCC effects in a murine model of HCT for kidney cancer [94]. Trials evaluating adoptively infused allogeneic NK cells and other novel methods to direct or target alloimmune cells against RCC in humans undergoing HCT for metastatic RCC will likely be explored in the near future.

The identification of transcripts derived from a HERV-E restricted to clear cell kidney tumors that encode immunogenic peptides capable of inducing cellular immune responses in patients with RCC after transplantation remains a promising area of investigation for the development of transplants targeting donor immunity against the tumor. Peptides derived from the *env* region of this HERV-E, which is also expressed in clear cell RCC, have recently been identified that have high binding affinity for HLA-A*0201, are immunogenic in vitro, and stimulate antigen specific CD8+ T cells that recognize ccRCC cells in an HLA-A*0201- restricted fashion [95]. These data suggest that antigens derived from this newly discovered HERV-E envelope could represent excellent targets for T-cell-based immunotherapy for kidney cancer. Because HERV-E expression appears to be restricted to kidney cancer, one could conceive of a future approach where donor T cells, stimulated ex vivo by HERV-E-derived peptides, are infused post-transplant to bolster a GVT effect specifically against the tumor.

A variety of novel "targeted" agents have recently been approved for the treatment of several malignancies. Several inhibitors of the VEGF pathway are now available for the treatment of metastatic RCC. Although unlikely to be curative, these agents have been shown to prolong progression-free survival. Since heavy disease burden and rapidly progressive tumors can significantly detract from the ability to generate clinically meaningful GVT responses, incorporating the aforementioned disease-stabilizing antiangiogenic agents into HCT strategies might be beneficial and should be explored.

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CHAPTER 63

Hematopoietic Cell Transplantation for Neuroblastoma

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Introduction

Neuroblastoma is a malignancy of young children derived from embryonic neural crest cells of the peripheral sympathetic nervous system. It is the most common extracranial solid tumor of childhood, with approximately 730 new cases per year in the United States, and accounts for 15% of cancer-related deaths in children [1]. Some of its unique features, such as the ability to secrete and store catecholamines, its inherent genetic heterogeneity, and its propensity to undergo differentiation either spontaneously or with various stimuli, have led to novel diagnostic and therapeutic approaches. As therapy has become more tailored to biologic and clinical risk groups, a higher proportion of children with neuroblastoma are surviving. However, nearly half of all children with neuroblastoma present after 1 year of age with metastatic disease; of these, less than 40% will survive more than 5 years, even with aggressive combination therapeutic approaches [2]. The goals of future therapy are to increase the specificity of treatment in order to improve the survival of children with advanced disease, while reducing therapy-related toxicity.

Clinical presentation and staging

Neuroblastoma can originate from any site in the sympathetic nervous system, presenting as a mass in the abdomen, mediastinum, neck, or pelvis. The most common primary site (in 70% of cases) is the abdomen (often arising from the adrenal gland); mediastinal tumors are more common in infants than in older children. Masses arising from the organ of Zuckerkandl in the pelvis can present with constipation and bladder dysfunction. High thoracic or cervical masses can cause Horner syndrome [3]. Epidural or intradural extension of tumor occurs in 5–16% of neuroblastoma cases. These patients may have symptoms associated with spinal cord compression. Prompt administration of chemotherapy appears to be effective for treating intraspinal neuroblastoma, and it may avoid some of the long-term sequelae associated with either radiation or surgical resection and laminectomy [4].

Metastatic neuroblastoma can classically present with proptosis and periorbital ecchymoses, and bone pain resulting in irritability and limp. Rapid enlargement of liver metastases can result in respiratory compromise, particularly in neonates. Skin lesions, seen almost exclusively in infants, have a bluish hue and have been given the nickname "blueberry muffin" lesions. Rarely, neuroblastoma will present with a paraneoplastic syndrome, including either watery diarrhea caused by secretion of vasoactive intestinal peptide, or opsoclonus–myoclonus–ataxia syndrome, characterized by jerky multidirectional eye movements, loss of balance, and intermittent muscle jerks [5]. The latter syndrome is thought to be caused by the production of antineuronal antibodies that cross-react with normal neural epitopes [6].

The previous International Neuroblastoma Staging System (INSS) [7] was based on extent of surgical excision, but the newly agreed upon International Neuroblastoma Risk Group Staging System (INRGSS) is based on the presence or absence of image-defined risk factors, which generally consist of encasement or invasion of vital organs, nerves, and vessels [8]. Staging alone is predictive of event-free survival (EFS) at 5 years (Table 63.1).

In addition to diagnostic studies such as bone marrow aspiration, histology, quantification of urine or serum catecholamines, and imaging by computed tomography, iodine-123-labeled *m*-iodobenzylguanidine ([^{123}I]MIBG) scintigraphy is mandatory for all patients, and should be performed prior to tumor excision. MIBG is a guanethidine derivative that is an analog of norepinephrine, and taken up in 90% of neuroblastomas. One unequivocal MIBG-positive lesion at a distant site is sufficient to define metastatic disease. Technetium-99m bone scintigraphy has largely been supplanted by [^{123}I]MIBG, but may be required if MIBG positivity of the primary tumor cannot be confirmed, although now [^{18}F]deoxyglucose positron emission tomography is preferred as superior to bone scan [9]. MIBG scans should be performed in all high-risk patients at diagnosis, prior to high-dose myeloablative therapy, and before and after therapy given for minimal residual disease (MRD). Interpretation of MIBG scans relies on a standardized semiquantitative scoring system, which allows for inter-institutional comparison. The Curie extension score is currently being used in large prospective phase III Children's Oncology Group (COG) trials for high-risk neuroblastoma in North America [9].

Tumor molecular biology

Amplification of the *MYCN* oncogene on chromosome 2p24, found in 25% of neuroblastoma cases, is the most consistent unfavorable genetic change, and is independent of age, stage, and other biologic characteristics. It is an essential component of risk assessment that can easily be measured by fluorescent in situ hybridization (FISH) on primary tissue or bone marrow tumor. Chromosomal gain at 17q without extensive amplification may

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be found in neuroblastoma in 50–60% of cases, and may portend a worse outcome [10].

Neuroblastomas may be diploid or hyperdiploid, and chromosomal content by measurement of DNA index has established that near-diploid DNA content is less favorable and associated with other adverse prognostic markers [11]. Allelic losses of 1p and 11q are common in neuroblastomas, and both predict adverse outcome [12]. The presence of any segmental chromosomal alterations [as detected by array comparative genomic hybridization (CGH)] has now been shown to be an independent adverse risk factor in neuroblastoma [13]. In addition, linkage analysis studies of familial neuroblastoma have identified germline mutations in the anaplastic lymphoma kinase (ALK) gene, and ALK mutations or amplifications have subsequently been identified in approximately 8% of all sporadic neuroblastoma tumors. Germline mutations in the ALK tyrosine kinase may be a main cause of familial neuroblastoma, and may represent a potential therapeutic target [14].

Risk classification and treatment approach

In an effort to optimize risk stratification internationally, the INRG classification schema was published in 2009 [15]. This system takes into account the optimal age cut-off of 18 months, image-defined INRGSS staging, *MYCN* amplification, histologic category, tumor grade, ploidy, and genetic aberrations of 11q (Table 63.2). Very low-risk tumors are generally managed with surgery alone, unless symptomatic cord compression or respiratory compromise necessitates a short course of chemotherapy. Patients in the low-risk group with Stage 1 or 2 disease have an expected 4-year survival of >95% with surgery only [16], whereas infants with Stage 4S disease have >90% survival with supportive care or a short course of chemotherapy [17]. The smaller intermediate-risk group generally requires multiple cycles of a modest outpatient chemotherapy regimen and often requires surgical resection, but the chemotherapy can safely be reduced to 3–6 cycles while maintaining a 3-year EFSl of >90% [18]. With aggressive therapy, long-term survival for high-risk neuroblastoma has only recently risen above 15% to a 3-year EFS of 30–40%. Despite advances, much still needs to be done to improve outcome (Figure 63.1).

Table 63.1 International Neuroblastoma Risk Group staging system

Stage	Description	Estimated 5-year EFS (%)
L1	Localized tumor not involving vital structures as defined by the list of image-defined risk factors and confined to one body compartment	90
L2	Locoregional tumor with presence of one or more image-defined risk factors	78
M	Distant metastatic disease (except stage MS)	35
MS	Metastatic disease in children younger than 18 months with metastases confined to skin, liver, and/or bone marrow	83

Source: Cohn et al. 2009 [15]. Reproduced with permission of the American Society of Clinical Oncology.

Treatment of high-risk neuroblastoma

Therapy for high-risk neuroblastoma is currently divided into three phases: intensive induction treatment, marrow ablative therapy, and management of MRD. The goal of induction therapy is to achieve maximum reduction of tumor burden, including reduction of bone marrow tumor (in vivo purging), within a time frame that will minimize the risk of developing resistant tumor clones and clinical progression. Subsequently, very high-dose myeloablative therapy may be used to try to overcome residual and potentially resistant tumor and is followed by autologous hematopoietic cell transplantation (HCT). The relapse rate of >40% even after such treatment [19] has led to the approach of using tumor-targeted therapies following high-dose conditioning in an effort to eliminate microscopic resistant clones (MRD).

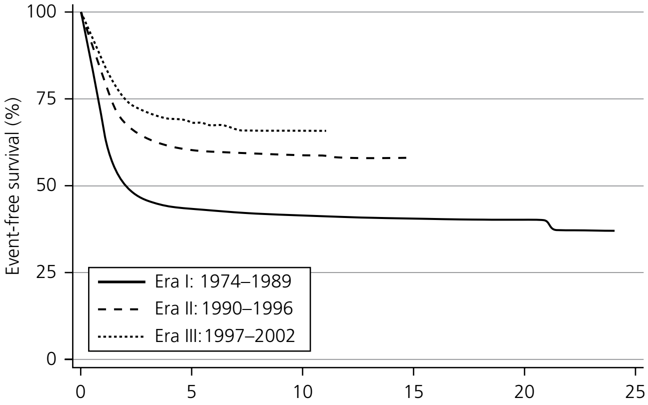
Induction therapy

Current standard induction chemotherapy usually includes 5–7 cycles of combination chemotherapy, including high-dose alkylators and a platinum derivative, along with surgery to bulky tumor sites. Induction regimens used in large cooperative studies have shown overall response rates, including complete and partial remission (CR + PR), ranging from about 60 to 90% at the end of 5–6 months of treatment [2,20]. Most regimens also include surgery to residual disease, although the overall impact of complete resection on survival in Stage 4 disease is still uncertain.

Table 63.2 Current Children's Oncology Group neuroblastoma risk classification

INRG stage	Age (months)	Histologic category	Grade of tumor differentiation	*MYCN*	11q aberration	Ploidy	Pretreatment risk group	
L1/L2		GN maturing; GNB intermixed					A	Very low
L1		Any, except GN maturing or GNB intermixed		NA			B	Very low
				Amp			K	High
L2	<18	Any, except GN maturing or GNB intermixed		NA	No		D	Low
					Yes		G	Intermediate
	>18	GNB nodular; neuroblastoma	Differentiating	NA	No		E	Low
					Yes		H	Intermediate
			Poorly differentiated or undifferentiated	NA				
				Amp			N	High
M	<18			NA		Hyperdiploid	F	Low
	<12			NA		Diploid	I	Intermediate
	12 to <18			NA		Diploid	J	Intermediate
	<18			Amp			O	High
	>18						P	High
MS	<18			NA	No		C	Very low
					Yes		Q	High
				Amp			R	High

Source: Monclair et al. 2009 [8]. Reproduced with permission of American Society of Clinical Oncology.



At risk					
Era I: 2207	915	702	261	48	0
Era II: 5035	2487	519	0	0	0
Era III: 3795	600	1	0	0	0

Figure 63.1 Event-free survival of children <21 years of age by era of diagnosis. Source: Moroz et al. 2011 [37]. Reproduced with permission of Elsevier.

Single institutional studies have demonstrated that further dose intensification of alkylating agents and cisplatin resulted in complete and very good partial remission (VGPR) rates of up to 85% [21,22]. This Memorial Sloan Kettering Cancer Center (MSKCC) N7 induction regimen consisted of cyclophosphamide, doxorubicin, and vincristine, alternating with cisplatin and etoposide, with good response in 21/24 patients. These excellent results however, could not be reproduced by a UK pilot study of 47 patients [23] nor by the French Society of Pediatric Oncology [24].

The large cooperative COG trial A3973 using the same regimen in 489 patients also resulted in a CR/VGPR rate of only 52%, with PR 26%, for overall response rate similar to those with previous protocols [25]. In comparison, the European study comparing a dose-intensive induction (rapid COJEC) with standard induction therapy (OPEC/OJEC) showed that although there was no detectable improvement in overall survival (OS), the 5-year EFS was significantly higher in patients receiving rapid COJEC induction (30.2% vs. 18.2%, $p = 0.02$) [26].

Some studies have also investigated newer single agents such as carboplatin and topotecan in newly diagnosed neuroblastoma, using the “up-front phase 2 window.” Following two courses of single-agent therapy prior to induction treatment, response rates (CR + PR) of >30% have been observed, with no evidence that the window design adversely affected outcomes [27]. A pilot induction regimen incorporating pharmacokinetically guided dose-intensive topotecan together with cyclophosphamide prior to four cycles of multiagent therapy, tested in 31 patients, demonstrated that the regimen was well tolerated and produced a tumor response in 84% of patients following induction; 3-year EFS and OS were 37.8 and 57.1%, respectively [28]. This pilot then provided the basis for the recently completed phase 3 COG ANBL0532 study.

Local control

Despite surgical resection of the primary tumor, recurrence in the local or regional area of primary disease is a component of relapse in a large proportion of children with high-risk neuroblastoma, at rates ranging from 20 to 80% in reports that often include local radiotherapy and myeloablative therapy [2,19,29]. Studies in Stage 4 disease utilizing radiotherapy administered to the primary tumor bed either before or following myeloablative conditioning suggest a lower local recurrence rate using radiotherapy in retrospective analysis, but the variability in extent of resection makes these difficult to interpret [29]. Retrospective analysis of the local control in the CCG 3891 study showed a benefit for local radiotherapy to residual disease for patients undergoing HCT.

Pilot studies have also been reported using higher focal radiation via intraoperative radiotherapy (IORT) in order to spare normal organs [30]. One retrospective study has lent support to the therapeutic value of IORT while avoiding the associated toxicities of external beam radiotherapy [31]. However, this benefit must be balanced by the occasional occurrence of hypertension and vascular stenosis. More recently, pilot studies have reported the use of proton beam radiotherapy, which adds precision but has the potential disadvantage of incomplete treatment of margins [32]. In addition, pilot studies in Europe have suggested that treatment prior to chemotherapy induction with the targeted radiopharmaceutical [^{131}I]MIBG rapidly reduces primary tumor bulk and facilitates later resection [33]. Although the particular modality may be debated, the current standard therapy of high-risk neuroblastoma continues to include radiotherapy to the primary tumor bed and residual metastatic sites [34,35].

Autologous hematopoietic cell rescue

The observed linear–log relationship between drug dose and tumor cell cytotoxicity for alkylating agents suggested that if the drug dose could be increased without increasing toxicity, then a multiple log increment in tumor cell killing could be achieved. The demonstration that hematopoiesis could be restored with autologous hematopoietic cells allowed the use of much higher doses of chemotherapy with autologous bone marrow support for the treatment of solid tumors. Early pilot studies showed that responses were seen in children with resistant neuroblastoma after high-dose chemoradiotherapy and bone marrow reinfusion [36]. Comparison of long-term survival for children with neuroblastoma in the premyeloablative era, and after 1990, a time when high-dose therapy with HCT became routine for many of these patients, showed a significant difference in outcome (Figure 63.1) [37].

Subsequent single-arm studies in the United States and Europe verified an apparent improvement in outcome for autologous HCT compared retrospectively with results for chemotherapy. The single-arm studies containing more than 20 patients that have been reported are summarized in Table 63.3. The 3-year EFS in these studies varied from 24 to 50%. Comparison between studies in this table is fraught with pitfalls, because EFS is sometimes estimated from time of transplant, which differs from 4 to 10 months from diagnosis, and in a few studies from time of diagnosis. The patient groups also differ in whether they were comprised of only Stage 4 patients >1 year at diagnosis or also included other high-risk patients.

Non-randomized comparisons of autologous HCT with chemotherapy

Several cooperative pediatric groups have attempted statistical non-randomized comparisons of outcomes for groups of patients treated either with conventional doses of chemotherapy or high-dose conditioning chemotherapy, total body irradiation (TBI), and purged

Table 63.3 EFS for high-risk neuroblastoma in first remission using myeloablative therapy and autologous HCT for studies of >20 patients

Ref.	Year	Myeloablative regimen	*n*	Toxic deaths	3-year EFS[a] (%)
[90]	1987	BCNU, teniposide, melphalan[c]	33		49 (2 yr)[b]
[91]	1991	Melphalan, TBI	54	7	32
[39,92]	1991, 1996	Cisplatin, teniposide, doxorubicin, melphalan, TBI	45	7	42
		Cisplatin, etoposide, melphalan, TBI	54	5	50
		Carboplatin, etoposide melphalan, TBI	48	4	41
[93]	1991	Vincristine, melphalan, TBI	62	13	30[b]
[94]	1991	Vincristine, melphalan, TBI	34	1	29
[95]	1991	Cisplatin, BCNU, melphalan (or thioTEPA), etoposide	25	6	40
[96]	1995	Etoposide, melphalan or cisplatin, etoposide, THP–Adriamycin, melphalan, with (*n* = 6) or without TBI	31	3	50
[97]	1996	Teniposide/etoposide, thioTEPA, TBI	27	4	41
[98]	1997	Melphalan ± etoposide, vincristine, cisplatin, BCNU	39	7	35[b]
[82]	1998	Cyclophosphamide, thioTEPA	51	1	48
[2]	1999	Carboplatin, etoposide, melphalan, TBI	129	12	34[b]
[29]	1999	Carboplatin, etoposide, melphalan, local radiation	77	4	62
[47]	1999	Busulfan, melphalan	116	7	47[b]
[99]	2001	Cyclophosphamide, carboplatin	49	4	33[b]
[100]	2002	Triple tandem: Carboplatin + etoposide, Carboplatin + etoposide, ThioTEPA +etoposide[d]	25	1	57[b]
[40]	2005	Melphalan	32	2	38 (5 yr)
[42]	2005	Melphalan, carboplatin[e]	75	5	56[b]
[49]	2006	Double tandem: Etoposide + carboplatin + cyclophosphamide, Melphalan + TBI[f]	97	5	61
[63]	2008	Carboplatin, etoposide, melphalan	489		40[b]
[48]	2011	Busulfan, melphalan	1577		49
		Carboplatin, etoposide, melphalan			33

[a] Unless stated otherwise, EFS measured from time of transplantation.

[b] Denotes EFS is calculated from time of diagnosis. All others are from time of transplantation.

[c] One course only *n* = 15, Two courses *n* = 18.

[d] Single transplant *n* = 2, double *n* = 2, triple *n* = 17.

[e] Variations in myeloablative therapy: melphalan alone *n* = 3, melphalan + cyclophosphamide *n* = 3, melphalan + busulfan *n* = 9.

[f] Single transplant *n* = 7, double *n* = 82.

Source: Adapted from Matthay et al. 2000 [89].

autologous HCT, with differing conclusions. In an effort to avoid the bias introduced by comparing therapies from two different time periods, outcomes from two contemporaneous Pediatric Oncology Group (POG) studies were compared: POG 8441 (surgery plus conventional chemotherapy) and POG 8340 (elective autologous HCT pilot protocol). There was no significant prognostic benefit of switching in remission from the chemotherapy protocol to the transplant protocol ($p = 0.91$) [38].

The Children's Cancer Group (CCG) examined the outcome of Stage 4 patients >1 year of age treated with identical induction chemotherapy on a CCG pilot protocol, who then either continued on the same chemotherapy ($n = 73$) for 1 year (CCG-321P2), or proceeded to trial CCG-321P3 with myeloablative chemotherapy, TBI, and purged autologous HCT ($n = 94$) [39]. The decision to use autologous HCT was non-random and depended on parental, investigator, and institutional choice. The advantage for autologous HCT versus chemotherapy was significant for the group as a whole, with 3-year EFS of 40 and 19%, respectively, different from the findings of the POG studies. The advantage for autologous HCT was greatest for certain very high-risk subgroups, including those >2 years of age at diagnosis, those with bone or bone marrow metastases, those with *MYCN* gene amplification, and those who had only a PR rather than CR to the first 4–6 cycles of induction chemotherapy. These and other studies, and also the overall improvement in survival for high-risk neuroblastoma seen in the era after introduction of HCT, led to several randomized clinical trials.

Randomized comparison of autologous HCT with standard-dose chemotherapy

The European Neuroblastoma Study Group (ENSG) performed the first randomized study comparing high-dose conditioning treatment with conventional chemotherapy. This study, open from 1983 to 1985, was a comparison of high-dose melphalan with no further therapy after induction, and showed a significant advantage in progression-free survival (PFS) for those patients with Stage 4 disease >1 year of age at diagnosis undergoing high-dose therapy (5-year EFS 33% vs. 17%, $p = 0.01$; 5-year OS 46% vs. 21%, $p = 0.03$). However, only 59% of patients were randomized, so the group as a whole excluded the highest risk patients, and bias may have been introduced. Long-term follow-up of the ENSG-1 was published in 2005 for a median of 14.3 years from randomization [40]. The median time to relapse for the melphalan versus the "no melphalan" group was unaltered at 18 months versus 3 months. Despite the limitations of the study, the survival improvement achieved at 5 years appears sustained at 14 years (Figure 63.2a).

At the time of publication of the initial promising European results, the CCG launched a larger randomized study in the United States comparing high-dose chemotherapy and TBI followed by purged autologous HCT with intensive non-myeloablative chemotherapy as the continuation regimen following induction, surgery, and radiation [2]. The results clearly showed a significant improvement in EFS for the patients randomly assigned to autologous HCT, both by an intent-to-treat analysis and also by treatment received (Figure 63.2b). As in the previous CCG non-randomized comparison, the highest

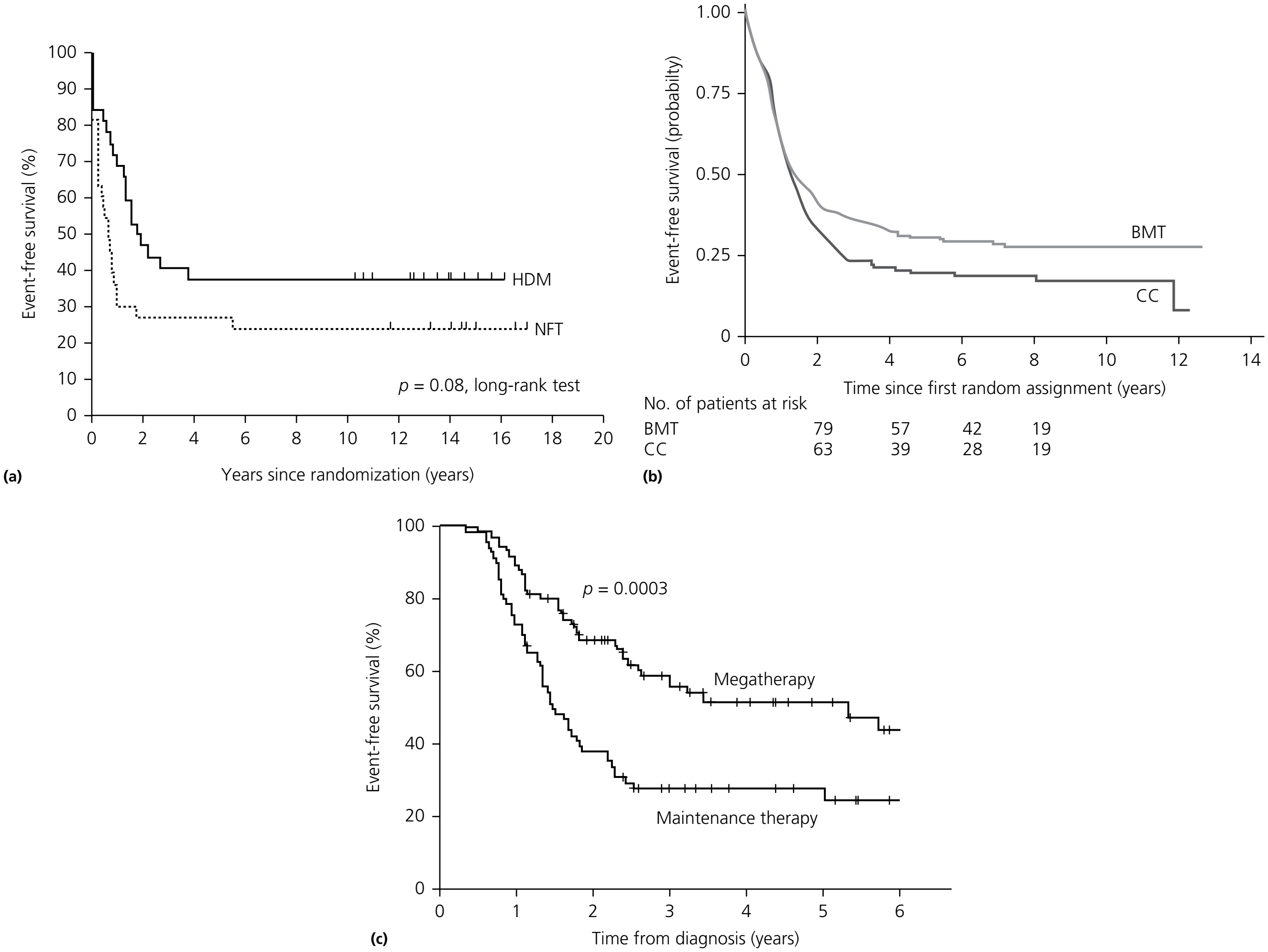


Figure 63.2 Outcomes of randomized trials comparing hematopoietic cell transplantation to no further therapy or chemotherapy for high-risk neuroblastoma. (a) High-dose regimen: high dose melphalan. Transplant group: $n = 32$ with 5-year EFS = 38% (95% CI 21–54%). No melphalan group: $n = 33$ with 5-year EFS = 27% (95% CI 12–42%); $p = 0.08$. Source: Pritchard et al. 2005 [40]. Reproduced with permission of John Wiley & Sons, Ltd. (b) High-dose regimen: carboplatin, etoposide, melphalan, TBI. Transplant group: $n = 189$ as randomized with a 5-year EFS of 30% (±4%). Continued chemotherapy group: $n = 190$ as randomized with a 5-year EFS of 19% (±3%); $p = 0.0434$. Source: Matthay et al. 2009 [41]. Reproduced with permission of the American Society of Clinical Oncology. (c) High-dose regimen: melphalan and carboplatin. Transplant group: $n = 75$ as randomized with a 3-year EFS 56% (95% CI 44–68%). Maintenance chemotherapy group: $n = 70$ as randomized with a 3-year EFS 28% (95% CI 17–38%); $p = 0.0003$. Source: Berthold et al. 2005 [42]. Reproduced with permission of Elsevier.

risk patients, those with *MYCN* amplified tumors, or those >2 years of age at diagnosis had the most significant benefit. In addition, there was no significant difference in toxic deaths between patients randomized to either arm, and the hospital days were identical, helping to validate the cost-effectiveness of this treatment. More recent analysis of continued follow-up of a median of 8 years on this study showed a significant advantage in 5-year survival for the patients randomized to the autologous HCT arm ($p = 0.004$) [41].

The most recent randomized study to compare myeloablative therapy against continued chemotherapy was a German cooperative study that enrolled 339 patients from 1997 to 2002 [42]. Patients ($n = 295$) were randomized to receive either myeloablative "megatherapy" and autologous HCT or maintenance with oral cyclophosphamide. The myeloablative regimen consisted of melphalan, etoposide, and carboplatin. This was followed by either immunotherapy with chimeric anti-GD2 monoclonal antibody given for 1 year or oral isotretinoin for 6 months. In accordance with the CCG study, EFS was significantly increased in the patients receiving myeloablative therapy and autologous stem-cell rescue (Figure 63.2c). Potential confounders in this study included variable time of randomization, variations in treatment regimens prior to transplant, timing of surgery and stem-cell collection, and high crossover rates.

High-dose conditioning regimens

The first high-dose regimen used in the treatment of neuroblastoma was single-agent high-dose melphalan. This progressed to various combinations of other agents, including cisplatin, etoposide and doxorubicin, melphalan with busulfan, and melphalan with carboplatin and etoposide. Other centers used a thioTEPA (*N*-triethylenethiophosphoramide) base, coupled with cyclophosphamide or etoposide, or busulfan combined with cyclophosphamide. More recently, attempts have been made to incorporate topotecan into the conditioning regimen in combination with thioTEPA and etoposide [43]. A retrospective analysis by the EBMT failed to show any difference in EFS using different high-dose regimens [44–46].

On the other hand, an analysis by the Institut Gustave-Roussy showed that patients treated with busulfan and melphalan appeared to have better EFS than those on other conditioning regimens at a single institution [47]. These data may depend on the chronology of the protocols and also the fact that the busulfan regimen was compared with a combination of a variety of other regimens. The ENSG is currently conducting a large, multinational cooperative randomized study (the HR-NBL1 trial) comparing the COG regimen of carboplatin, etoposide, and melphalan (CEM) with the busulfan and melphalan (BuMel) regimen [48]. Preliminary results demonstrated a significant difference in 3-year EFS from time of transplant in favor of the BuMel regimen (49% vs. 33%, $p < 0.001$). This was primarily related to the decreased incidence of relapse and progression observed with this regimen. In addition, despite the occurrence of sinusoidal obstructive syndrome (18% incidence) associated with the BuMel regimen, the severe toxicity rate (ICU and toxic deaths) was significantly higher for the CEM than the BuMel regimen. Owing to the superiority of BuMel that was demonstrated in this trial, it has become the recommended conditioning regimen administered to high-risk neuroblastoma patients prior to autologous HCT in Europe and is now being tested in North America in pilot studies.

Tandem transplants

Tandem transplantation uses repetitive high-dose therapies followed by HCT to provide further increases in dose intensity. Several groups have investigated whether this translates to improved outcomes in high-risk neuroblastoma. George et al. reported an updated compilation of the limited institution experience with a tandem transplantation protocol [49]. The first high-dose therapy employed a regimen of etoposide, carboplatin, and cyclophosphamide, and the second included melphalan and TBI. An average of 7.2×10^6 $CD34^+$ cells/kg were available to support each cycle. Ninety-seven high risk patients were treated with five cycles of chemotherapy followed by two courses of myeloablative therapy and hematopoietic cell rescue. The 5-year PFS from diagnosis for all patients was 47% [95% confidence interval (CI), 36–56%].

Following this, a pilot study by the POG tested the feasibility and safety of tandem transplantation in a cooperative group setting using alternating courses of cisplatin/etoposide, cyclophosphamide/doxorubicin/vincristine, and ifosfamide/etoposide during induction, followed by etoposide, carboplatin, and cyclophosphamide conditioning for autologous HCT #1, and thioTEPA and cyclophosphamide (Thio/CY) conditioning for autologous HCT #2. The results of this study showed that this regimen is feasible in terms of transplant-related mortality and stem-cell collection. PFS from diagnosis was 24.2 ± 7.5% at 5 years [50]. To address better the question of whether two tandem transplants produce superior outcomes compared with a single transplant, the COG phase 3 randomized trial ANBL0532 compares the efficacy of a single transplant using CEM with two tandem transplants using Thio/CY followed by CEM. The study closed to accrual in 2012 and data are being collected.

Targeted radionuclides as part of high-dose therapy

The use of targeted radionuclides allows the delivery of very large radiation doses to the tumor with less radiation to normal organs. Targeted radioisotope therapy using anti-GD2 antibody or MIBG for delivery of radiation in the form of iodine-131 has been tested extensively in clinical trials in relapsed neuroblastoma [21]. [^{131}I]MIBG has been widely shown in European and US studies to elicit a 30–40% response rate for refractory and relapsed neuroblastoma in phase I and II studies [51,52]. The main toxicity has been hematologic, which has been abrogated with stem-cell infusion [53]. Several recent studies have investigated MIBG at higher dose levels and combined with other agents, including standard chemotherapy and myeloablative therapy, demonstrating the feasibility of this approach.

The results of these studies argue strongly for the incorporation of [^{131}I]MIBG therapy into regimens that include chemotherapeutic agents. A phase I study incorporated escalating doses of [^{131}I]MIBG followed by carboplatin, etoposide, and melphalan with hematopoietic stem-cell infusions [54]. The combination appeared feasible and effective at a dose of 12 mCi/kg of [^{131}I]MIBG. A phase I study by the NANT (New Approaches to Neuroblastoma Therapy) Consortium combining irinotecan, vincristine, and escalating doses of [^{131}I]MIBG showed that MIBG is tolerable and active (six responses in 24 evaluable patients) when given at a dose of 18 mCi/kg with standard doses of vincristine and irinotecan [55]. The COG pilot study ANBL09P1 is testing the administration of 18 mCi/kg [^{131}I]MIBG as the last cycle of induction prior to first stem-cell infusion in newly diagnosed patients, followed by BuMel conditioning prior to second autologous HCT.

Allogeneic transplantation

Allogeneic HCT has been proposed as an alternative to autologous HCT with the hope that the allogeneic cells will provide a further "graft-versus-neuroblastoma" effect, and also decrease any risk of tumor cell reinfusion. To date, lack of evidence of immunologic benefit, coupled with the problems of frequent lack of an human leukocyte antigen (HLA)-compatible sibling donor and the significantly higher toxic death rates, have discouraged extensive use of allogeneic transplantation. Non-relapse death rates were generally higher than those reported for autologous transplants, in the range 15–30%. A CCG study compared two concomitant groups of patients in a pilot study who received the same induction and conditioning regimen; 36 patients went on to receive an autologous purged bone marrow transplant (BMT), and 20 similar patients with HLA-compatible sibling donors received allogeneic BMT [56]. There was no significant difference in relapse rate but there was an apparently higher risk of toxicity associated with the allogeneic group; the estimated PFS was 25% for the allogeneic group versus 49% for the autologous group ($p = 0.051$).

Ladenstein et al. [57] performed a case–control study using the EBMT Solid Tumor Registry to investigate the potential advantage of allogeneic BMT in high-risk neuroblastoma patients without prior disease progression. Seventeen allogeneic and 34 autologous BMT cases were studied, and PFS was not significantly different: 35% for autologous and 41% for allogeneic BMT at 2 years, respectively.

Currently, autologous HCT continues to be the preferred method of support for high-dose therapy, and allogeneic HCT is reserved for specific clinical trials testing immunomodulatory effects. One example is the use of allogeneic HCT for patients relapsing after autologous HCT (NCT01156350). Similarly, the use of escalating doses of natural killer (NK) cell infusions from allogeneic or haploidentical donors is also currently under investigation in phase I trials (NCT01287104, NCT01576692) [58].

Hematopoietic cell source and purging

Bone marrow involvement by neuroblastoma is extremely common in children with metastatic disease, present by light microscopy in 60–80% of children with INSS Stage 4 disease at diagnosis. Detection of tumor by immunocytology using a mixture of monoclonal antibodies reactive at the cell surface has been shown to detect tumor reliably with a sensitivity that may vary from 1/10^4 to 1/10^6 nucleated bone marrow cells, depending on the method used [59–61]. Circulating tumor cells in blood are detectable by immunocytology at diagnosis in up to 50% of children with Stage 4 neuroblastoma. Furthermore, tumor cell concentration in bone marrow has been associated with poorer outcomes. The occasional reports of miliary lung relapse after autologous HCT, the site one would expect to be involved after intravenous infusion of tumor cells, supports the importance of tumor-free hematopoietic cell grafts [62].

Therefore, purging of hematopoietic cells has been studied, either by positive selection of CD34$^+$ cells or by immunomagnetic purging with antineuroblastoma tumor antibodies. Although earlier reports suggested that purging of bone marrow may be important, the widespread use of peripheral blood as the stem-cell source may have different implications. Immunomagnetic purging of neuroblastoma cells was evaluated in a group-wide phase III COG study (A3973), and did not confer a significant advantage in 2-year EFS or OS in the context of dose-intensive chemoradiotherapy and biologic therapy in first response [63]. Although all peripheral blood stem cells (PBSCs) infused were shown to be tumor free by immunocytology, more sensitive detection methods using quantitative reverse transcription polymerase chain reaction (qRT-PCR) had shown that tumor cells may still be present in a significant number of PBSC grafts, and purging may not have removed all neuroblastoma cells [64]. There is still uncertainty whether the lack of benefit of purging is due to reinfusion of microscopic amounts of tumor in the PBSC or to the fact that other areas of resistant microscopic residual disease in the patient have not been eliminated.

Minimal residual disease

Despite improvements in EFS using high-dose therapy, the relapse rate, even for patients transplanted in CR, remains high [2,19]. Immediately after HCT, when disease is likely to be minimal, provides the ideal window of time to eradicate resistant clones that are still present using novel therapies not dependent upon standard cytotoxic mechanisms. It has been demonstrated that children undergoing HCT who have residual disease by [^{123}I]MIBG scan (a Curie score of >0) have a significantly worse EFS after HCT [65]. Minimal residual disease in bone marrow by qRT-PCR after HCT also is predictive of higher risk of relapse [66]. Even when residual disease is undetectable by these sensitive tests, relapse after myeloablative therapy and HCT is frequent, especially in bones and bone marrow.

In vitro, both all-*trans*-retinoic acid and isotretinoin (13-*cis*-retinoic acid) cause decreased proliferation and differentiation in neuroblastoma cell lines, including some established from refractory tumors [67,68]. After some pilot studies, a phase III randomized trial of children with high-risk neuroblastoma completing consolidation chemotherapy or autologous HCT showed that the 3-year EFS was significantly better for the patients randomized to isotretinoin (Figure 63.3a) [41]. Long-term follow-up showed improvement in OS. The apparently contradictory report from the ENSG showed no advantage for using isotretinoin to treat MRD after myeloablative therapy [69]; this is likely attributable to the low isotretinoin dose employed in that study.

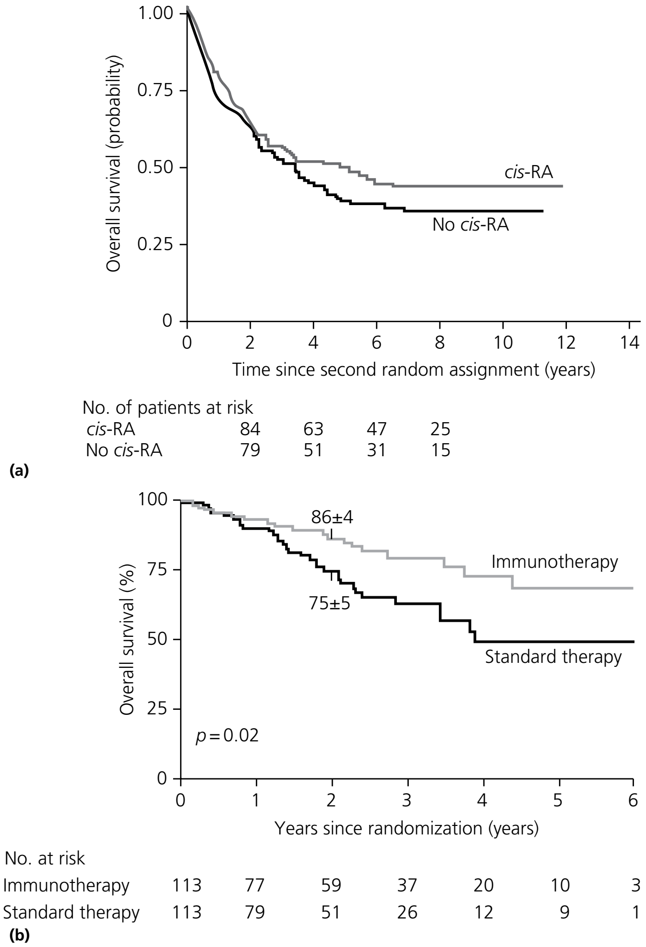


Figure 63.3 Treatment of minimal residual disease with isotretinoin and immunotherapy. (a) Use of isotretinoin in the treatment of minimal residual disease after HCT for high-risk neuroblastoma. Follow up began at the time of randomization, 34 weeks after diagnosis, with no significant difference in OS 5-year overall survival (p = 0.19). Source: Matthay et al. 2009 [41]. Reproduced with permission of the American Society of Clinical Oncology. (b) Use of immunotherapy (Ch14.18, IL-2, and GM-CSF) improves EFS after HCT for high-risk neuroblastoma. The 2-year estimate for overall survival was 86 ± 4% in the immunotherapy group and 75 ± 5% in the standard-therapy group (p = 0.02). Source: Yu et al. 2010 [76]. Reproduced with permission of the Massachusetts Medical Society.

Other retinoids such as fenretinide are currently under investigation for MRD. In contrast to isotretinoin, fenretinide does not induce maturational changes, but is cytotoxic and induces both apoptosis and necrosis [70]. In a phase II study, 62 patients with recurrent or refractory neuroblastoma were treated with 7 days of fenretinide 2475 mg/m^2/day divided three times daily every 21 days for a maximum of 30 courses [71]. Prolonged stable disease (SD) was seen in 14/59 evaluable patients, but this did not meet the study criteria for efficacy. Pharmacokinetic studies suggest that variably low bioavailability may have contributed to the modest effect. A study by the NANT Consortium investigated a new liquid fenretinide preparation in lipid to enhance absorption (fenretinide/LYM-X-SORB), and was able to demonstrate increased plasma levels at much lower doses, with four complete responses and six more with stable disease in 29 patients treated with 4–26 courses of therapy [72].

Another approach is the use of antibody-targeted therapy. Antibody therapy in relapsed neuroblastoma using monoclonal anti-GD2 antibodies has provided promising responses [73–75]. A randomized prospective trial assigned 226 patients in remission after high-dose therapy and HCT to receive isotretinoin alone, or the retinoid plus chimeric anti-GD2 antibody (Ch14.18) with granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin-2 (IL-2). Toxicities were significant but manageable. Both 2-year EFS and OS were superior in the immunotherapy group (66% vs. 46% and 86% vs. 75%; Figure 63.3b) [76]. Further improvements are being tested using a fusion protein of the humanized form of the antibody, Hu14.18, and IL-2 [77].

Another avenue of study includes the inhibition of angiogenesis. Testing in animal models suggests responsiveness to angiogenic inhibitors, particularly in minimal disease states. Much investigation has focused upon new and novel antiangiogenic small molecules and antibodies, with promising results in cell lines, in vitro models, and xenografts. Vascular endothelial growth factor (VEGF) inhibitors alone and in combination with chemotherapeutics showed promise [78]. Early studies of multitargeted tyrosine kinase inhibitors to VEGF, platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) are also under way [79].

Other studies have focused on harnessing the patient's immune system to target residual tumor cells [80]. The use of new immunomodulatory agents such as lenalidomide in combination with anti-GD2 antibody is under investigation by the NANT Consortium (N11-04). Cellular therapy for neuroblastoma and genetically engineered cellular vaccines to generate immune responses are also under investigation, including cytotoxic T-lymphocytes (CTLs) specifically engineered to recognize neuroblastoma proteins in the context of MHC I. Invariant NKT cells are a subtype of lymphocyte capable of enhancing antitumor activity through rapid and copious inflammatory cytokine release at the site of tumor; however, clinical trials attempting to enhance NKT function in neuroblastoma patients have not yet been performed. NK cells in particular may play a vital role in tumor surveillance post-transplant. Favorable combinations of NK cell killer immunoglobulin-like receptor (KIR) and HLA genotypes have been correlated with superior outcomes post-transplant [81].

Acute and late complications of HCT in neuroblastoma

The complications of high-dose therapy and HCT for neuroblastoma are similar to those of other high-dose regimens, and vary with the particular conditioning regimen (see Section 7). The most common acute complications with most preparative regimens for neuroblastoma include the toxic effects of the high-dose therapy, with frequent mucositis, sinusoidal obstruction syndrome (SOS), fever, and infection. Potentially fatal complications include gastrointestinal hemorrhage, pneumonitis and acute respiratory distress syndrome (ARDS), severe SOS with renal failure, or, rarely, intracranial hemorrhage or cardiac failure. Toxic death rates generally have ranged from 5 to 20% on autologous HCT protocols (Table 63.3). With increasing expertise, more rapid engraftment with peripheral blood hematopoietic cells, and the removal of TBI from most regimens, the acute toxic death rate appears to be decreasing to less than 5% [29,47,82].

With more patients surviving, later complications of high-dose therapy are becoming noticeable. One common complication particular to neuroblastoma is the frequent occurrence of significant hearing loss post-transplantation, seen in 10–20% of children. These patients receive large doses of platinum compounds during induction chemotherapy and again with many of the preparative regimens. They also are exposed to other ototoxins, including aminoglycoside antibiotics, diuretics, and noise exposure, all of which may exacerbate the effects of platinum compounds [83]. Newer studies are investigating the role of gene polymorphisms that may predispose certain patients to more severe platinum-induced ototoxicity; this may lead to the development of strategies to reduce hearing loss in some patients [84]. Another late effect seen because of the young age of the children with neuroblastoma is abnormal dental development and growth delay [85].

Earlier studies of transplant in neuroblastoma reported a low incidence of second malignancies, with only one patient in 129 in the CCG study [2] and two of 509 patient in the EBMT Registry report [44]. However, secondary malignancies may increase in frequency, as the high doses of alkylators and etoposide in both the induction and conditioning regimens have been shown to result in secondary leukemia [86]. Some children have now been reported to develop secondary leukemia after radiopharmaceutical therapy for neuroblastoma [52,87]. In addition, some unusual second malignancies have been reported after transplantation for neuroblastoma, such as renal cell carcinoma [88].

Conclusion

The use of myeloablative chemotherapy has been shown to improve EFS and OS for children with advanced neuroblastoma over the last three decades. It is vital now to increase the number of patients achieving response during induction with better chemotherapy and targeted agents, as approximately 15–20% of patients will progress early and become ineligible for HCT. The tumor reduction during the transplant conditioning may be further improved by using radio-targeted therapy, and integrating new non-cross-resistant agents into current protocols. It has also been shown that therapy for MRD post-transplantation is a critical component of the improvement in outcome for neuroblastoma. New targeted approaches to MRD deserve study, including inhibitors of key genetic pathways, differentiating agents, antiangiogenic agents, and immunologic approaches. Lastly, it is critical to refine the therapy of these children to reduce the serious late complications of treatment, and to study health-related quality of life in the survivors.

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CHAPTER 64

Hematopoietic Cell Transplantation for Other Pediatric Solid Tumors

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Introduction

Chemosensitivity

As a result of ongoing improvements in diagnosis, multimodal therapy, and supportive care, the treatment outcome of children with cancer has steadily improved since 1960. For instance, the 5-year survival of children with Wilms tumor has risen from under 45% to over 90%, for rhabdomyosarcoma from under 20% to 70%, and for Ewing sarcoma from 10% to 60% (reviewed in [1]). Improvements were possible, in part because the most frequent pediatric solid tumors (neuroblastoma, Wilms tumor, rhabdomyosarcoma, retinoblastoma, germ cell tumor, osteosarcoma, and Ewing sarcoma) are all chemosensitive.

However, subsets of patients have been defined who face a poor prognosis despite having initially chemoresponsive disease. Examples include patients over 18 months of age with metastatic neuroblastoma (see Chapter 63), and patients with metastatic Ewing sarcoma (see below). For such patients treated conventionally, the prognosis for disease-free survival (DFS) 3 years from diagnosis remains less than 20%.

In addition, the prognosis for recurrent or refractory pediatric solid tumors is dismal, and few patients have experienced prolonged survival. Most pediatric solid tumor patients are treated with intensive chemotherapy, and most recurrences occur during treatment or within 1 year of its completion. Rapid recurrence indicates the presence of tumor cells resistant to the chemotherapeutic agents used. Tumor cells often manifest substantial cross-resistance to multiple antineoplastic agents, including drugs to which the cells were never exposed.

Dose–response

For radiation and most classes of chemotherapeutics, including alkylators, anthracyclines, vinca alkaloids, and antimetabolites, there is a steep linear relationship between drug concentration and the log tumor cell kill assessed by in vitro colony formation and limiting dilution assays [2]. This steep log–linear dose–response relationship translates into important effects of chemotherapy dose on tumor response and survival in experimental tumor-bearing animals. In studies of the treatment of animals with osteosarcoma and mammary adenocarcinoma, sharp reductions in complete response (CR) and partial response (PR) rates accompany small (~15%) reductions in the dose of single agents of all classes, including antimetabolites (5-fluorouracil and 6-mercaptopurine), anthracyclines (doxorubicin and daunorubicin), antimicrotubule agents (vincristine), and alkylators [melphalan and cyclophosphamide (CY)]. In this system, the only curative single agent is CY, given at the dose that causes lethal toxicity in 30% of animals (LD30). Various combinations of chemotherapy can produce cures at <LD10, but even so, modest (~33%) dose reductions eliminate cures [3]. Of great relevance to pediatric oncology is a rhabdomyosarcoma xenograft model demonstrating a strong effect of the dose of melphalan on the tumor response of six primary tumor cell lines, including one from a CY-resistant tumor. Importantly, melphalan at adequate doses is much more active than the standard chemotherapy combination for rhabdomyosarcoma, vincristine, actinomycin D, CY, and doxorubicin [4].

Modeling of experimental data indicates that the likelihood of cure correlates best with dose intensity, whereas the duration of PR correlates best with the total dose of chemotherapy. The maximum cumulative dose of alkylators and anthracyclines that can be administrated safely does not increase when the dose intensity is reduced. It is therefore advantageous to maximize dose intensity; the maximum dose intensity is achieved by giving a single, very large course of drug [5].

Dose limiting myelotoxicity

For agents such as melphalan, carmustine, and carboplatin, whose major dose limiting toxicity is myelosuppression, hematopoietic cell transplantation (HCT) may permit 3–10-fold dose escalation. Given a steep dose–response relationship, this approach may translate into several log more cell kill and improved durability of response.

Indications and outcomes

Ewing sarcoma family of tumors

Epidemiology and etiology

The Ewing Sarcoma Family of Tumors (ESFT) encompasses Ewing sarcoma, primitive neuroectodermal tumor (PNET), Askin tumor, and peripheral neuroepithelioma, diseases previously considered distinct. The recognition that these tumors are all characterized by a reciprocal chromosomal translocation involving a gene on chromosome 22 known as EWS with a fusion partner that is an *ets* family member transcription factor has resulted in their reclassification as a distinct, molecularly defined tumor type. Although clearly a single group of tumors, there is a spectrum of differentiation

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within this group: the typical undifferentiated Ewing sarcoma lies at one end of the spectrum and PNET with neural differentiation lies at the other. The ability to characterize these tumors molecularly and the growing reliance upon evaluation of chromosomal abnormalities as the definitive means of establishing a diagnosis are emblematic of the era of molecular oncology.

ESFT are the second most common type of malignant bone tumor, behind osteosarcoma. These tumors account for 2% of childhood cancers, according to data from the Surveillance, Epidemiology and End Results (SEER) section of the National Cancer Institute. SEER data, however, are stratified for age, and therefore make epidemiologic understanding of these tumors that affect both adolescents and young adults difficult. Moreover, SEER data do not break down adult sarcoma incidence by histology, making a true understanding of the epidemiology of ESFT in the United States impossible. A review of the M.D. Anderson Cancer Center tumor registry evaluated the epidemiology of sarcomas in the adolescent and young adult (AYA) population without restrictions based on age or histologic subtype [6]. In this study, ESFT accounted for 25% of the bone sarcomas in the AYA population, whereas osteosarcoma accounted for 57%. These data allow an estimation of new cases in the United States every year of 390 ESFT of bone and 170 of soft tissue.

ESFT incidence peaks in the second decade of life, and is predominantly a disease of the AYA population. About 64% of cases occur in the second decade of life, compared with 27% in the first decade and only 9% in the third [7]. The M.D. Anderson data corroborate that finding, as chondrosarcoma was the second most frequent bone sarcoma histology in the population as a whole (28% of cases), but ESFT was more frequent in the AYA group. Interestingly, ESFT is overwhelmingly a disease of White children. In New York, ESFT represents 22% of primary bone cancers in Whites, but only 7% in Blacks [8].

ESFT are not commonly associated with other congenital diseases, or with skeletal abnormalities or other cancers. No constitutional chromosomal abnormalities have been reported in patients with ESFT, and the concordance rate in siblings is very low. ESFT are rarely reported as second malignant neoplasms in patients previously treated for cancer.

Molecular and cellular biology

These tumors are defined molecularly by the presence of a reciprocal translocation t(11;22)(q24;q12) that fuses a gene known as EWS with the ETS family transcription factor FLI1 and is found in 85% of such tumors [9]. Alternative reciprocal translocations, such as t(21;22)(q22;q12), t(7;22)(p22;q12), t(17;22)(q12;q12), and t(2;22)(q33;q12), all of which result in a fusion between EWS and an *ets* family transcription factor, have been reported in the remaining 15% [10]. Several studies have suggested that EWS–FLI1 is oncogenic, and is sufficient to initiate and maintain the transformed phenotype [11]. In fact, inhibition of the fusion gene in cell lines inhibits growth in vitro [12]. In addition to the defining translocation, numerous other cytogenetic abnormalities have been described in ESFT, and Sandberg and Bridge provided a comprehensive overview [10].

Controversy still surrounds the most basic cellular biological question related to ESFT, namely the cell of origin. There is cytologic and ultrastructural evidence of neural maturation in the more differentiated ESFT tumors [13] that were formerly classified as PNET. An attempt to establish the histogenesis of ESFT cell lines by analysis of intermediate filament expression, however, suggested an epithelial phenotype, on the basis of expression of cytokeratins 8 and 18 [14]. More recently, DNA microarrays have been employed to attempt to address this question. Gene expression profiling experiments have shown that these tumors are related to both neuronal and endothelial lineages [15]. Interestingly, expression of EWS–FLI1 in human embryonic kidney cells is insufficient to induce the entire ESFT gene expression profile, suggesting that the cell being tested impacts the process of differentiation. In contrast, Riggi et al. demonstrated that forced expression of EWS–FLI1 in bone marrow-derived mesenchymal progenitor cells induces the formation of tumor cells with all of the essential hallmarks of ESFT [16]. Thus, support can be found in the literature for neuronal, epithelial, endothelial, or mesenchymal origin of these tumors. Future work will be required to clarify the histogenesis of ESFT.

Clinical description

A detailed description of the clinical presentation of ESFT can be found elsewhere [1]. In brief, pain and swelling are the most common presenting symptoms of ESFT, occurring in 84 and 63% of patients, respectively. These symptoms are often longstanding, with a median delay between onset of symptoms and diagnosis in excess of 6 months. Although tumors can arise in any bone in the body and in soft tissue, these tumors show a predilection for the lower extremity (46%) and pelvis (20%). ESFT is a very aggressive malignancy, with 25% of patients presenting with overt metastases. Of these, half have metastases to the lungs, one-quarter to other bones, and one-fifth to bone marrow. Metastases to other sites are rare, except in late-stage disease.

Non-transplant approaches

For these tumors, the primary adverse risk factor is the presence of metastatic disease. A more recently recognized prognostic factor is histologic response to chemotherapy. Data from the Rizzoli Institute showed 95% DFS for patients with localized disease and complete tumor necrosis, compared with 34% for patients with macroscopic viable tumor at the time of definitive surgery. Pelvic primary disease or a large tumor size (>8 cm in maximal diameter or >100 mL in volume) historically carried a poor prognosis for survival. Through intensification by the addition of ifosfamide and etoposide, EFS has improved from 54 to 69% for patients with localized disease and eliminated pelvic primary location and large tumor size as prognostic factors [17]. Unfortunately, even with this addition, which has now become standard of care, the 22% EFS of patients with metastatic disease has not improved. The recently published Children's Oncology Group (COG) study tested increasing dose intensity by randomizing patients to receive this same treatment regimen of alternating cycles of vincristine–doxorubicin–cyclophosphamide and ifosfamide–etoposide with cycles given at 2-week intervals versus the standard 3-week intervals. The 5-year EFS was 65% for patients with localized disease treated at 3-week intervals, but 73% for patients treated with the time-compressed regimen [18], lending further support to the importance of dose and time intensity for the cure of pediatric solid tumors.

In addition to systemic chemotherapy, adequate local control is essential to eradicate the disease. Local control measures include radiation therapy and surgery. The relative efficacy of radiotherapy compared with surgery remains controversial. Studies utilizing dose-intensive chemotherapy showed little difference in survival between patients treated with surgery and patients treated with radiation [19], with respect to both local recurrence and overall survival (OS). In a single-institution study of 76 patients with localized disease, the local failure rate was identical in the patients treated with radiation, surgery, or both [20]. Interestingly, in a multivariate analysis, only the use of chemotherapy was a prognostic factor for local control (the 10-year local control rate for patients treated with chemotherapy was 83.7%, compared with 51.1% for patients who did not receive chemotherapy), supporting the concept that systemic treatment contributes to local control.

Hematopoietic cell transplantation

Patients with high-risk ESFT, including patients with metastatic or recurrent disease, have a very poor prognosis. Dose-intensive chemotherapy has not improved the outcome for patients with metastatic disease, and patients with recurrent disease are not cured with standard chemotherapy. For these patient populations, HCT has been investigated as a way to improve outcomes. One of the earliest and largest reports of high-dose therapy investigated the use of total body irradiation (TBI) with autologous peripheral blood progenitor cell (PBPC) support as consolidation therapy for high-risk ESFT patients. Over a 5-year period, 91 patients were treated on three successive chemotherapy protocols at the National Institutes of Health (NIH), and remissions were consolidated in 65 of the patients with 8 Gy of TBI [21]. Nineteen patients were not given TBI, because they failed to achieve remission or relapsed prior to treatment, and seven refused. Twenty of the 65 treated patients became long-term survivors (31%), a higher rate than expected with chemotherapy alone in this group of patients, and superior to a group of contemporary patients treated without high-dose therapy. However, only patients who did not progress after chemotherapy were eligible for HCT, so OS for the total patient population was only 22%, no better than results with chemotherapy alone. Subsequent studies confirmed that HCT appears to offer a survival advantage to select patient groups, such as those with chemosensitive metastases limited to the lungs (Figure 64.1) [22].

More current results from European Bone Marrow Transplant Registry (EBMTR) data combined with data from the Pediatric Registry for Stem Cell Transplantations (PRST), Asia Pacific Blood and Marrow Transplantation (APBMT) and Meta European Intergroup Cooperative Ewing Sarcoma Study (MetaEICESS) suggest several improvements. Although the data are from non-randomized patients, a 5-year OS of 44% was reported for patients treated with a busulfan-containing regimen, compared with 23% for patients treated without this drug [23].

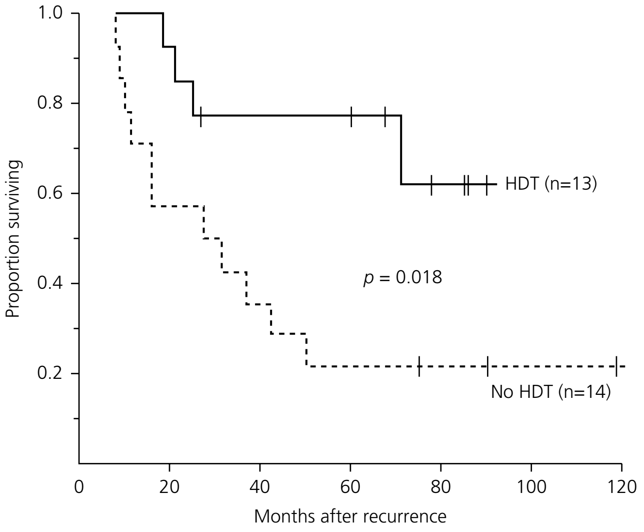

Figure 64.1 Survival from time of first recurrence for patients with recurrent ESFT who responded to salvage induction chemotherapy, by use of high-dose therapy (HDT). Source: Barker et al. 2005 [22].

A follow-up study published from the previously mentioned EBMTR data, included 87 patients, mostly (n = 69) from the EBMTR, who had a molecularly confirmed diagnosis of ESFT, and the outcome was assessed based on transplant regimen used. Other than a higher incidence of relapse in the reduced-intensity conditioning group, there were no differences in OS rates, failing to address this question [24].

All of the studies summarized above share the same weakness – none was prospective and randomized. This limits the confidence with which HCT can be recommended as "standard of care" for ESFT patients. An ongoing study, Euro-Ewing 99, was designed to address this deficiency. In this study, patients are assigned to one of three risk groups, based on the presence or absence of metastases, the size of the primary tumor, and response to neoadjuvant therapy. Patients in the lowest risk group are treated with 14 cycles of chemotherapy. Patients judged to have intermediate-risk disease (PR, rather than CR to neoadjuvant therapy, a tumor >200 mL, or pulmonary metastases) are randomized to consolidation with chemotherapy versus high-dose therapy with busulfan and melphalan. The patients with the highest risk (extrapulmonary metastases) are treated with either of two different preparative regimens for their HCT according to physician discretion, although the recommended treatment is a busulfan–melphalan-based regimen. For the highest risk patients, the results have been published and showed a 3-year event-free survival (EFS) of 27% and an OS of 34% [25]. Although several favorable prognostic factors were identified in this otherwise very high-risk group (such as isolated bone marrow metastasis, smaller tumor size, and fewer sites of metastasis), the utility of HCT in this population remains unclear. Results from the remaining arms of the study are expected to resolve some of the controversies surrounding the utility of HCT for patients with ESFT, especially patients with intermediate-risk disease.

Rhabdomyosarcoma

Epidemiology and etiology

Rhabdomyosarcoma is the most common soft tissue sarcoma of childhood, accounting for 5–8% of childhood cancer [7]. About 250 new cases of rhabdomyosarcoma are diagnosed in the United States annually, slightly more frequently in boys than in girls. Little is known about the etiology of rhabdomyosarcoma. These tumors sometimes arise as second malignant neoplasms in patients previously treated with ionizing radiation, but radiation exposure accounts for a very small minority of cases.

Molecular and cellular biology

There are two major histologic variants of rhabdomyosarcoma: embryonal and alveolar. Embryonal rhabdomyosarcoma, named for its resemblance to immature skeletal muscle, has been subdivided into solid and botryoid variants. Embryonal histology accounts for 57% of cases of rhabdomyosarcoma and usually affects children between 3 and 12 years of age. Alveolar histology, named for its resemblance to normal lung parenchyma, accounts for 19% of cases of rhabdomyosarcoma, and usually strikes patients between 6 and 21 years old. Embryonal rhabdomyosarcoma carries a more favorable prognosis than alveolar.

As in ESFT, recurrent translocations have been identified in rhabdomyosarcoma, although only in the alveolar subtype. In 55%

of cases, a translocation between chromosomes 2 and 13, t(2;13)(q35;q14), is seen, and in 22% of cases a similar translocation involving chromosome 1, t(1;13)(p36;q14), is identified [26]. These translocations involve related transcription factor genes, PAX3 and PAX7, respectively. The PAX3–FKHR translocation appears to carry a poorer prognosis than PAX7–FKHR. In each case, the DNA-binding domain of the PAX gene is fused to the transactivation domain of the FKHR gene. Disruption of PAX genes leads to abnormal muscle development [27], suggesting a direct etiologic relationship between the translocation and the development of malignancy. Moreover, ectopic expression of these chimeric genes can transform cells. It is probably the translocation oncogene, rather than histology, that dictates the course of disease. In fact, tumors histologically classified as alveolar rhabdomyosarcoma but which are fusion gene negative behave similarly to embryonal rhabdomyosarcoma, both clinically and molecularly [28], and based on this observation, molecular staging systems have recently been proposed [29].

Clinical description

Signs and symptoms present at diagnosis depend on the site of the primary tumor, the involvement of surrounding normal organs, and the presence of distant metastases. Typically, patients present with an asymptomatic mass, although involvement of cortical bone may cause pain, and genitourinary disease may cause hematuria or urinary retention. Different subtypes of rhabdomyosarcoma arise in different sites. Embryonal rhabdomyosarcoma typically arises in the head and neck region or the genitourinary tract, while alveolar rhabdomyosarcoma tends to arise in the extremities and the trunk [1]. Head and neck sites, including orbit and parameningeal primaries, account for 40% of cases, trunk and extremity sites 25–30% of cases, and genitourinary sites 20% of cases. The most common sites of metastasis are the lungs, lymph nodes, cortical bone, and bone marrow.

Non-transplant approaches

Rhabdomyosarcoma is treated with multimodality therapy, including chemotherapy, radiation therapy, and surgery. Surgery alone is insufficient to cure even localized disease. In the era before the development of effective systemic chemotherapy, OS for patients with localized disease was 25% or less, but with current multimodality therapy, failure-free survival rates for patients with localized disease are as high as 90% [30].

The most important predictor of treatment failure is the clinical group identified in early Intergroup Rhabdomyosarcoma Study Group (IRSG) studies (Table 64.1). In addition to classification by clinical group, rhabdomyosarcoma is also staged using a specific TNM staging system [30] that takes into account not only tumor size, lymph node status, and distant metastases, but also incorporates information regarding the site of the primary tumor, reflecting prognostic differences between favorable and unfavorable anatomic locations. Patients are then classified as high, intermediate, or low risk based on clinical group and stage, and treatment is based on this assessment of risk. Although inclusion of molecular information seems to be promising, fusion gene status has not yet been incorporated into formal staging systems [29].

Table 64.1 Clinical group definitions for rhabdomyosarcoma patients

Group	Definition
I	Localized disease completely resected
IIa	Gross total resection with microscopic residual disease
IIb	Regionally involved lymph nodes, completely resected with the primary
IIc	Regional disease with involved nodes, totally resected with either microscopic residual disease or histologic evidence of involvement of the most distant lymph node in the dissection
III	Incomplete resection
IV	Distant metastases

The principles of non-transplant therapy for rhabdomyosarcoma, as established by the IRSG clinical trials, include local control (consisting of surgery, radiation therapy, or both) and systemic therapy to address either gross or microscopic metastatic disease. Primary surgical resection is indicated, unless the tumor is deemed unresectable. In this case, radiation therapy is employed, and can provide definitive local control for patients with unresectable rhabdomyosarcoma [31]. The specifics of adjuvant systemic chemotherapy vary depending on risk stratification. In general, the chemotherapy used for low-risk patients is based on a vincristine–dactinomycin–cyclophosphamide backbone, while intermediate- or high-risk patients utilize the same backbone with a significantly higher dose of cyclophosphamide and addition of other active agents such as irinotecan, ifosfamide, and etoposide. Unlike osteosarcoma or Ewing sarcoma family tumors, patients with rhabdomyosarcoma undergo surgical resection prior to the institution of systemic therapy, as reflected in the staging system, which takes into account the extent of resection. Upon recovery from surgery, patients are treated with chemotherapy with or without radiation, which is usually delivered relatively early in the treatment regimen.

Hematopoietic cell transplantation

The role of high-dose therapy and HCT in high-risk rhabdomyosarcoma is not established. Several retrospective analyses have been reported. Between 1982 and 1994, the EBMTR received reports of 98 transplants performed in children and young adults with relapse or progression of initially localized rhabdomyosarcoma. The proportion surviving disease free is approximately 20%, and median survival remains short, only 8.3 months from HCT [32]. The German–Austrian Pediatric BMT Group in 1997 published the results of 36 transplants performed for metastatic or recurrent rhabdomyosarcoma between 1986 and 1994. Thirty-four of the 36 patients received high-dose melphalan-based regimens, usually augmented with etoposide and carboplatin. The estimated EFS at 2 years after HCT was 36 ± 7% [33]. This rate is better than expected, but the small number of patients and possible effects of selection bias preclude conclusions about the efficacy of HCT for rhabdomyosarcoma.

The European Collaborative MMT4-91 trial was the first prospective trial to evaluate the efficacy of HCT as consolidation therapy in first remission for patients who presented with metastatic rhabdomyosarcoma. This study resulted from the amendment of MMT4-89 for patients with newly diagnosed metastatic rhabdomyosarcoma, so that institutions could chose to substitute HCT with high-dose melphalan for the fourth and final 9-week cycle of chemotherapy in patients who had achieved CR before the third cycle of therapy. Hence there was a nearly contemporaneous control population of patients either enrolled on MMT4-89 or enrolled on MMT4-91 at centers not participating in HCT. Fifty-three patients in CR after six courses (two cycles) of chemotherapy were treated with high-dose melphalan, either alone or in combination with other agents such as carboplatin, etoposide, thioTEPA, and/or busulfan. Outcomes were compared with those for 44 patients who were also in CR after six identical cycles of chemotherapy but went on to receive further conventional dose chemotherapy rather than HCT. Although patients were not randomized to HCT or conventional therapy, decisions to administer HCT consolidation were made at the center level, reducing the potential for selection bias. Unfortunately, the patient population treated with HCT ($n = 53$) in fact had poorer prognostic factors: lymph node involvement was more common (56% vs. 34%), alveolar histology was more common (44% vs. 30%), fewer patients were <10 years old (60% vs. 68%), and more patients had large tumors >5 cm in diameter (73% vs. 61%) than in the control population ($n = 44$). Despite these differences and the small sample size, the median time from the end of therapy to relapse was significantly longer for the HCT group than the conventional chemotherapy group (168 vs. 104 days, $p = 0.05$), but there was no difference in EFS or OS [34]. Single-institution protocols for high-risk rhabdomyosarcoma that incorporate HCT consolidation showed similar results, with DFS for Stage IV patients ranging from 14 to 28% at 3 years or more [35–38].

Wilms tumor

Epidemiology and etiology

Wilms tumor is the most common renal malignancy of childhood, with an annual incidence in the United States of 8.1 cases per million children under age 15 years, with a slight female predominance. Children with bilateral disease have an earlier age of onset, 23.5 months for males and 30.5 months for females, than those with unilateral disease, 36.5 for males and 42.5 months for females. This age difference for laterality was a key observation in the development of Knudson's classic two-hit model of tumorigenesis [39]. The additional variation in age of onset by gender and ethnicity suggested heterogeneity in the pathogenesis of Wilms tumor [40].

Molecular and cellular biology

Molecular correlates of these epidemiologic observations have subsequently been identified. In most normal tissue, imprinting of the insulin-like growth factor 2 gene, IGF2, silences the maternal allele, so only the paternal allele is expressed. In a study of 36 informative Wilms tumor specimens, loss of imprinting that led to expression of the normally silent maternal allele was strongly associated with perilobular nephrogenic rest histology and a 2.2-fold increased expression of IGF2 [41]. Conversely, mutation of the Wilms Tumor 1 (*WT1*) suppressor gene is associated with earlier age at onset, stromal-predominant histology, intralobular nephrogenic rests, and poorer response to therapy [42]. Other genetic abnormalities identified in Wilms Tumor include mutation of the WTX gene and also mutations in CTNNB1 [43], which are both components of the Wnt signaling pathway. Molecular markers are increasingly being recognized as prognostic predictors, and loss of heterozygosity of 1p and 16q is associated with decreased EFS and OS in otherwise favorable histology Wilms tumor [44].

Clinical description

Children with Wilms tumor typically present with a painless abdominal mass or abdominal distention. Other findings include gross hematuria and fever. Hemorrhage into the tumor can result in a rapidly expanding abdominal mass and anemia. Compression of renal vasculature can produce hypertension. Radiographic findings can usually distinguish Wilms tumor as an intrinsic renal mass from neuroblastoma arising in the adrenal gland and displacing the kidney. Patterns of spread include local extension beyond the tumor pseudocapsule into the renal sinus, the renal vasculature, and lymphatics. The tumor can be locally aggressive, violating the renal capsule, and can disseminate through lymphatics. Hematogenous dissemination is primarily to the lungs, and less commonly to the liver.

Non-transplant approaches

Wilms tumor is the exceptional childhood solid tumor that is often curable by conventional chemotherapy when metastatic at diagnosis, and even after relapse. However, high-risk groups can be defined. The National Wilms Tumor Studies NWTS-2 and NWTS-3 accrued 2757 untreated patients aged 15 years or younger, with Stage I–IV disease. The prognosis of the 367 patients (14%) who relapsed after achieving an initial CR was analyzed in 1989. Histology was an important predictor of outcome, with unfavorable histology defined by the presence of diffuse anaplasia (Figure 64.2) [45]. In NWTS-3, 3-year survival after relapse was 42% with favorable histology versus 16% with unfavorable histology. Similar numbers of patients relapsed at <6, 6–11, and >12 months from diagnosis. Their respective 3-year survival rates were 18, 30, and 41%, respectively. Among patients with favorable histology, the 3-year survival by initial stage was Stage I 57%, II/III 36%, and IV 17%; for patients with unfavorable histology, 3-year survival was

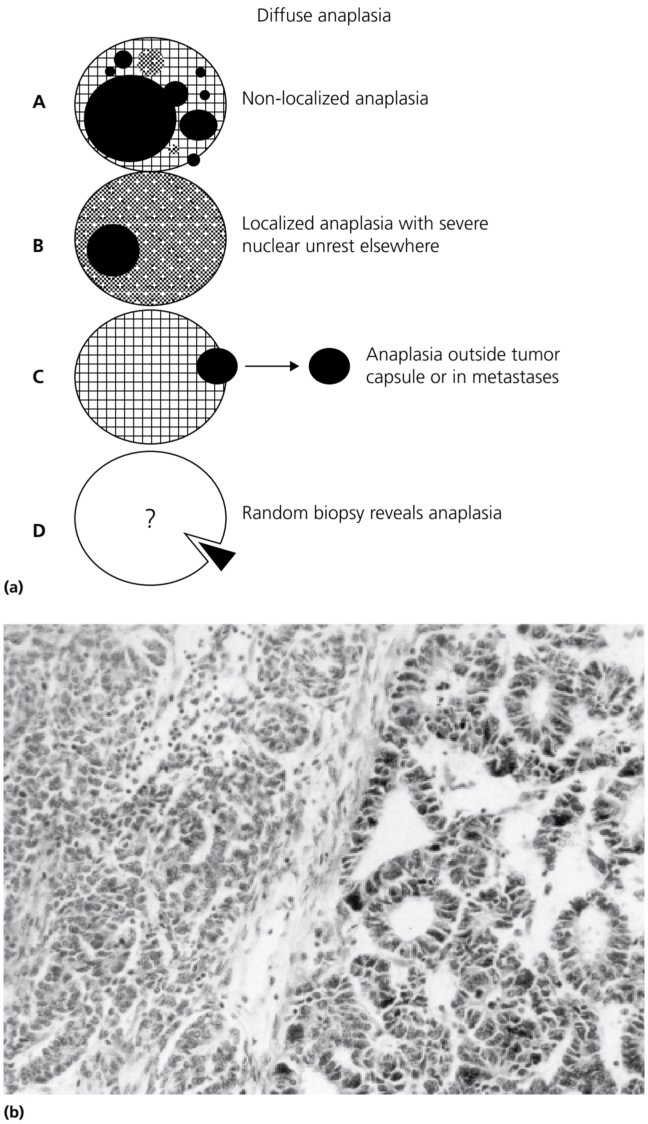
Diffuse anaplasia
A
Non-localized anaplasia
B
Localized anaplasia with severe nuclear unrest elsewhere
C
Anaplasia outside tumor capsule or in metastases
D
?
Random biopsy reveals anaplasia
(a)
(b)

Figure 64.2 (a) Patterns of anaplasia within Wilms tumor that constitute diffuse anaplasia. (b) Anaplastic focus on the right, sharply demarcated from adjacent non-anaplastic Wilms tumor on the left. Source: Faria et al. 1996 [45]. Reproduced with permission of Lippincott Williams & Wilkins.

Stage I 17%, II/III 14%, and IV 7%. Among Stage II/III patients with favorable histology, those randomized to receive three-drug initial therapy had a 3-year survival from relapse of 16%, versus 42% among those initially treated with two-drug regimens. Intra-abdominal relapse was unfavorable when this field had been previously irradiated, precluding further radiation therapy to the site of relapse [46].

An analysis of the long-term survival outcome of patients relapsing on the UK Childrens' Cancer Study Group (CCSG) Wilms Tumor 1 trial, which accrued 381 patients from 1980 to 1986, confirmed the negative prognostic importance of unfavorable histology, high initial stage, and early relapse, given salvage with ifosfamide or cisplatin and etoposide [32]. Taken together, these results led to the accepted definition of a high-risk subgroup of relapsed Wilms tumor patients whose expected 3-year survival is under 20%, including those with any of the following features: unfavorable histology, relapse within 6 months of diagnosis, failure of a three-drug regimen, and involvement of sites other than lung and abdomen, or involving the abdomen after irradiation.

However, the National Wilms Tumor Study 5 (NWTS-5) treated patients who relapsed after vincristine and dactinomycin therapy without prior irradiation on a uniform regimen consisting of vincristine, doxorubicin, cyclophosphamide, and etoposide, combined with radiation to involved organs. Of 72 patients enrolled, 68 were evaluable, and the 58 patients aged 18 years and younger with unilateral disease at initial diagnosis were analyzed. Only 10 of the patients had no adverse prognostic features, 32 had one, and 16 had two. Nevertheless, the 4-year EFS was 71% and the 4-year OS was 82% [47], showing that aggressive conventional therapy can overcome adverse prognostic factors in minimally pretreated patients.

In contrast, results of the NWTS-5 study for patients who relapsed after initial therapy with vincristine, dactinomycin, and doxorubicin were less favorable. A total of 103 patients were enrolled in this stratum, and 91 were evaluable. The 60 patients with initially unilateral disease who relapsed after achieving a CR were analyzed. For these 60 patients, the 4-year EFS was 42% and the 4-year OS was 48%. Twenty-five patients had only one adverse prognostic factor, 22 had two or three, and 13 had four or more. More adverse prognostic factors, male gender, and sites of relapse other than the lungs conferred significantly poorer EFS [48]. In summary, there is room for improvement over the results of non-transplant chemotherapy in patients with high-risk relapses following three-drug initial therapy.

HCT for high-risk relapse of Wilms tumor

Limited published information is available regarding high-dose therapy for patients with high-risk relapse of Wilms tumor. The experience collected by the EBMTR has been reviewed [49]. Twenty-five patients with Wilms tumor received high-dose therapy over a 7-year period from 1984 to 1991. Twenty-one of these children had had one to four relapses, and four patients had Stage IV disease refractory to first-line therapy. Of 11 patients transplanted in CR2, 10 had one or more high-risk features as defined above. Twenty of the 25 patients received high-dose melphalan-based regimens, although seven different regimens were used. Eight of 17 children transplanted in CR became long-term disease-free survivors. Of patients with measurable disease, 5/8 achieved a CR and 1/8 achieved a PR to high-dose therapy, demonstrating the activity of these regimens in refractory Wilms tumor. However, only one of eight children with measurable disease at the time of HCT became a long-term survivor [49].

These results are sufficiently encouraging to warrant prospective evaluation, first in phase II trials to identify active regimens and then in a randomized comparison with chemotherapy for patients with high-risk disease. The French Society of Pediatric Oncology (Société Française de l'Oncologie Pédiatrique, SFOP) completed the first prospective trial of HCT for high-risk relapsed Wilms tumor [50]. From 1988 to 1994, 31 patients underwent HCT, including 29 relapsed (CR2, 16; PR2, 4; CR3, 3; PR3, 5; CR5, 1) and two Stage IV anaplastic Wilms tumor patients transplanted in CR1. All patients had at least one high-risk feature, and were heavily pretreated with five or six chemotherapy drugs before HCT. The preparatory regimen consisted of melphalan 180 mg/m^2, etoposide 1000 mg/m^2, and carboplatin, dosed to achieve an area under the curve of 20 mgmin/mL over 5 days. Radiation therapy was delivered to sites of bulky metastatic disease after recovery from HCT. Seven patients sustained renal tubular damage, one developed sinusoidal obstruction syndrome (SOS) of the liver but recovered fully, and three developed interstitial pneumonitis. Only one of nine evaluable patients failed to achieve CR after HCT. Sixteen patients relapsed at a median of 8.5 months (range 3–53 months) after HCT, and 12 patients remained in continuous CR a median of 48.5 months (range 36–96 months) after HCT. DFS was 50 ± 17% and OS was 60 ± 18% at 3 years [50]. The only statistically significant prognostic factor was the number of disease progressions before HCT: In second response, DFS was 63.1 ± 20%, compared with 22.2 ± 24% in third response or beyond. The results are better than historical results for high-risk patients with such advanced disease. However, over the same period, 15 patients in the participating centers with at least one adverse prognostic factor failed to undergo consolidation because of uncontrolled progressive disease (PD), and would need to be included in an intention-to-treat analysis to estimate the effect of HCT on survival from relapse in an unselected population.

The German Cooperative Wilms Tumor Studies confirmed these results, treating 23 patients with HCT from 1992 to 1998 because of defined high-risk criteria including relapse, progression, or incomplete response of initial Stage IV disease [12], second or subsequent relapse [9], relapse within a radiation field [4], bone or brain metastases [3], relapse <6 months after nephrectomy [3], or relapse of a tumor with unfavorable histology [1]. The preparative regimen was identical with that of the SFOP in 19 patients. One patient developed renal failure requiring dialysis for 1 month, and six developed tubular dysfunction. For the entire cohort, the EFS was 48% and OS 61%, but disease status at the time of HCT strongly predicted outcome: 11/13 patients in CR but only 3/10 patients in PR survived at a median follow-up of 58 months [51].

The most recently published large data set from the EBMTR was reported in 2008 and included 343 transplants for 305 patients during 1985–2005. OS for the patients in CR1, CR2, and SR (sensitive relapse: patients who had chemotherapy-responsive disease but never achieved a CR) were 71, 40, and 34%, respectively, and it seems that disease status does predict outcome although the optimum timing of HCT remains unclear [52]. The selection of a subgroup of patients who would benefit the most from HCT is important from not only the tumor response and disease-related mortality standpoint, but also from the standpoint of therapeutic toxicity, considering that many Wilms tumor patients have only one kidney. A meta-analysis published in 2013 identified 19 studies that included patients with relapsed or refractory Wilms tumor who received either conventional therapy or autologous HCT [53]. Although not conclusive owing to the difficulties in comparing patients treated with different upfront therapies, it appears that in very high-risk patients (defined as risk group III, which includes initial Stage IV with unfavorable histology, those who received at least four chemotherapy agents, and those with PD after first relapse and salvage therapy), there is a small survival advantage to HCT.

The NWTS-5 pediatric salvage protocol was initially designed to incorporate HCT for patients who failed to achieve a CR after two courses of salvage induction chemotherapy. However, the study was amended to eliminate HCT. Although the results of this salvage protocol are not yet mature, the St. Jude institutional experience and the results of the Children's Cancer Group pilot salvage protocols suggest that newer chemotherapy approaches have improved the outcome of patients with high-risk relapse even without HCT [54,55], and it would therefore be appropriate to launch an international trial to assess the value of HCT compared with conventional-dose chemotherapy in high-risk recurrent Wilms tumor, with the greatest benefit expected in patients in second response (Figure 64.3).

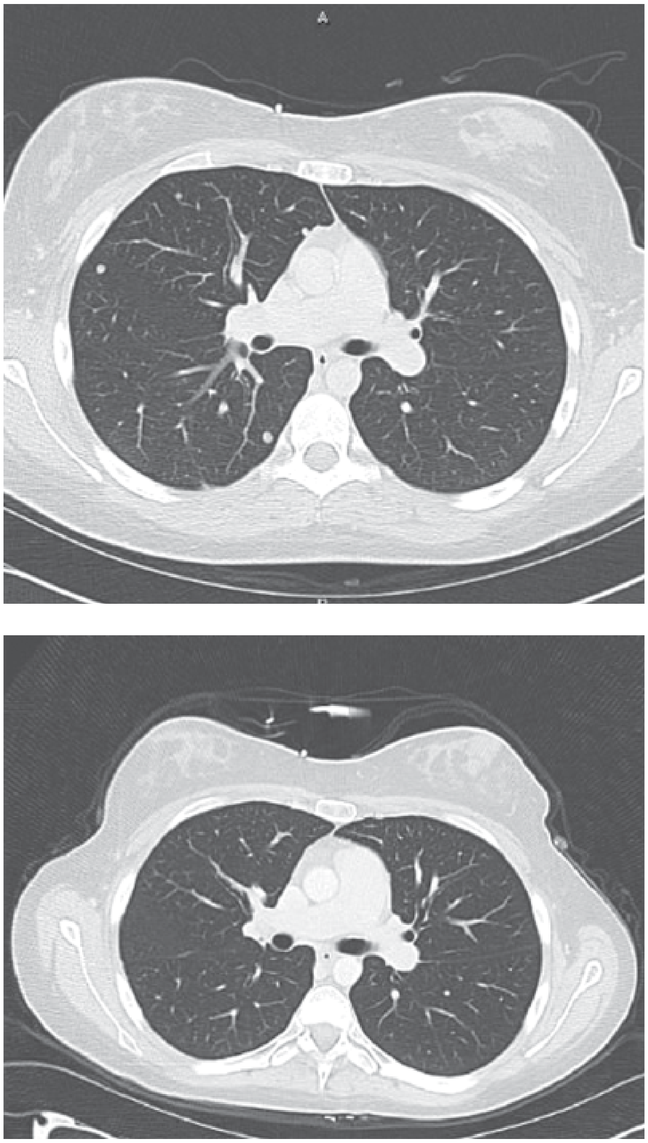

Figure 64.3 Metastatic recurrent Wilms tumor. CT scans showing first chemoresponsive relapse subsequently consolidated with autologous HCT.

HCT as consolidation for Wilms tumor

Because of the excellent curability of Wilms tumor with conventional chemotherapy, few patients have been treated with HCT for Wilms tumor in first response, all with Stage IV disease. All five patients reported by the EBMT, including two with unfavorable histology, survived disease-free at a median of 62 months (range 14–67 months) after HCT [49]; both patients with Stage IV anaplastic Wilms tumor transplanted by the SFOP died of PD [50], whereas the one similar patient treated by the German Cooperative Wilms Tumor Studies survived [51].

Osteosarcoma

Epidemiology and etiology

Osteosarcoma is the most common primary bone tumor of childhood and adolescence, and usually involves the long bones [1]. The incidence of osteosarcoma is three new cases per million per year, or 5.6 cases per million Caucasian children younger than 15 years in the United States. The peak age of onset is during the second decade of life, during the adolescent growth spurt, somewhat earlier in girls than in boys. Boys are affected more frequently than girls, a finding speculated to reflect the larger bone volume in boys compared with girls. A second incidence peak after the age of 50 years may reflect tumors induced by environmental exposures.

The only proven exogenous risk factor for the development of osteosarcoma is exposure to ionizing radiation [56]. Unlike ESFT, however, osteosarcoma has been associated with several inherited syndromes, including bilateral retinoblastoma, Li–Fraumeni syndrome, Bloom syndrome, Rothmund–Thomson syndrome, Werner syndrome, and multiple exostoses. Osteosarcoma is also associated with Paget disease. These findings have led to the identification of numerous genetic changes involved in the development of this tumor. Hence a good deal is known about the molecular biology of osteosarcoma.

Molecular and cellular biology

The recognition that patients with bilateral retinoblastoma are at increased risk of developing osteosarcoma implicates the *RB1* gene in the pathogenesis of osteosarcoma. As expected from this observation, loss of *RB1* at chromosomal locus 13q14 is a frequent finding in sporadic osteosarcoma [57]. Osteosarcoma is one of the defining tumors of Li–Fraumeni syndrome, and this has led to investigation of mutations in p53 in spontaneous osteosarcoma. Mutations in p53 occur in 40–60% of cases [58]. Both of these genes, *TP53* and *RB1*, are implicated in cell cycle regulation and proliferation. Numerous other cell cycle regulatory genes have also been implicated in this disease, including *MYC*, *CDKNA2*, *CDKN2A*, *CDKN2B*, and *MDM2* [58–60].

Chromosomal instability appears to play a key role in the molecular pathogenesis of osteosarcoma. No recurrent chromosomal translocation has been identified, but numerous loci with amplification, point mutation, or loss of heterozygosity have been reported (reviewed in [61]). This is consistent with the observation that osteosarcomas are diagnosed with increased frequency in patients with Bloom syndrome, Rothmund–Thomson syndrome, and Werner syndrome, disorders caused by germline mutations in RecQ helicases, which are involved in maintaining genomic stability [62].

In addition to these general regulatory genes, loss of function of genes implicated in bone differentiation has also been seen in osteosarcoma. Deregulated bone differentiation is commonly seen in osteosarcoma [63]. *Runx2* is a primary regulator of bone development [64] and is upstream of other regulatory genes, including OSX

[65] and TWIST [66]. The TWIST locus, 7p21, was found to be rearranged in 31 cases of osteosarcoma, deleted in 22, and amplified in nine out of a total of 68 cases examined [67]. Additionally, high Ezrin expression has been correlated with a poor outcome in osteosarcoma patients [68], a protein implicated predominantly in metastatic disease [69]. Further investigation into the molecular biology of osteogenesis will undoubtedly uncover further genes that contribute to the development of osteosarcoma.

Clinical description

The most common presenting complaint is pain [1]. Swelling and pathologic fracture are also often seen. Radiographic findings typically include a mixed osteolytic–sclerotic lesion with disorganized soft tissue calcification. At diagnosis, the disease is localized in 80% of cases, with metastases, most commonly to the lung, seen in the other 20%. Bone is the other common site of metastatic disease, with metastases rarely seen in lymph nodes. Osteosarcoma arises most frequently in the limbs (80% of cases), the femur being the most common bone. It occurs predominantly in the metaphysis of long bones, arising from the medullary cavity and invading into the epiphysis, even in the presence of a growth plate.

Non-transplant approaches

The mainstays of therapy for osteosarcoma remain surgery and chemotherapy. Until the 1970s, surgery was the only available therapy, but complete resection cured only 20% of patients. Three out of four patients died within 2 years of diagnosis, almost exclusively due to pulmonary metastases. The introduction of adjuvant chemotherapy has increased OS to almost 70% for patients with localized disease [70], although the cure rate for patients with metastatic disease remains unacceptably low. The first combination chemotherapy regimens consisted of methotrexate, doxorubicin, and cisplatin, and this combination remains the standard 40 years later. Neoadjuvant chemotherapy was introduced in the late 1970s to facilitate limb salvage surgery [71], and led to the recognition that histologic response to therapy was a strong prognostic feature. Patients with a good histologic response (>90% tumor necrosis) had 68% OS at 5 years, compared with 52% in poor responders [72]. Based on this finding, there have been several attempts to improve survival by augmenting chemotherapy for the poor responders. No such attempt has shown a statistically significant benefit [73]. The most recent COG study, AOST0331 (EURAMOS-1), randomized patients after determining histologic response to neoadjuvant chemotherapy. Poor responders were randomized to either have standard MAP (high-dose methotrexate, cisplatin, and doxorubicin) or MAP combined with ifosfamide and etoposide, and good responders were randomized to either standard MAP or MAP followed by maintenance -nterferon-α. The study closed to accrual in June 2011, and is expected to have primary end-point results around 2015. For now, the current standard therapy for osteosarcoma remains the same today as it was in 1980 – surgical resection and a three-drug regimen of methotrexate, doxorubicin, and cisplatin – and is the same for patients with localized or metastatic disease, despite the vast difference in prognosis between these groups.

Hematopoietic cell transplantation

HCT is not commonly used to treat osteosarcoma, despite the lack of effective therapy for relapsed patients and the poor prognosis of patients who present with metastases. In fact, the EBMT received reports of only seven patients transplanted for osteosarcoma as of 1992. Of the five patients with measurable disease, one died of toxicity, three had no response and one transplanted in refractory relapse achieved a PR that was improved to CR by surgical excision of lung metastases, and remained in second CR 18 months post-HCT. The two patients transplanted in second CR relapsed 8 and 11 months after HCT [74].

The Cooperative German–Austrian–Swiss Osteosarcoma Study Group retrospectively analyzed 15 patients who received HCT for recurrent osteosarcoma. These patients had all achieved a CR when initially treated. Their sites of relapse were lung in nine patients, lung plus local in two, mediastinal in two and local only in two. All underwent resection of their recurrent disease before HCT; only two had macroscopic residual disease, and neither responded to the high-dose preparative regimen. The preparative regimens all incorporated a combination of etoposide with melphalan or carboplatin, or both. Six patients received two courses of HCT, first with thioTEPA and cyclophosphamide and then with melphalan, but two of these patients died of toxicity. The 3-year OS was 29% and DFS was 20%. Both disease-free survivors received melphalan-based preparative regimens [75].

The Italian Sarcoma Group reported the results of a trial of surgery and tandem autologous HCT for patients with relapsed osteosarcoma. Thirty-two patients were treated. PBPCs were mobilized with cyclophosphamide and etoposide. Fourteen patients had surgery before chemotherapy and 11 had surgery at the completion of all therapy. Eleven of 32 patients were in CR before their first course of high-dose therapy, while 21 were treated with gross disease. At the end of treatment, 25 patients (78%) were in CR, six were in PD, and one had died of toxicity. Despite this excellent response rate, 3-year OS was only 20%, and 3-year DFS was only 12% [76].

These few studies suggest that there is little if any benefit to high-dose chemotherapy with HCT for relapsed osteosarcoma. In light of the lack of benefit to treatment intensification for patients with a poor response to neoadjuvant therapy, this suggests that future improvements in the treatment of patients with relapsed or refractory osteosarcoma will not come from treatment intensification, and that new approaches are required.

Retinoblastoma

Epidemiology and etiology

Retinoblastoma affects one child of every 18,000 live births in the United States in the first 5 years of life [77]. Approximately 25–30% of cases are bilateral, and follow an autosomal dominant inheritance pattern; in addition, approximately 10–15% of unilateral cases are hereditary. The observation that hereditary cases occur earlier in life and are bilateral or multifocal was key in the development of Knudson's two-hit model of carcinogenesis [39]. Knudson proposed that patients who inherit one mutation need only a single somatic mutation in a given cell for malignant transformation, while sporadic cases required the accumulation of two independent somatic mutations to transform a single cell.

Molecular and cellular biology

The postulated inactivation of a tumor suppressor gene was confirmed by the demonstration of loss of heterozygosity at 13q14 [78]. The cloning of the *RB1* tumor suppressor gene [79] was a pivotal step in understanding the pathogenesis of retinoblastoma and also a tumor suppressor gene involved in many other malignancies. The RB1 gene product is commonly absent in both sporadic and heritable retinoblastoma, as predicted by the two-hit model. Normally, RB1 mRNA is ubiquitously expressed, and encodes a nuclear protein with DNA binding sequences [80]. The protein is unphosphorylated in quiescent cells, but is phosphorylated in proliferating cells [79] and regulates the cell cycle in its unphosphorylated form by binding the E2F1 transcription factor [81]. In addition to loss of function of both alleles of the *RB1* gene, amplification of *MDMX* (65%) or *MDM2* (10%) occurs in the majority of human retinoblastomas, inactivating p53 and promoting the development of retinoblastoma [82].

Clinical description

Retinoblastoma typically presents as leukocoria when the tumor becomes visible through the pupil or causes retinal detachment or vitreous hemorrhage. Patients may lose central vision, leading to strabismus. Less commonly, heterochromia can result from neovascularization of the iris. In developing countries, the diagnosis may not be made until the eye becomes enlarged or the orbit is invaded, but extensive disease is uncommon in the United States. In patients with germline *RB1* mutations, new primary tumors may appear in the retinas for the first 3–5 years of life. In addition to bilateral retinoblastoma, these patients are at risk for primitive neuroectodermal tumors in the pineal and suprasellar regions, a presentation termed trilateral retinoblastoma.

Non-transplant approaches

Early detection of retinoblastoma is the norm, and the treatment outcome of patients with localized disease is excellent. Enucleation is curative for early solitary unilateral lesions, while adjuvant chemotherapy is indicated for patients with pathologic risk factors such as extension beyond the cribriform plate. Orbital extension is curable in 60–85% of patients with multimodal therapy, including external beam irradiation and systemic chemotherapy. In patients with bilateral disease, additional methods are used in an effort to preserve useful vision, including brachytherapy, cryotherapy, laser photocoagulation, and systemic and also subconjunctival or intra-arterial chemotherapy. In contrast, advanced central nervous system (CNS) involvement and "trilateral retinoblastoma" [83] are incurable: multimodal therapy including systemic and intrathecal chemotherapy and cranial irradiation has produced remissions, which, however, have not been durable [84]. Hematogenous dissemination to bone, marrow, and viscera and involvement of soft tissue and lymph nodes carry an extremely poor prognosis. Only ifosfamide and CY have efficacy as single agents and have produced CRs when used in two- and three-drug combinations [85].

HCT as consolidation for disseminated disease

There are several individual case reports and small case series [86–89] of HCT for patients in second CR after metastatic recurrence in bone or marrow. These reports describe a total of 25 patients, of whom only five experienced further progression of their disease. The remaining patients had no evidence of disease 18–107 months after relapse.

The SFOP formally evaluated HCT for patients with high-risk retinoblastoma, using a single-arm protocol active from 1989 to 1994 [90]. The target population was patients with extraocular disease or histologic evidence of tumor at the cut end of the optic nerve or its subarachnoid space. Measurable extraocular disease had to be chemosensitive in order for patients to be eligible to proceed to HCT. During the study period, 34 high-risk patients were identified: eight with microscopic residual optic nerve involvement, 10 with extraocular disease confined to the orbit, 11 with extraocular involvement including distant bones or marrow, and five with CNS involvement. Nine patients did not proceed to HCT because of CNS progression [6], parental refusal [2], or toxicity [1]. Therefore, 25 patients (six with optic nerve, seven with orbital disease, eight with distant bone or marrow disease, and four with CNS involvement) proceeded to HCT with a preparatory regimen of carboplatin 1250–1750 mg/m^2, etoposide 1750 mg/m^2, and cyclophosphamide 3200 mg/m^2. Five of six patients who had measurable disease at the time of HCT achieved CR. On an intention-to-treat basis, five of eight patients with optic nerve involvement, six of 10 patients with orbital involvement, five of 11 patients with bone or marrow involvement, and one of five patients with CNS involvement survived with no evidence of disease. Compared with historical results using much longer courses of conventional chemotherapy, this approach produced comparable survival for patients with optic nerve or orbital involvement, and was superior for patients with distant bone or marrow involvement [90]. More recently, the group from the Memorial Sloan Kettering Cancer Center reported their results intensifying conventional therapy with high-dose chemotherapy and stem-cell support for patients with stage 4a and 4b metastatic retinoblastoma [91,92]. Fifteen consecutive patients who presented with stage 4a retinoblastoma were treated on a protocol that included an alkylator-based induction chemotherapy regimen followed by high-dose chemotherapy with autologous stem-cell support. Of the 13 patients who completed this therapy, 10 remained disease free with a median follow-up of 103 months. For stage 4b retinoblastoma patients, a retrospective review identified eight patients from six centers presenting between October 2000 and January 2006. Of the five patients who were able to receive autologous HCT, two were long-term survivors at 40 and 101 months.

One potential benefit to this approach for advanced-stage disease is the possibility of avoiding bilateral enucleation and external beam radiation therapy, which carry a significant risk for secondary malignancies and also facial deformities. Lee et al. reported a regimen utilizing tandem autologous HCT for bilateral retinoblastoma without external beam radiation therapy. Of 14 patients who presented with a bilateral retinoblastoma, nine underwent tandem autologous HCT. The regimen was well tolerated and all nine patients survived with at least one eye after treatment [93].

Based on these promising data in a condition with otherwise dismal prognosis, the current COG study, ARET0321, aims to determine the efficacy of autologous HCT in a multicenter collaborative study. Patients with metastatic disease without CNS involvement (stage 4a) and metastatic disease with CNS involvement (trilateral or stage 4b) will receive high-dose chemotherapy consolidation with carboplatin, thioTEPA, and etoposide if there is more than partial response after an induction regimen including cisplatin, vincristine, etoposide and cyclophosphamide. This will be followed by an autologous stem-cell infusion, then response-adapted radiation therapy.

Desmoplastic small round cell tumor

Epidemiology and etiology

Desmoplastic small round cell tumor (DSRCT) is a highly malignant abdominal small round blue cell tumor with epithelial, mesenchymal, and neural characteristics, initially described in 1991 [94]. It occurs in children and young adults and only 101 cases were reported between 1989 and 1996. There is a strong male predominance and a median age at diagnosis of 25 years. There are no known associations with exposure to carcinogens [95].

Molecular and cellular biology

A balanced translocation, t(11;22)(p13;q12), results in *EWS–WT1* fusion transcripts considered diagnostic of DSRCT. As with the *EWS–FLI-1* fusion transcripts of Ewing sarcoma, these fusion transcripts retain the amino-terminal effector domain of EWS and replace its DNA binding domain with that of its fusion partner. The fusion transcripts are heterogeneous both within and between tumors with respect to the use of specific EWS and WT1 exons, the presence of internal deletions, and insertion of heterologous DNA. However, the splice variants are in-frame and the chimeric proteins bind *WT1* response elements [96].

The transcriptional regulatory activity of EWS–WT1 differs from that of WT1. The platelet-derived growth factor A chain (PDGFA) is directly induced by EWS–WT1, and may contribute the stromal content of DSRCT [97]. In addition, expression of insulin-like growth factor-1 receptor (IGF-1-R), which has mitotic and antiapoptotic functions, is normally repressed by WT1, but is activated by EWS–WT1 chimeras [98]. The interleukin-2/15 receptor beta chain promotes cell growth by downstream activation of STAT3 and STAT5 [99]. The brain-specific angiogenesis inhibitor 1-associated protein 3 (BAIAP3) when expressed ectopically in tumor cells enhances anchorage-independent and low serum growth and may assist the secretion of FGF-1 and -2 to produce the desmoplastic reaction characteristic of the tumor [100]. The leucine-rich repeat containing 15 (*LRRC15*) gene normally expressed only in placenta in the trophoblast cell layer responsible for implantation of the embryo, is hypothesized to contribute to the invasiveness of DSRCT [101].

Clinical description

Patients typically present with abdominal distension and pain, with constipation a common associated finding [102]. DSRCTs most commonly grow in the peritoneal cavity, with large masses and multiple implants along serosal surfaces, and may metastasize to lymph nodes, liver, lung, bone, spleen, kidney, and pleura. Characteristic computed tomography (CT) findings are of bulky intra-abdominal soft tissue masses without a distinct organ of origin [103]. Unusual sites of primary involvement include kidney, lung, bone, and pancreas. Exploratory laparotomy reveals large, firm, multinodular tumors with smooth surfaces. Conventional microscopy reveals sharply demarcated clusters of small round tumor cells embedded in a dense desmoplastic stroma. Immunohistochemical staining reveals expression of epithelial (cytokeratin, epithelial membrane antigen), mesenchymal (vimentin), myogenic (desmin), and neural (neuron-specific enolase, CD56) markers [104,105]. WT1 immunoreactivity results from expression of the EWS–WT1 fusion transcript. CD99 (MIC2), commonly used to identify Ewing family tumors, is also commonly positive [106].

Non-transplant approaches

This tumor is often initially responsive to chemotherapy such as combinations of doxorubicin, cisplatin, CY, etoposide, and fluorouracil. However, the outcome has been dismal, with median survival of only 17 months (range 3–72 months); as of 1996, only 7/101 reported patients were alive a median of 24 months (range 4–48 months) from diagnosis [107]. Several more extensive series have been reported from single institutions [95,102,108,109]. Cumulatively, these series suggest that complete resection of desmoplastic small cell tumors contributes to long-term survival, but these tumors are generally not resectable at initial presentation; they are usually initially chemosensitive, but the short duration of responses warrants attempts at consolidation with intensified therapy.

Hematopoietic cell transplantation

There are a few reported cases of HCT as salvage therapy for recurrent or refractory desmoplastic small cell tumor, but none resulted in long-term survival [107,110,111]. In the Memorial Sloan Kettering Cancer Center series, the intention was to consolidate first responses of patients with initially unresectable disease with HCT, but two of five patients did not proceed to HCT because of the toxicity of the initial chemotherapy. Three patients with initially unresectable disease received HCT with thioTEPA and carboplatin in first response, two in CR, and one in PR. The patient transplanted in PR progressed at 15 months. The patients transplanted in CR were alive without evidence of disease 13 and 34 months from initiation of therapy [108]. The St. Jude DSRCT series included four patients who underwent autologous HCT. Two patients who received HCT after radiation therapy died of toxicity. Of the two other patients, the one transplanted in CR and treated with radiation after HCT remained alive without disease 16 months after completion of therapy. In an adult population, entered on a protocol with intent to transplant in Milan, of 10 patients with DSRCT, only five achieved a PR to induction therapy with ifosfamide, vincristine, and etoposide and proceeded to HCT with a melphalan-based preparative regimen. None of the patients were converted to CR or cleared their EWS–WT1 fusion transcripts after high-dose melphalan, and all progressed within 12 months [112]. One of these patients subsequently received an allogeneic HCT after reduced-intensity conditioning, and cleared his fusion transcript after developing grade III acute graft-versus-host disease (GVHD) [112], suggesting the presence of a graft-versus-DSRCT effect. Success has been achieved in small series exploring more intensive alkylator-based approaches. All four patients with DSRCT treated in first partial ($n = 2$) or complete ($n = 2$) response with busulfan, melphalan, and thioTEPA, with or without amifostine remained alive after 1.1–6.4 years (median 3.2 years) of follow-up, of whom only one had progressive disease [113]. Three patients with DSRCT treated with VACIME with repeated PBPC support became long-term survivors with no evidence of disease 25–66 months from diagnosis [114]. In the European RMS4.99 protocol, patients were treated with autologous HCT after initial surgical staging and induction chemotherapy. Seven out of 10 patients showed a partial response to HCT and three achieved a CR. Only three of these 10 were reported as alive, including one patient who was only 7 months since discharge [115]. More recently, the Center for International Blood and Marrow Transplant Research (CIBMTR) reported their experience with high-dose chemotherapy with autologous stem-cell support for DSRCT. A total of 36 patients underwent high-dose chemotherapy with autologous stem-cell support between 1999 and 2007. Treatment-related mortality (TRM) was low with one death in the first 100 days, while the DFS for patients who were in CR pre-transplant was 92% at 1 year and 75% at 3 years, compared with 35% at 1 year and 9% at 3 years for patients who were not in CR. The median OS for patients who were not in CR was 21 months, which is slightly longer then historic controls, suggesting some benefit to autologous HCT [116]. In summary, the role of HCT remains to be defined for patients with DSRCT, but the few long-term survivors have been patients who have obtained complete surgical resections and autologous HCT may contribute to survival in both patients who have achieved CR and those who did not achieve CR after conventional chemotherapy.

HCT for other rare pediatric solid tumors

Pleuropulmonary blastoma is a rare tumor, approximately 100 cases of which have been reported. The primary therapy is surgical excision, without adjuvant therapy. Because of the histologic resemblance to soft tissue sarcoma and Wilms tumor, chemotherapy regimens designed for these entities have been used. Such combination chemotherapy has produced 30–40% DFS [117]. There are very few reports of the use of HCT in patients with pleuropulmonary blastoma. Melphalan-based HCT was used for progressive disease [118], one for chemotherapy-sensitive microscopic residual disease [117] for consolidation after complete surgical resection in first relapse [119], and one for refractory metastatic disease [120]. The patients with bulky refractory disease progressed soon after HCT, but the patients with microscopic residual disease and complete surgical resection were alive in continuous CR at 12 and 31 months respectively after HCT.

Esthesioneuroblastoma is a rare tumor, arising from olfactory epithelium; of over 240 cases in the literature, only 21% are pediatric. The behavior of this tumor may be more aggressive in childhood. It has been suggested that esthesioneuroblastoma may be a member of the Ewing sarcoma family on the basis of a t(11;22) in 2/3 cell lines derived from metastatic esthesioneuroblastoma cases. However, unlike Ewing tumors, esthesioneuroblastomas do not express MIC-2, and primary esthesioneuroblastomas are characterized by trisomy 8, not t(11;22) [121]. In a single-institution series, long-term survival was achieved in 3/5 adult patients salvaged with CY-containing HCT preparatory regimens versus 4/17 salvaged with conventional chemotherapy [122]. One adolescent was treated for a cervical nodal recurrence with modified radical neck dissection, and consolidation with high-dose carboplatin, etoposide, melphalan, and HCT, and had no evidence of disease 1 year later [123]. In a case series from Japan, 12 patients with esthesioneuroblastoma were diagnosed between 2000 and 2003, including four who received HCT following induction chemotherapy, who were long-term survivors [124]. Although this was an uncontrolled study that also included patients who were transplanted after surgery, it is a promising lead for patients with relapsed and recurrent esthesioneuroblastoma.

Future directions

Patterns of failure

After HCT for Ewing tumors, the majority of relapses are metastatic, most commonly in lung and secondly in bone [21,125]. A more recent analysis indicated that bony failures often occur in involved bones outside the local radiation field, and in lesions detected by magnetic resonance imaging (MRI) or positron emission tomography (PET) scan, but not bone scan [126]. Possible solutions to this problem include irradiating the lungs and the entire involved bone system prophylactically and incorporating the use of MRI and/or PET scans in staging before HCT.

In contrast, failures after HCT for rhabdomyosarcoma tend to occur early, at a median of 4 months after HCT, almost always (23/26 cases) in previously known sites [33]. This observation argues for the use of local radiation to all previously known sites of disease.

Regimens

Role of total body irradiation

For all but the most radiosensitive tumors, the dose of radiation therapy that can be delivered as TBI is inadequate for the control of bulky tumors. Doses of adjuvant radiation demonstrated to control the majority of microscopic residual disease (typically 10^6–10^8 cells) exceed the maximum tolerable doses of TBI [127]. However, the relationship between the dose of radiation and the percentage reduction in risk of recurrence appears to be linear. Extrapolating from these dose–risk data, zero reduction in risk occurs at a dose between 0 and 5 Gy, indicating that there is a low threshold, if any, for tumor cell killing by radiation [128]. Therefore, the modest doses of radiation feasible as TBI may be expected to control very small micrometastases (perhaps 10^2–10^4 cells).

The question of whether TBI is a necessary or desirable component of the high-dose cytoreductive regimen has been addressed retrospectively by the EBMT. For rhabdomyosarcoma patients, addition of TBI to melphalan appears only to increase toxicity [32]. A retrospective analysis of the German–Austrian Pediatric BMT Group also found no benefit of 12 Gy hyperfractionated TBI added to melphalan, etoposide, and carboplatin [33]. For Ewing sarcomas, the EBMTR observed that patients receiving TBI fared worse ($n = 30$, 19% EFS) than those receiving high-dose chemotherapy alone ($n = 33$, 34% EFS); the best results were obtained with chemotherapy combinations that included busulfan (51% EFS) [129].

Tandem transplants

A substantial number of tandem transplants, in which two or more HCTs are performed with minimal recovery time between transplants, have already been performed in an attempt to improve tumor control. This approach is most likely to be effective if there is more than one active and non-cross-resistant cytoreductive regimen, without cumulative toxicity. Even if a single regimen must be used twice, if a similar log cell kill may be obtained in a second course, repeated application may overcome problems of delivery of drug to the core of a bulky tumor. Whether tandem HCTs as delivered so far have been beneficial is controversial. The results of several institutional series indicate longer responses in patients receiving multiple courses of high-dose therapy [130,131]. However, the cumulative experience of the EBMT provides no convincing evidence in favor of double transplants in Ewing sarcoma [129]. In addition, at least with carboplatin and etoposide, no patient improved their response with a second course [132].

The use of PBPCs instead of bone marrow may improve the efficacy of tandem transplants because faster hematopoietic recovery may allow greater treatment intensity. Two courses of melphalan 100 mg/m^2 could consistently be administered with PBPC rescue within 21–34 days (median 24 days) with no change in pharmacokinetics or pharmacodynamics [133]. A limited-institution pilot study demonstrated the feasibility of tandem PBPC transplant for metastatic neuroblastoma or sarcoma, delivering CY 3600 mg/m^2, etoposide 2400 mg/m^2, and carboplatin 2000 mg/m^2, followed within 28–42 days by melphalan 180 mg/m^2 and TBI 12 Gy in 46 of 51 eligible patients [134,135]. Of the five patients who received only one HCT, three were by patient request and two were ineligible to proceed because of liver toxicity. There were four toxic deaths (9%). Three courses of HCT have been administered to 17 of 22 patients, with one toxic death in the acute transplant period [136]. Although short-term toxicity may be acceptable, this pilot trial shows the importance of long-term follow-up, because three patients developed pancytopenia as a late complication, two with graft failure and one with monosomy 7-associated myelodysplastic syndrome.

A novel variation is the use of double high-dose therapy with only one HCT procedure [137]. Because the dose-limiting toxicity of melphalan and thioTEPA in the setting of HCT is mucosal, and the affected tissues recover relatively quickly, two cycles can be given 1 week apart, and patients rescued with a single PBPC product after the second cycle. This strategy allows the safe delivery of thioTEPA 1000 mg/m^2 and melphalan 280 mg/m^2 over 9 days. The indications for HCT were heterogeneous in this phase I/II trial, but

10/13 patients entering HCT in CR and 6/13 patients entering HCT with residual disease were alive with no evidence of disease 15–59 months (median 35 months(after transplantation. Of particular interest is that all three patients with hepatoblastoma who entered HCT with persistent α-fetoprotein elevation achieved long-term CR [137].

Targeted therapy

Targeted therapeutics refers to treatments designed to exploit a biologic feature of the tumor to eradicate it. Examples of targeted therapies include tyrosine kinase inhibitors such as imatinib mesylate and ligand-targeted monoclonal antibodies such as rituximab. There are two significant ongoing applications of targeted therapy for pediatric solid tumors that involve HCT: [^{131}I]-*m*-iodobenzylguanidine (MIBG) for the treatment of neuroblastoma (see Chapter 63) and [^{153}Sm]ethylenediaminetetramethylenephosphonic acid (EDTMP) for the treatment of osteosarcoma. Each of these treatments utilizes a fundamental characteristic of the tumor to direct a radiopharmaceutical agent specifically to the tumor, and ameliorates the myelosuppression with autologous hematopoietic cell support.

[^{153}Sm]EDTMP consists of a radioisotope (^{153}Sm) conjugated to a tetraphosphonate compound that localizes to sites of bone turnover, such as a metastatic bone lesion. Because [^{153}Sm]EDTMP targets bone lesions by a mechanism similar to that of the radiotracer used in a bone scan, lesions that are visible on bone scan, including both primary osteosarcoma and metastatic deposits, are targeted by this agent. Radioactive decay of the ^{153}Sm yields a medium=-energy electron (β particle) and a low-energy photon. The electron has a very short pathlength (1–2 mm), thus providing exquisitely precise targeting of the cytotoxic energy. The photon can be detected using the same instrument as used for a diagnostic bone scan, allowing uptake at a target tumor to be confirmed and the amount of radiation delivered to the tumor to be measured. The half-life of ^{153}Sm is very short (46 hours), facilitating handling. The compound was originally tested in adult patients with bone metastases from carcinomas, such as breast and prostate cancer, and is approved by the US Food and Drug Administration (FDA) for palliation of painful metastases. In 2002, Anderson et al. published the results of a phase I study of [^{153}Sm]EDTMP for the treatment of high-risk osteosarcoma patients [138]. Thirty patients were treated with 1–30 mCi/kg of [^{153}Sm]EDTMP. Because this compound significantly depresses bone marrow function, patients were rescued with an infusion of autologous PBPCs 14 days after treatment. Other than low blood counts, the only side effect was transient hypocalcemia in patients treated with the highest dose. All of the patients began the trial requiring narcotics for pain relief, and every patient experienced a decrease or elimination of their narcotic requirement. In an attempt to improve efficacy, this group subsequently treated patients with gemcitabine, a chemotherapeutic agent that is thought also to act as a radiation sensitizer [139]. Fourteen patients were treated with high-dose [^{153}Sm]EDTMP followed 1 day later by gemcitabine. After 2 weeks, autologous PBPCs were reinfused. At 6–8 weeks of follow-up, there were six partial remissions and two mixed responses, but none of these responses were durable. In another study, patients with recurrent or refractory osteosarcoma were treated with tandem doses of [^{153}Sm]EDTMP. The first dose was low, allowing count recovery within 6 weeks, and the second was higher and required peripheral blood stem-cell support. Although none of the patients had better than disease stabilization by RECIST 1.1 criteria, there was a dose–response relationship between tumor-absorbed radiation dose and change in tumor volume, suggesting that this approach can deliver cytotoxic, targeted radiation safely, and that finding a way to increase dose might improve treatment response [140]. Hence [^{153}Sm]EDTMP shows promise for treating osteosarcoma, but further work is required to determine its optimal use.

Hematopoietic cell grafts

The source of the graft: peripheral blood hematopoietic cells versus bone marrow

It is now recognized that mobilizing and collecting PBPCs is feasible in children despite their small size and the intensity of the pediatric chemotherapy regimens, and PBPCs produce faster engraftment [141] and immune reconstitution [142] than bone marrow. Although tumor cells can frequently be detected in mobilized PBPC grafts [143,144], the question of whether the purging of autologous hematopoietic cells can improve outcome remains to be answered. Few data are available regarding the relationship between tumor contamination of the graft and survival outcomes, but long-term DFS can occur in patients with Ewing sarcoma who receive PBPC grafts containing detectable fusion transcript [145]. In rhabdomyosarcoma, the major determinant of long-term survival after HCT was whether CR was achieved prior to HCT [32], and 23 relapses occurred in previously known sites, compared with only three in new metastatic sites [33], suggesting that tumor control in the body, not reinfusion of tumor cells in the graft, is the major problem.

Allogeneic HCT

The role of allogeneic HCT in pediatric solid tumors is being explored. Allogeneic transplantation carries the risk of GVHD, but its potential benefits include the absence of tumor cells in the graft and perhaps an immunologic antitumor effect. For rhabdomyosarcoma, the German–Austrian Pediatric BMT Group found no evidence of benefit from allogeneic HCT, as none of five allogeneic HCT recipients survived [33]. In addition, donor lymphocyte infusion was reported to be ineffective in a case of relapsed rhabdomyosarcoma [146], but since the patient did not have measurable disease at the time of donor lymphocyte infusion and did not develop GVHD, this case did not address the activity of an effective immune response. A CR after reduced-intensity matched-sibling peripheral blood HCT for a patient who had failed two prior lines of therapy including autologous HCT suggests benefit from an allogeneic effect [147]. For Ewing tumors, there was a trend towards better outcome with allogeneic HCT (50% survival versus 23% survival of the entire group of 27 allografted patients at a median of 56 months) in the single-institution experience with (TB)I/ME(C) of Vienna, from 1984 to 1996 [148]. With longer follow-up, incorporating the experience from Düsseldorf, DFS after allogeneic HCT was not better than after autologous HCT for Ewing tumors because of higher rates of non-relapse mortality [149]. In the multicenter study published in 2011 from the EBMT, APBMT, PRST, and MetaEICESS, outcomes of 87 patients who received an allogeneic HCT for Ewing sarcoma were reported. Interestingly, there was no difference in OS between reduced-intensity ($n = 50$) and high-intensity ($n = 37$) conditioning. Although the reduced-intensity group had a higher risk of relapse, the high-intensity group had a higher risk of fatal complications, leading to comparable OS. Although this result suggests that there is no clinically relevant graft-versus-tumor effect in Ewing sarcoma, there are informative case reports of patients with recurrent, metastatic Ewing sarcoma who underwent allogeneic HCT and whose disease responded to withdrawal of immune suppression [147] or institution of interleukin-2 (IL-2) therapy [150] with subsequent development of acute GVHD.

Because sarcoma patients coming to transplant are always heavily pretreated, TRM is a substantial concern. This prompted the investigation of reduced-intensity conditioning regimens for these patients. The Pediatric Oncology Branch at NIH recently reported results of 23 patients with ultra-high-risk sarcomas who underwent reduced-intensity allogeneic HCT. All patients engrafted promptly with no peri-transplantation mortality. Three of the seven patients transplanted without overt disease (42%) remained alive, with a median survival of 29.0 months, compared with a median survival of 14.5 months for those transplanted with radiographically evident disease [151]. This approach was feasible in this very high-risk patient population, and may provide a survival benefit for those who go to transplant in remission.

Overall, few data are available regarding allogeneic HCT for pediatric solid tumors. This probably results largely from the role of neuroblastoma as the prototypical poor prognosis pediatric solid tumor and the clear consensus that there is no role for allogeneic transplantation in neuroblastoma [152,153]. The lack of benefit from allogeneic HCT indicates that tumor contamination of autologous grafts is not a major source of relapse, and that alloreactive cytotoxic T-lymphocytes (CTLs) are not substantially effective against high-risk pediatric solid tumors. However, a potentially important biologic difference between neuroblastoma and sarcomas is that in vitro, neuroblastoma cells are poor targets for CTLs, whereas sarcoma cells are excellent CTL targets [154]. Hence the role of HCT for ESFT and other sarcomas remains an unanswered question worthy of further study.

Immunotherapy

Rhabdomyosarcomas and Ewing tumors are targets both for natural killer (NK) cells and for CTLs [155], and tumor-infiltrating lymphocytes isolated from osteosarcomas can lyse allogeneic osteosarcoma cell lines [156]. The possibility that these immunologic effector mechanisms may be clinically important in high-risk Ewing tumors was suggested in the pilot trial of hyperfractionated TBI with melphalan and etoposide conducted in Vienna and Düsseldorf. In their initial cohort of 17 patients with very high-risk Ewing tumors, 3/4 allograft recipients were disease-free survivors, along with 3/5 autograft recipients treated with IL-2 versus 2/8 autograft patients without IL-2 [157]. The advantage of IL-2 after autografting persisted in a larger EICESS cohort treated using the same approach: DFS was 52% for patients receiving IL-2 after HCT versus 22% without IL-2 ($p < 0.05$) [126]. The possible effector mechanisms involved in patients receiving IL-2 after HCT have been explored, and expansion of T and NK cell numbers, but not NK/T cells, was demonstrated. NK activity was enhanced, in spite of increased activation of the CD94 inhibitor receptor [158].

The National Cancer Institute has conducted a pilot trial of tumor-specific vaccination for Ewing sarcoma and alveolar rhabdomyosarcoma, based on pulsing dendritic cells with the unique EWS/Fli-1 and PAX3/FKHR fusion peptides associated with the t(11;22) and t(2;13) translocations characteristic of these tumors [159]. Sixteen patients were enrolled, and 15 received at least one vaccination. Unfortunately, most patients experienced tumor progression during the first 6-week vaccination cycle, so only four patients received two or more cycles of vaccination. One of these four patients had a mixed response, and another had a fusion peptide-specific lymphocyte proliferative response. This study is important not only because of the encouraging suggestion of some immunologic activity against tumor, but also because it identified several pitfalls of immunotherapy. These patients had severely impaired immunity and large tumor burdens, and the immature circulating dendritic cells used may actually suppress immune responses [159]. Moreover, a single peptide antigen was used, with uncertain human leukocyte antigen (HLA) restriction and cell-surface expression, and the tumor itself may have induced tolerance or anergy in autologous lymphocytes.

An alternative strategy of generating antitumor CTL cell lines ex vivo has been explored in Ewing sarcoma patients. Specific antitumor CTLs were generated from three patients, all of whom had undergone prior autologous HCT, after two or more cycles of stimulation of autologous PBMCs with autologous peripheral blood-derived dendritic cells, $CD4^+$ lymphocytes, and irradiated early passage autologous tumor cells [160]. Although the approach is labor intensive, potential advantages include the response to both unknown and previously identified tumor antigens and the reduced potential for escape by loss of antigen expression. The clinical utility of such cell lines has yet to be demonstrated.

Another strategy in maximizing the antitumor effect after allogeneic transplant has been to manipulate NK cells. NK cell alloreactivity depends on inhibitory killer immunoglobulin-like receptor (KIR) mismatch [161]. In a pilot study, three patients with refractory solid tumors received a haploidentical HCT, and two patients who were known to have a KIR mismatch with the donor demonstrated tumor response (although one eventually progressed and died of disease) [162]. Multiple trials are currently being conducted with various methods to maximize this antitumor effect of NK cells, such as IL-15 activation and KIR mismatch. The development of reduced-intensity allogeneic HCT offers an alternative avenue to immunotherapy for pediatric solid tumors, but novel approaches will be required to demonstrate activity if this approach is beneficial only in minimal residual disease.

Conclusion

The prognosis of patients with metastatic, recurrent, or refractory pediatric solid tumors is dismal. High-dose preparatory regimens with HCT have demonstrated activity against a broad spectrum of the pediatric solid tumors. HCT has produced promising results in the treatment of Ewing tumors. Prospective randomized trials of HCT versus conventional chemotherapy are in progress for patients with poor-prognosis Ewing tumors and are being considered for high-risk recurrent Wilms tumor. For less common indications, phase I and II trials are appropriate. Approaches now being investigated to improve the efficacy of HCT for pediatric solid tumors include tandem HCT, targeted therapy, chemoprotection, and targeted immunotherapy, possibly via allogeneic HCT.

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CHAPTER 65

Hematopoietic Cell Therapy for Human Immunodeficiency Virus Infection

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Introduction

In the two decades since Holland and Saral first suggested a potential role for HCT in eliminating the HIV reservoir, [1], the field has evolved to a point at which hematopoietic stem/progenitor cell (HSPC) transplantation (HSCT) has a credible potential in treating patients with human immunodeficiency virus type 1 (HIV-1) infection. The predominant place for the use of HSCT in the setting of HIV-1 infection is, of course, in the treatment of patients with acquired immune deficiency syndrome (AIDS) who have hematologic malignancies. In this regard, the standard of care now provides autologous HSCT as successful salvage treatment in AIDS patients with high-risk or refractory lymphoma. More importantly, however, allogeneic HSCT for leukemia has established this approach as a potentially curative treatment of AIDS [2]. The future may show a role for HSCT even in the management of the HIV-1-infected patient without malignancy.

The single case report of allogeneic transplantation of HSPCs, using a matched unrelated donor with genetic resistance to HIV-1, resurrected the question of whether there is a curative role of allogeneic HSCT for HIV/AIDS [2]. Even without intrinsic cellular resistance of donor cells, it has recently been suggested that the allogeneic effect following HSCT could alter the HIV-1 reservoir in ways that allow combination antiretroviral therapy (cART) to control infection effectively [3]. There will undoubtedly be more interest in the use of HSCT in HIV/AIDS, hence it is increasingly important to understand how to manage the HIV-1-infected patient during this procedure. At the same time, the potential for genetic modification of autologous HSPCs to reproduce HIV-1 resistance of the immune system continues to develop as another stem-cell-based strategy [4]. This chapter highlights the current understanding of the pathogenesis of AIDS and the rationale for gene therapy of HIV/AIDS, and presents a review of recent studies of both autologous and allogeneic stem-cell transplantation in HIV/AIDS patients. Guidelines for the management of the HIV-1-infected patient during HSCT are presented, recognizing that these stem-cell-based therapies for AIDS patients provide new information leading to better control of HIV-1 infection.

The pathogenesis of HIV-1 infection and AIDS

HIV-1 infection

HIV-1 infection is associated with three phases: primary infection, a chronic phase, and a terminal phase of AIDS [5]. Following primary infection, which lasts 3–6 weeks, there is an immune response to the virus and establishment of a relatively stable level of HIV-1 RNA in plasma and in fixed tissue-based reservoir. The chronic phase of infection can last 10 years or more, but eventually the immune system fails, the virus develops a tropism for T cells, and a terminal phase in which there is more rapid loss of CD4$^+$ lymphocytes, more profound immunodeficiency, and the development of infections or malignancies that define AIDS.

The initial HIV-1 infection follows contact with infectious body fluids and gains entry into susceptible cells following interaction of the viral envelope proteins and a primary cellular receptor, CD4 (reviewed by Shaw and Hunter [6]). Of hematopoietic interest, chemokine receptors, primarily CXCR4 and CCR5, are coreceptors required for viral entry [7,8]. These chemokines are from a diverse family of peptides that bind to receptors and activate immune and/or inflammatory responses [9]. The C–C chemokine receptor, CCR5, is the major coreceptor for macrophage-tropic (R5) HIV-1, and a C–X–C chemokine receptor, CXCR4, is used for entry of T-tropic (X4) strains of HIV-1 [6]. Other chemokine coreceptors can fulfill this coreceptor function in vitro, but CCR5 and CXCR4 are most critically important in vivo.

The actual entry of virus into a cell follows the formation of a heterotrimer complex, consisting of the HIV-1 envelope, the CD4 receptor, and the chemokine coreceptor, that forces an alteration of the membrane that exposes a peptide domain contained within the HIV-1 fusion protein (gp41) [10]. The engagement of the gp41 fusion peptide with the target cell membrane culminates in penetration of HIV-1 into the cell with subsequent movement into the nucleus. Infection can then be influenced by natural cell factors, many of which are only beginning to be understood [11]. For example, the native ligands for CCR5, such as RANTES and MIP-1α, and stromal-derived factor 1 for CXCR4, can compete with HIV-1 for binding to these virus coreceptors and inhibit HIV infection [12,13]. A meta-analysis of patient cohorts has shown that only the CCR5-Δ32 and the CCR2-64I mutations are associated with strong protective effects from progression of HIV-1 infection [14]. In addition, there are cellular proteins that act as restriction factors to inhibit HIV-1 replication and alter pathogenesis, such as APOBEC3G, TRIM5α, tetherin, SAMHD1, and others [15]. Undoubtedly, additional targets for future

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treatment of HIV-1 will derive from the genome-wide analyses that uncover new cellular inhibitors of HIV-1 [11].

HIV-1-infected mucosal tissues are areas rich in CCR5$^+$ dendritic cells (DCs) and T lymphocytes (T cells). HIV-1 can cross the mucosal barrier by various mechanisms, including epithelial barrier breach, transcytosis, and transport in infected DCs [5], and, once across the mucosa, the virus infects CCR5$^+$ DCs, which propagate the infection locally to T cells and to draining lymph nodes [16]. DCs of the myeloid lineage are infected predominantly with R5 strains of HIV-1, although infection with dual-tropic isolates of HIV-1, exhibiting the capacity to use CCR-5 and/or CXCR-4 for entry, can infect DCs. Expression of the coreceptors is regulated by a complex cytokine network that influences HIV-1 replication in these cells [17]. Therefore, factors that stimulate cytokine release, such as co-infection with other microbes and microbial antigen translocation from the gut [18], can influence the homeostatic activation or inhibition of DCs and serve as important factors that will affect HIV-1 pathogenesis to AIDS.

As shown in primate models and later confirmed in humans [5,19], the primary infection with HIV-1 results in a massive reduction in CD4 cells in the gut-associated lymphoid tissue (GALT). When subsets of CD4$^+$ and CD8$^+$ T cells are studied after primary HIV-1 infection, the major loss of lymphoid elements is in the CCR5$^+$CD4$^+$ T cells [19]. Moreover, this depletion is specific to CD4$^+$ T cells having activated CD69$^+$CD38$^+$HLA-DR$^+$ memory phenotype, and a likely explanation for selective memory T-cell loss with functional disturbance observed in HIV-1-infected humans [5,20].

This preferential depletion of mucosal CD4$^+$ lymphocytes in the GALT, relative to the loss of peripheral blood CD4 cells, remains even after cART treatment brings the HIV-1 viremia under control with near-complete immune recovery of the peripheral blood CD4$^+$ cell population [5]. During the chronic phase of HIV-1 infection, continuing immune activation induces apoptotic processes that lead to the bystander effect producing continued loss of uninfected CD4$^+$ T cells [21]. This further supports a process that maintains the infected cell reservoir [18]. Hence the continued activation of the immune system is thought to drive the persistent loss of CD4$^+$ lymphocytes and is a critical component of disease pathogenesis [22].

The HIV-1 reservoir

The observation that HIV-1 can be isolated from the blood of patients during cART therapy when the HIV-1 RNA levels are below the level of clinical detection suggested a site from which infection could always be recovered [23,24]. The tissue repository of HIV-1-infected cells from which this active replicating HIV-1 is derived is considered the HIV-1 reservoir [25,26]. These cells form a pool from which HIV-1 replication can be rekindled following drug withdrawal or HIV-1 activation. In cases where anti-HIV-1 chemotherapy has been suspended during analytical treatment interruption, a rebound of replicating virus occurs within a few weeks in most patients studied [27]. This rapid reappearance of virus indicates that HIV-1 eradication has not occurred and implies that continuous combination therapy must be maintained at all times, possibly for the life of the patient, in order to maintain control of HIV-1.

The latently infected, resting CD4$^+$ memory lymphocyte, carrying integrated HIV-1 DNA is the primary component of this reservoir [28]. In patients with undetectable infection [<50 genome copies (gc)/mL] on cART, a fraction of the CD4$^+$ cells, usually 0.1–10 infectious units per million (IUPM) cells, contain integrated HIV-1 DNA from which virus can be isolated upon cell activation using a virus outgrowth assay that is the gold standard for measuring the HIV-1 reservoir [25]. Assays for HIV-1 proviral DNA from these cells, including integrated and unintegrated HIV-1 DNA, serve as surrogate measures of the reservoir [29]. Strategies for curing HIV/AIDS target this reservoir and approaches consisting of cytoreductive chemotherapy, immunotherapy, and cell-based treatments are being considered [4,30].

Allogeneic transplantation in HIV/AIDS patients

Chief concerns regarding allogeneic HSCT in the HIV-1-infected patient derive from the need for chronic immunosuppression for graft-versus-host disease (GVHD) prophylaxis in an already immunodeficient patient and the use of cART in a patient receiving such prophylactic therapy. Solid organ transplantation (SOT) laid to rest some of the concerns about graft rejection and demonstrated the feasibility of maintaining HIV-1 infected patients on chronic immunosuppression [31]. The underlying HIV-1 infection is usually not worsened during SOT if patients remain on cART. Recently, with the observations of apparent modification of the HIV-1 reservoir after matched unrelated HSCT, the question has been raised as to whether GVHD might even have a therapeutic affect on the HIV-1 reservoir, and this question is currently under intensive investigation [3].

In the pre-cART era, allogeneic HSCT was resoundingly unsuccessful in an HIV-1-infected recipient, in large part due to procedure-related mortality from infection. Yet a review of multiple case reports reported in the past 25 years indicates that at least 56 patients with HIV-1 infection had undergone allogeneic HSCT by 2010 [32]. Hassett et al. are credited with the first reported use of allogeneic HSCT in AIDS with the unsuccessful treatment of two AIDS patients using matched unrelated donors [33]. A retrospective study by the European Group for Blood and Marrow Transplantation (EBMT) included 30 patients transplanted between 1987 and 2003 and confirmed the negative outcome pre-cART [34]. However, with the introduction of cART, survival post-HSCT appears to have improved [32].

Among these early experiences was a series of HIV-1-infected patients transplanted at Johns Hopkins University [1]. In one patient with relapsed lymphoma, following a fully ablative total body irradiation (TBI)-based conditioning regimen, HSCT was performed using intravenous antiretroviral therapy with the best available agent at the time, zidovudine. The patient engrafted by day +17 but died of lymphoma-related complications by day +47. Of note, the peripheral blood polymerase chain reaction (PCR) was negative for detectable virus post-transplant and analysis of multiple tissues at autopsy did not demonstrate detectable virus [35]. This was the basis for the suggestion by Saral and Holland [1] that combined HSPC therapy and effective antiretroviral therapy might be effective in controlling HIV-1 infection.

With the availability of cART, more successful outcomes were then reported. The first of these was in a 2002 case report of a 33-year-old female with acute myelogenous leukemia, hepatitis C virus infection, and HIV-1 infection who underwent a fully ablative CD34$^+$-selected allogeneic transplant from a human leukocyte antigen (HLA)-matched sibling [36]. The patient was on cART prior to the transplant and had an undetectable viral load, and her post-transplant course was complicated by grade II GVHD that was treatment responsive. At the time of the report, 39 months post-transplant, the patient remained in remission and with undetectable HIV-1 viral loads.

The pattern of immune recovery following allogeneic stem-cell infusion remains incompletely understood, but case reports suggest that the CD4$^+$ lymphoid compartment does have some capacity to recover in HIV-1 patients post-HSCT [32]. The outcome in two HIV-infected recipients of reduced-intensity conditioning (RIC) for allo-HSCT demonstrated that reconstitution of tCD4$^+$ T cells was similar to that observed in other non-myeloablative transplants in HIV-1-negative recipients [37]. Of note, the HIV-1 naïve donor cells developed that ability to target HIV-1 epitope specificities that were different from pre-transplant patterns of the recipient. In this patient, who achieved full donor chimerism, there was also loss of detectable HIV-1 proviral DNA. This may be suggestive that the pool of latently infected lymphocytes was eliminated after transplantation, a phenomenon not seen in the autologous transplant patients [38]. Adding to this suggestive but anecdotal data is a series from Ohio State University that showed that all HIV-1-infected allogeneic HSCT recipients of RIC remained alive, in remission, off immunosuppression but on cART, at the follow-up time of 1 year [39]. Two of these patients remained HIV-1 RNA negative by clinical PCR assay. The first systematic study of the HIV-1 reservoir in the setting of RIC for allo-HSCT in HIV-1-infected recipients was recently reported by Henrich et al. [3]. Here, surrogate markers for the HIV-1 reservoir size, namely ultrasensitive PCR assays for HIV-1 RNA and DNA, HIV-1 tropism assays, and HIV-1 antibody were followed for up to 3.5 years while the recipients were continuously on cART. HIV-1 was undetectable by virus outgrowth assay, and RNA and DNA were negative in peripheral blood mononuclear cells (PBMCs), CD4$^+$ T cells, and plasma. Simultaneously with immune recovery and GVHD, the HIV-1 antibody decreased and, with the inability to detect HIV-1, there was the suggestion that under the cover of cART, the procedure was associated with a reduction in the HIV-1 reservoir [3]. These patients were taken off cART without a rebound in HIV-1 RNA (oral report, 2013 International AIDS Society Meeting, Kuala Lumpur). An ongoing study of allogeneic HSCT for treatment of hematologic malignancies by the Blood and Marrow Clinical Trials Network (NCT01410344) should resolve the question of whether an allogeneic HSCT can eliminate the HIV-1 reservoir and could be a curative strategy.

An alternative allogeneic approach is the use of cord blood HSCT-cb that has been screened for HIV-1 resistance factors [40,41]. It is estimated that there are as many as 400,000 cord blood units worldwide [40], and this could be developed as a donor source for this population. There is a small cord blood bank of CCR5-Δ32/Δ32 units, and these have been proposed as an alternative to matched unrelated donor transplants for AIDS-related hematologic malignancies; however, the statistical probability of finding a suitable match within such a cord blood bank is limiting [41]. The delayed immune recovery post-conventional HSCT-cb may also be problematic, but there is as yet no adequate experience in AIDS patients.

Autologous stem-cell transplantation in the AIDS patient with lymphoma

AIDS-related lymphoma

Although the era of cART has improved overall AIDS patient survival [42], cancers remain a continuing concern as a source of mortality [43]. In recent times, there has been a decrease in AIDS-defining cancers such as non-Hodgkin lymphoma (NHL) and a rise in HIV-1-related Hodgkin lymphoma (HL) [44]. Diffuse large cell lymphoma and Burkitt lymphoma are similar to those of HIV-negative patients, but plasmablastic and primary effusion lymphoma are more unique to HIV-1 infection.

The treatment of AIDS-related lymphomas changed after the introduction of cART, and what was once the standard approach using low-dose chemotherapy was supplanted by more aggressive therapies used in HIV-negative patients, such as R-CHOP [rituximab, cyclophosphamide, hydroxydaunomycin, vincristine (Oncovin), prednisone] for HIV NHL and ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) for HL. HIV-1 response to cART is associated with improved survival following lymphoma treatment [45], with remission rates after R-CHOP approaching rates seen in HIV-negative lymphoma patients [46]. Recent studies found that short-course R-EPOCH [rituximab, etoposide, prednisone, vincristine (Oncovin), doxorubicin hydrochloride] gave a 5-year progression-free survival (PFS) and overall survival (OS) of 84 and 68%, respectively [47]. The National Cancer Institute (NCI) pioneered an approach of short-course R-EPOCH that attempted to achieve the goal of antilymphoma efficacy while preserving immune function [47]. In this paradigm, starting after two cycles of R-EPOCH, patients were restaged with computed tomography (CT) and [^{18}F[fluorodeoxyglucose positron emission tomography (FDG PET) scans after each cycle until they achieved a complete remission or stable disease. The minimum number of cycles administered was three, and side effects of chemotherapy were generally similar to those seen in HIV-1-negative lymphoma patients. Mixed opinion persists about whether to suspend cART during chemotherapy due to concerns that pharmacodynamic or pharmacokinetic drug–drug interactions could lead to more toxicity or to varying levels of antiviral drug levels that could induce HIV-1 resistance.

Salvage therapy for AIDS-related lymphoma using autologous HSCT

Autologous stem-cell transplantation (ASCT) in HIV-1-positive patients was initially reported in the pre-cART era [48]. In this case study, a 40-year-old male with HIV-1 infection and relapsed NHL was treated with the standard high-dose conditioning BEAM [BCNU (carmustine), etoposide, cytosine arabinoside (cytarabine), melphalan] and autologous HSCT while on zidovudine and zalcitabine antiretroviral therapy. The post-transplant course was complicated by multiple opportunistic infections, including cytomegalovirus viremia, mycobacterium pneumonia and cryptosporidiosis, underscoring the risk that AIDS-related immune dysfunction conferred. Nonetheless, it also demonstrated the feasibility of mobilizing stem cells and successful engraftment in the bone marrow milieu of active viral replication. This effort was followed by a larger French study of autologous transplantation that included AIDS patients with refractory or relapsed lymphoma, generally with undetectable HIV-1 plasma levels at study entry on cART [49]. Using conditioning regimens consisting of either radiation plus chemotherapy or chemotherapy alone, four of eight were alive and in complete remission at the time of reporting, three died of lymphoma within 3 months post-ASCT, and one died of a late opportunistic infection.

The City of Hope experience included patients with less advanced lymphoma and specifically excluded chemotherapy-refractory patients, similar to the institutional practice with HIV-negative patients [50]. The initial series of 20 patients with either HL or NHL were required to have HIV-1 viral levels in plasma <10,000 gc/mL on cART, and the majority of patients received high-dose conditioning chemotherapy consisting of CBV [cyclophosphamide, BCNU (carmustine), etoposide]. Engraftment times were similar to those with HIV-1 negative patients, except for one patient who had delayed engraftment until day 23. This patient engrafted after the discontinuation of the zidovudine component of the cART. Attempts were made to continue cART throughout the transplant period, but in reality 11 of 20 patients were unable to tolerate these oral medications because of nausea or mucositis. PFS and OS were superior to those in the French series (85 and 81%, respectively), likely due to the inclusion of only chemosensitive patients. The cohort was expanded to 29 patients and with a median of 41 months follow-up, the PFS remained high at 78% [51] (Figure 65.1). The 2-year disease-free survival (DFS) and OS were also similar [75% and 75% in the HIV-1-infected group versus 56% (p = NS) and 75%, respectively, in the HIV-negative group]. The non-relapse-related mortality was also similar between the groups at 11% [95% confidence interval (CI) 4–28%] in the HIV-1 positive patients versus 4% (95% CI 1–25%) in HIV-negative controls. Infectious complications did differ between the groups, with more opportunistic infections in the HIV-positive patients without impacting OS.

A retrospective case–control study comparing 53 HIV-1-infected lymphoma patients with 53 HIV-1-negative controls matched for histology, non-age-adjusted International Prognostic Index (IPI), and disease status at the time of transplant demonstrated the minimum effect of HIV-1 infection on outcome [52]. OS was 61.5% (95% CI 47–76%) for HIV-1-positive patients and 70% for controls (p = NS). There was a trend towards delayed platelet engraftment in the HIV-1 cohort. Similarly, a non-statistically significant difference in non-relapse mortality was seen in the HIV-1-infected cohort, primarily due to the higher rate of bacterial infections. Interestingly, although there was no difference in relapse rates between groups, there was a favorable tendency for lower rates in the HIV lymphoma group, similar to the City of Hope case–control series.

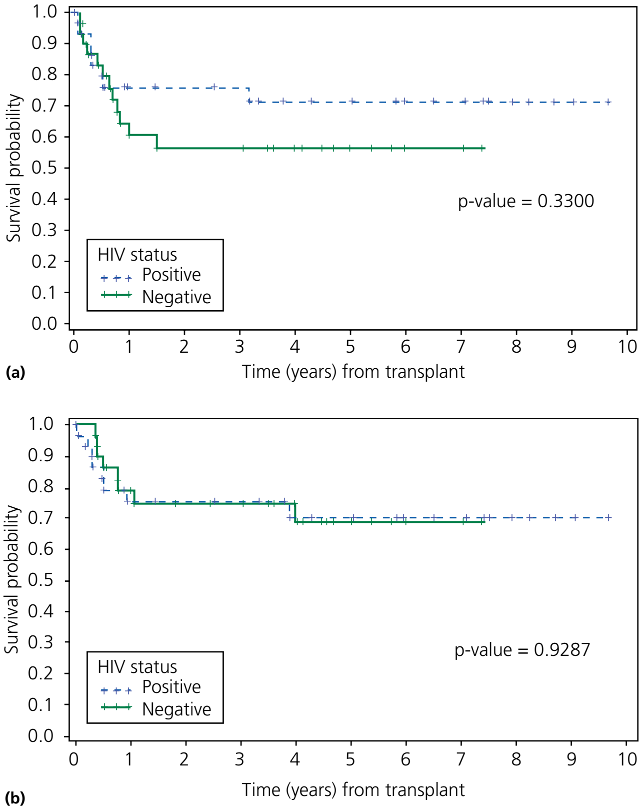

Figure 65.1 Probability of disease-free survival (a) or overall survival (b) by HIV-1 status (solid line, HIV-negative; dashed line, HIV-1 positive) in patients with non-Hodgkin lymphoma treated with dose-intense therapy followed by autologous HSCT. Source: Krishnan et al. 2010 [51]. Reproduced with permission of Elsevier.

The Italian Cooperative Group on AIDS and Tumors (GICAT) reported their long-term results on 50 patients with HIV-1 infection and relapsed/refractory NHL or HL [53]. Forty-six patients were already on cART and four started cART at the time of study enrollment or stem-cell mobilization. In contrast to the City of Hope requirements, any HIV-1 plasma RNA level was allowed, and viral loads at study entry ranged from 204,000 to 750,000 gc/mL. Thirteen patients were withdrawn before stem-cell collection due to toxic death (n = 2), progressive disease (n = 10), or patient refusal (n = 1). Of the remaining 37 patients, 31 adequately mobilized sufficient CD34$^+$ cells after either debulking chemotherapy (n = 19) or cyclophosphamide (4 g/m^2) + GCSF (n = 12). Ultimately, after early disease progression in four patients, 27 of the initial 50 patients underwent autologous HSCT after BEAM chemotherapy, and all engrafted at a median time of 10 days post-ASCT. The 3-year PFS for these patients was 76.3% [53], similar to the City of Hope experience. Multivariate analysis for prognostic factors for survival showed that marrow involvement, performance status <2, and CD4 count <100 cells/μL were significantly associated with poor outcome.

An EBMT retrospective multicenter experience with 68 patients from 20 institutions reported outcome with autologous HSCT involving high-risk patients in first CR (n = 16), with relapsed disease (n = 44), or chemorefractory disease (n = 8) [54]. Relapse or progression post-autologous HSCT occurred in 29.5% of patients, and transplant-related mortality was 7.5%, mainly due to bacterial infections. PFS was 56% , and analysis of prognostic factors demonstrated that histology, other than diffuse large-cell lymphoma, the use of more than two pretreatment regimens, and not being in clinical remission at the time of transplant were significantly associated with a higher risk of post-HSCT relapse [54]. Table 65.1 summarizes published outcomes of autologous HSCT for HIV lymphoma.

Given the favorable outcome of such salvage therapy in AIDS lymphoma, the question is whether the myeloablative conditioning affects the HIV-1 reservoir. To address this, Cillo et al. reported a retrospective analysis of frozen PBMC specimens from transplant survivors who remained on cART with no detectible HIV-1 RNA in plasma [38]. Specimens of blood, studied pre-transplant and at one other post-transplant time point, used assays for HIV RNA and DNA having single-copy sensitivity, a surrogate measure of the HIV-1 reservoir. Despite the absence of detectable HIV-1 RNA in plasma by routine assays, with the more sensitive assay 9/10 patients were viremic post-transplant while on cART, with a range of <0.15 to 26 HIV-1 genome copies/mL, and 9/10 were found to have detectable HIV-1 DNA. The stem-cell infusion product undoubtedly was contaminated by HIV-1-infected cells, and the transplant recipients were likely "re-infected" with endogenous virus post-transplant, perhaps during the cessation of cART. Nevertheless, there was no suggestion that myeloablative chemotherapy followed by cART had any substantive effects on the HIV-1 reservoir. Based on this, it could be concluded that the method used was not sufficient for eliminating HIV-1 infection.

Table 65.1 Outcome of autologous HSCT in high-risk AIDS lymphoma

Ref.	No. of patients	Conditioning regimen	PFS (%)	Median time to follow-up
Gabarre et al. 2004 [92]	14	BEAM/CY TBI/ other	29	49 months
Krishnan et al. 2005 [58]	29	CY/VP16/TBI CBV	76	24 months
Spitzer et al. 2008 [93]	20	BU/CY	50	23 weeks
Re et al. 2009 [53]	27	BEAM	76	24 months
Balsalobre et al. 2009 [54]	68	BEAM and variants	56	32 months

BEAM, BCNU (carmustine), etoposide, cytosine arabinoside (cytarabine), melphalan; BU, busulfan; CBV, cyclophosphamide, BCNU (carmustine), VP-16 (etoposide); CY, cyclophosphamide; CBV, cyclophosphamide, BCNU (carmutine), VP-16 (etoposide); TBI, total body irradiation.

Management of the AIDS patient during HSCT

Conditioning for allogeneic HSCT in hematologic malignancy

A variety of conditioning regimens have been used in the setting of HSCT for HIV-1 malignancy, and these are in general the same as those used in the non-HIV-1-infected population [32]. Even the use of antithymocyte globulin is tolerated in the HIV-1-infected patient [55]. Most of the differences in management of the HIV-1-infected patient undergoing HSCT occur during conditioning and are related to the antiretroviral therapy. In the now famous "Berlin" case of allogeneic transplant from a CCR5-Δ32/Δ32 matched unrelated donor, a fully ablative conditioning regimen was used consisting of amsacrin, fludarabine, and cytarabine for the first transplant and, following relapse, cyclophosphamide and TBI for the second [2].

Conditioning for autologous HSCT in hematologic malignancy

The City of Hope experience with autologous stem-cell transplantation for lymphoma utilized either CBV or FTBI (full total body irradiation) plus cyclophosphamide and etoposide for conditioning. As noted above, this case–control analysis compared HIV-1-negative and -positive patients with lymphoma and did not show any difference in transplant-related mortality between groups [51]. Other trials involving autologous transplant for HIV lymphoma have used BEAM- or FTBI-based regimens [53,54]. A large ongoing intergroup BMT CTN (Blood and Marrow Transplant Clinical Trials Network)-sponsored trial of autologous HSCT in AIDS-related NHL used BEAM, which is becoming the preferred conditioning regimen for this disease.

cART management during HSCT

The use of antiretroviral therapy in the HSCT setting is not different to that in the solid organ transplant setting. In solid organ transplantation, the major challenge is managing the drug interaction between antiretroviral drugs and immunosuppressive agents. Ritonovir-boost protease inhibitors are particularly problematic to administer during the HSCT conditioning regimen and post-transplant period due to extensive drug–drug interactions. Indeed, in one of the first case reports of a liver transplant, the patient developed toxic levels of cyclosporine due to its interaction with cART [56]. In the HSCT setting, there is the added concern of interaction between cART and the chemotherapy used for conditioning or for GVHD prophylaxis. Hence there must be a balance in management of the HIV/AIDS patient during HSCT; on the one hand, the cART must not negatively impact the required drugs needed for the transplant procedure, and on the other, the cART must be available to prevent any rebound in HIV-1 infection that might affect the outcome of the transplant. However, for practical reasons, oral cART is often interrupted due to patient intolerance from mucositis or GVHD. At present, subcutaneously administered enfuvirtide is the only parenteral anti-HIV-1 agent available, and use of this agent should be considered in the design of the cART regimen. The goal should be to minimize the cessation of cART since HIV-1 rebound can lead to infection that can affect the allogeneic graft, as shown in a study by Kang et al. in which a patient developed an acute febrile illness and a rise in HIV-1 viral load to 1,000,000 gc/mL [57]. The autologous HSCT recipient receiving myeloablative conditioning with CBV or TBI is more likely to be intolerant of cART early post-transplant and may not resume medication for 1–2 months post-transplant. Despite this, few long-term problems with HIV-1 drug resistance or with CD4 count recovery are seen [58]. However, BEAM conditioning has shown less oral medication intolerance and ability to continue cART during the early transplant period due to chemotherapy-related side effects [53]. Based on this and the emerging data on the potential effect of continued cART on the HIV reservoir, as seen in the allogeneic transplant setting [3], the current recommendation is to maintain strict adherence to cART coverage during either autologous or allogeneic transplant. This may require substitution of cART with the parenteral agent enfuvirtide in some patients.

The main clinical concern with cART management of the AIDS patient undergoing HSCT is drug–drug interactions. Although there is limited information in this area, case reports have provided information regarding the effect on the cytochrome p450 system to guide current therapy [32]. As shown in Table 65.2, recommendations from ongoing BMT CTN trial 0903 suggest modification of certain cART regimens prior to the conditioning period. As summarized here, for fludarabine/busulfan and fludarabine/melphalan regimens, for which liver metabolism occurs, both raltegravir-based integrase strand transfer inhibitor (INSTI) therapy and non-nucleoside reverse transcriptase inhibitor (NNRTI)-based cART may be continued as tolerated. However, ritonavir-boosted protease inhibitor (PI)-based cART requires a period of brief cART cessation or modification. Ritonavir strongly inhibits CYP3A4 activity [59], and this should increase busulfan levels. It is recommended, therefore, to remove ritonavir and substitute another agent, usually raltegravir, in the cART regimen. For cyclophosphamide-based conditioning regimens and GVHD prophylactic regimens that utilize post-transplant cyclophosphamide, the ritonavir-induced inhibition of CYP3A4 will likely lower the active metabolites of cyclophosphamide and potentially reduce the intended cytoreductive effect. However, NNRTI drugs induce CYP3A4 activity and should have the opposite effect on levels of active metabolites of cyclophosphamide. Until definitive studies are available, it is recommended to substitute for any ritonavir-boosted cART regimen and to use NNRTI-based cART with caution.

Fungal and GVHD prophylaxis in the HIV/AIDS patient after HSCT

Because of the complex interactions of the triazoles (voriconazole, itraconazole, and to a lesser extent posaconazole) with both cART and with tacrolimus/rapamycin regimens, the echinocandins (anidulafungin, caspofungin, micafungin) and amphotericin B are preferred for antifungal prophylaxis. In addition, the immunosuppressive medications used for GVHD prophylaxis must be managed

Table 65.2 Recommendations for cART use during conditioning regimen[a],[b]

Conditioning regimen	Metabolism (primary site)	Interaction potential [strength of evidence]	Recommendation
Flu/Bu • Fludarabine • Busulfan	Fludarabine (liver): • Rapid dephosphorylation to 2-fluoro-Ara-A, then phosphorylated to 2-fluoro-Ara-ATP intracellularly Busulfan (liver): • Metabolized hepatically; substrate of CYP3A4	High Concomitant administration of ritonavir-boosted PIs may increase the levels of busulfan. [Strength of evidence: LOW]	Raltegravir-based: may continue cART as tolerated NNRTI-based: may continue cART as tolerated PI-based: regimens including ritonavir should be discontinued a minimum of 48 hours prior to the administration of myeloablative regimens containing busulfan Antiretroviral therapy should not be resumed until, at least, 48 hours after the last dose of busulfan and once the patient is able to tolerate oral medications consistently
Flu/Mel • Fludarabine • Melphalan	Fludarabine (liver): • Rapid dephosphorylation to 2-fluoro-Ara-A, then phosphorylated to 2-fluoro-Ara-ATP intracellularly Melphalan (blood): • Chemical hydrolysis in the plasma to inactivate metabolites	Low The effects of ritonavir-boosted PIs on levels of fludarabine are unknown Efavirenz has been given in combination with fludarabine [Strength of evidence: MODERATE]	Raltegravir-based: may continue cART as tolerated NNRTI-based: may continue cART as tolerated PI-based: the potential for interactions with ritonavir-boosted PIs or NNRTIs is low. Antiretroviral therapy can be continued if the benefits clearly outweigh the risks
Cy/TBI • Cyclophosphamide • TBI	Cyclophosphamide (liver): Prodrug is converted by hepatic microsomal enzymes to the active form. The activation pathway involves CYP3A4 isoenzymes. • Substrate for CYP2A6 (minor), 3A4 (major) • Inhibits CYP3A4 (weak) • Induces CYP2B6 (weak), 2C8/9 (weak) Active metabolites include acrolein	High Complex interactions exist between ritonavir and cyclophosphamide or its metabolite, acrolein. Cyclophosphamide levels may be increased when combined with protease inhibitors, but the active metabolites may be decreased NNRTIs may increase levels of acrolein through CYP induction if similar to other CYP inducers such as phenytoin that can increase the AUC of active metabolites by 50% [Strength of evidence: LOW]	Raltegravir-based: may continue cART as tolerated NNRTI-based: regimens including NNRTIs should be used with caution and with frequent monitoring due to the increase in toxic metabolites caused by CYP450 induction PI-based: regimens including ritonavir should be discontinued a minimum of 48 hours prior to the administration of myeloablative regimens containing cyclophosphamide Antiretroviral therapy should not be resumed until, at least, 72 hours after the last dose of cyclophosphamide and once the patient is able to tolerate oral medications consistently

[a] cART combinations may not have been studied but the interaction potential has been predicted based on the metabolic pathways of the various agents.

[b] Recommendations summarized from the Bone Marrow Transplantation Clinical Trials Network protocol 0903, with permission.

differently in the setting of cART. Again, the potent inhibition of CYP3A4 by ritonavir will increase both tacrolimus and sirolimus blood levels. The NNRTI-based cART regimens have the opposite effect of reduced tacrolimus and sirolimus levels due to induction of CYP3A4, and this is especially true for efavirenz and nevirapine. Since many patients are unable to take the cART as oral medication during the acute transplant phase, when cART is reintroduced patients are already on an immunosuppressive regimen. As noted in Table 65.3, the immunosuppressant dose may need to be adjusted based on trough levels after the reinitiation of cART. To complicate the dosing further, sometimes the full induction of CYP3 A4 may take several weeks, hence the full extent of the cART effects may not be seen immediately. It should additionally be noted that dose adjustments based on drug toxicity may also be complicated by difficulty in accurately identifying the suspect agents. For example, patients on tenofovir may experience renal toxicity, a side effect also seen with tacrolimus. If patients have impaired renal function, in addition to reducing the immunosuppressant doses, one has to remember that nucleoside reverse transcriptase inhibitors (NRTIs) are usually excreted renally, and hence also may need dose adjustment. Good clinical judgment and availability of drug levels are essential in optimizing patient management.

Gene therapy for HIV/AIDS

The rationale of an HSCT-based strategy for HIV/AIDS is the generation of a new immune system that would control HIV-1 infection while at the same time destroying the endogenous reservoir of virus, leading to a cure of the infection. Success would be largely predicated upon the creation of a durable (HIV-1 resistant) immune system through transplantation of resistant HSPCs [60]. Approaches have used xenogeneic, allogeneic, and autologous blood stem cells. A single unpublished case of a baboon stem-cell transplant into an HIV-1-infected patient occurred in 1995, but no further attempts at xenogeneic treatment of HIV-1 are known. The feasibility of transferring HIV-1 resistance via HSPCs was best demonstrated when an AIDS patient with acute myeloid leukemia was transplanted with HLA-matched, unrelated donor HSPCs containing a homozygous 32-base pair deletion in the chemokine receptor 5 gene (CCR5Δ32/Δ32) [2]. Individuals who are homozygous at this locus do not express CCR5 on their cell surface, and therefore cannot be infected with CCR5 (R5) tropic HIV-1 [61]. The recipient attained complete hematopoietic reconstitution with the donor graft, suspended cART early after HSCT, and remained with undetectable HIV-1 RNA in blood and undetectable HIV-1 DNA in tissues using single-copy sensitive PCR methods for more than 5 years after treatment [62]. As noted above, the allogeneic effect itself could explain the favorable outcome in this case, but the use of mutant donor cells implies some potential contribution of cellular resistance to infection. Despite the success of this single case, the statistical improbability of a donor having both the homozygous CCR5Δ32/Δ32 mutation and HLA compatibility severely limits this approach. The frequency of homozygous CCR5Δ32/Δ32 genotype is <2% in the Western European Caucasian population and virtually absent in people of Asian and African descent [63]. Hence genetic modification of autologous HSPCs is addressed in an effort to create an alternative strategy.

Table 65.3 Guidance for dosing of tacrolimus and sirolimus during cART[a]

Concomitant medication	Effect on tacrolimus dosing	Effect on sirolimus dosing
Protease inhibitors [r = boosted]: • Atazanavir/ritonavir [ATZ/r] • Fosamprenavir/ritonavir [fAPV/r] • Indinavir/ritonavir [IND/r] • Lopinavir/irtonavir [LPV/r] • Saquinavir/ritonavir [SQV/r] • Tipranavir/ritonavir [TPV/r] • Atazanavir [ATZ] • Nelfinavir [NFV]	↑↑↑ Tacrolimus levels due to potent inhibition of CYp3A4 and P-glycoprotein, especially with ritonavir-boosted cART regimens [94,95] **Hold drug**; Target tacrolimus trough = 5–15 ng/mL Dose base on daily trough: <5 ng/mL: 1 mg PO or 0.25 mg IV 5–15 ng/mL: 0.5 mg PO or 0.125 mg IV >15 ng/mL: Hold tacrolimus until next level	↑↑↑ Sirolimus levels due to potent inhibition of CYP3A4 and P-glycoprotein, especially with ritonavir-boosted cART regimens [96] **Hold drug**; Target tacrolimus trough = 3–12 ng/mL Dose base on daily trough: <3 ng/mL: 0.4 mg PO 3–12 ng/mL: 0.3 mg PO >12 ng/mL: Hold sirolimus until next level
Non-nucleoside RT inhibitors: • Efavirenz [EFV] • Nevirapine [NVP] • Etravirine [ETV]	↓ Tacrolimus levels due to induction of CYp3A4 [94] **Continue drug**; Target trough = 5–15 ng/mL Avoid use with ETV; for EFV and NVP base daily tacrolimus dose on trough[c]: <5 ng/mL: increase dose by 25%[c] 5–15 ng/mL: no adjustment >15 ng/mL: decrease dose by 25%	↓ Sirolimus levels due to strong induction of CYp3A4[b] **Continue drug**; Target trough = 3–12 ng/mL Avoid use with ETV; for EFV and NVP base daily sirolimus dose on trough[c]: <3 ng/mL; increase dose by 25% 3–12 ng/mL: no adjustment >12 ng/mL: decrease dose by 25%
Nucleoside RT inhibitors: • Abacavir [ABC] • Didanosine [ddI] • Emtricitabine [FTC] • Lamivudine [3TC] • Stavudine [d4T] • Tenofovir [TDF] • Zidovudine [AZT, ZDV]	No effect known [97] No dose adjustment necessary but monitor tacrolimus trough 2–3 times per week Monitor renal function for patients on TDF[d]	No effect known No dose adjustment necessary but monitor sirolimus trough 2–3 times per week Monitor renal function for patients on TDF[d]
Integrase inhibitor: • Raltegravir [RLV]	No effect known[e] No dose adjustment necessary	No effect known[e] No dose adjustment necessary
Fusion inhibitor: • Enfuvirtide [ENF, T20]	No effect known[f] No dose adjustment necessary	No effect known No dose adjustment necessary
CCR5 antagonist: • Maraviroc [MVC]	No effect known No dose adjustment necessary	No effect known No dose adjustment necessary

[a] Based on information provided in Bone Marrow Transplantation Clinical Trials Network protocol 0903, with permission; recommendations may be based on known or theoretic effect of antiretroviral agent or class of retroviral agent on cytochrome P450 enzymes or P-glycoprotein 1.
[b] EFV is more potent than NVP as an inducer of CYP3A4; full induction can take up to 2 weeks.
[c] Recommendation based on theoretical considerations of the potent induction of CYP3A4 by ETV.
[d] For patients with renal impairment, adjust nucleoside RT inhibitor dose based on agent package information.
[e] Raltegravir-based regimens may be preferred over PI- or NNRTI-based regimens since metabolism is by glucuronidation and not by CYP3A4 metabolism.
[f] Enfuvirtide is administered subcutaneously and may be used at times when patients cannot take oral medications.

Molecular basis for gene therapy of HIV/AIDS

Gene-based strategies target viral or cellular elements necessary to suppress HIV-1 infection, to protect CD4⁺ T cells and other susceptible cells from further HIV-1 infection, and to restore immune function [4]. Gene therapy involves the transfer of genetic information (transgene) into human subjects to replace a missing or defective function or to produce an intracellular molecule (RNA, DNA, protein) for therapeutic purposes. Highlighted here are those strategies undergoing clinical evaluation and those in advanced preclinical development (Tables 65.4 and 65.5). Among genetic strategies are cell- and gene-based therapeutic approaches that can be used in concert with conventional antiretroviral drugs. This approach was made possible by the ability to copy human genes in vitro and then insert them into target cells of interest. Although simple at first glance, this process is highly complex.

First, the transgene must be cloned into a delivery vehicle (vector) that also provides the transcriptional regulatory elements required for gene expression. The delivery vehicle may be a viral or non-viral vector. Second, the vector must be delivered efficiently into the target cells. Most delivery methods employ a process whereby cells are removed from the patient, and the transgene is delivered to the target cells ex vivo. The modified cells are then expanded in culture and reinfused into the patient. Several approaches for direct delivery of genes to target cells in vivo, bypassing the need for expensive time-consuming ex vivo cell manipulations, are also in clinical studies, but at this point these are restricted to local tissue delivery such as skin, muscle, or lung. Third, except for strategies that edit the genome by transient exposure to nucleases, most strategies require long-term stable expression of the transgene in modified cells.

Both integrating and non-integrating vectors are being developed, with each having their own unique advantages and disadvantages [4,64]. Vectors that can integrate the transgene into host DNA promise to produce long-lived expression, whereas non-integrating vectors can be expected to have a shorter period of expression but will minimize the theoretical risk of insertional mutagenesis by the integrated DNA. Efforts to improve the technologies are directed towards the three critical problems facing this approach: the design of better strategies for efficient, safe, and cost-effective gene delivery, the design of new molecular targets for improved antiviral effect, and the development of clinical trials that test the feasibility and safety of these approaches. These three aspects of gene therapy for HIV-1 infection are described below, but for a more complete discussion the reader is referred to specific reviews of the field [64,65].

Molecular strategies for HIV-1 gene therapy

A diverse array of transgenes to suppress HIV-1 functions or block the infectious cycle have been designed and several of these have been tested in vivo (see Table 65.4). These can be broadly categorized into two groups: those coding for nucleic acid-based suppressors and those coding for protein-based suppressors. As in drug-based therapy, the rationale for antiviral gene therapy is that

Table 65.4 Anti-HIV gene therapeutics in clinical trials

Mechanism of antiviral	Target	Delivery of genes/cell transplantation	Sponsor	Phase	Status of trial	Ref.
HSPC-based gene therapy studies						
Ribozyme	Viral (tat–rev mRNA)	Retroviral (MMLV) into autologous CD34$^+$ HSPC	City of Hope Ribozyme Pharmaceutics	Pilot	Completed	Rossi 2000 [98]
RNA decoy	Viral (rev protein)	Retroviral (MMLV) into autologous CD34$^+$ HSPC	Children's Hospital Los Angeles	Pilot	Completed	Kohn et al. 1999 [67]
Ribozyme	Viral (tat–vpr mRNA)	Retroviral (MMLV) into autologous CD34$^+$ HSPC	Johnson & Johnson	I–II	Completed	Mitsuyasu et al. 2009 [78]
shRNARNA decoy (TAR) ribozyme	Viral (tat–rev mRNA) Viral (tat protein) Host (CCR5 mRNA)	Lentiviral vector (SIN HIV) into autologous CD34$^+$ HSPC	City of Hope, Benitec	Pilot	Ongoing	DiGiusto et al. 2010 [70]
C46 peptide siRNA-CCR5	Viral entry	Lentiviral vector (SIN HIV) into autologous CD34$^+$ HSPC	Calimmune	I	Ongoing	An et al. 2007 [99]
T-cell-based gene therapy studies						
Ribozyme	Viral (U5 and pol mRNA)	Retroviral (MMLV) into autologous CD4$^+$ T cells	UCSD	I	Completed	Wong-Staal et al. 1998 [100]
C46 peptide	Viral (env protein)	Retroviral (MMLV) into autologous CD4$^+$ T cells	University Medical Center Hamburg-Eppendorf	I	Completed	van Lunzen et al. 2007 [101]
Ribozyme	Viral (tat–vpr mRNA)	Retroviral (MMLV) into syngeneic CD4$^+$ T cells	Johnson & Johnson	Pilot	Completed	Macpherson et al. 2005 [102]
Antisense	Viral (env mRNA)	Lentiviral vector (LTR HIV) into autologous CD4$^+$ T cells	VIRxSYS	I–II	Ongoing	Levine et al. 2006 [66]
ZFN	Host (CCR5 DNA)	Adenoviral vector into autologous CD4$^+$ T cells	Sangamo Biosciences	I–II	Completed	Perez et al. 2008 [87]

Source: adapted from Burnett et al. 2012 [64].

Table 65.5 Anti-HIV gene therapeutics in pre-clinical animal studies

Type of HIV inhibitor	Transgene	Target	Delivery of genes	Cell delivery	Ref.
Gene editing	SB-728 ZFN	Host CCR5 gene	Adenovirus or nucleofection	CD34$^+$ HSPC	Li et al. 2013 [103]; Holt et al. 2010 [77]
Gene editing	Ad5/F35 X4-ZFN	Host CXCR4 gene	Adenoviral vector	CD4$^+$ T cells	Wilen et al. 2011 [104]
Protein-based	C46 peptide	Viral envelope	Lentiviral vector	CD34$^+$ HSPC	Trobridge et al. 2009 [105]
Protein-based	C46 peptide	Viral Envelope	Lentiviral vector	CD4$^+$ T cells	Kimpel et al. 2010 [106]
Protein-based	LEDGF/p75 KD LEDGF$_{325-530}$	Viral integrase	Lentiviral vector	CD4$^+$ T cells	Vets et al. 2012 [107]
Protein-based	TRIM5α	Viral capsid protein	Lentiviral vector	CD34$^+$ cells	Anderson et al. 2008 [108]
Combo	CCR5 shRNA TRIM5α TAR decoy	CCR5 gene Viral capsid protein Viral Tat protein	Lentiviral vector	CD34$^+$ HSPC	Walker et al. 2012[89]
Combo	C46 peptidetat/rev shRNA CCR5 shRNA	Viral envelope Viral mRNA Host CCR5 mRNA	Foamy virus vector	CD34$^+$ HSPC	Kiem et al. [109]
Protein-based	Neutralizing antibody	Viral entry	Adeno-associated virus	Direct intramuscular injection	Balazs et al. [110]
Protein-based and selection	C46 peptide Methylguanine methyltransferase	Viral envelope	Lentiviral vector	CD34$^+$ HSCs	Younan et al. [91]

Source: adapted from Burnett et al. 2012 [64].

inhibition of the virus at several critical steps of virus replication is likely to result in a more complete and effective suppression than a blockade at a single step of virus infection. Also, as in drug-based therapy, concerns for emergence of HIV-1 variants that resist or escape the gene-based strategy apply. For these reasons, current gene therapy strategies use combinations of different types of transgenes with different viral and cellular targets that aim to neutralize completely HIV-1 functions at different points in the infection cycle. The several genetic inhibitor strategies that have entered clinical trials are outlined in Table 65.4.

RNA-based suppressors

The binding of newly transcribed RNA-antisense molecules to mRNA sequences prevents translation of the encoded protein, thereby resulting in loss of gene function. The first lentivirus vector-based gene transfer in humans encoded an antisense to HIV-1 *env*, in the setting of T-cell immunotherapy, and was shown to be safe [66]. RNA decoy strategies attempt to compete with specific HIV-1 RNA elements that bind viral proteins as part of the replication cycle by overexpressing their RNA homologs. TAR (Tat response element) and RRE (Rev response element) are two such *cis*-acting factors that are necessary for proper function of the key HIV-1 regulatory proteins, Tat and Rev. Together, these and related studies provided the conceptual basis for a clinical trial of transduced and transplanted marrow-derived HSC in HIV-1-infected children [67].

Ribozymes are RNA molecules that can cleave RNA targets at specific sequences, a property that is being exploited to cleave and thus inactivate HIV-1 RNAs [64]. The first in vitro study suggesting the use of a "hammerhead" ribozyme to suppress HIV-1 was directed against the HIV-1 *gag* RNA sequence [68]. A "hairpin" ribozyme that cleaved at the leader sequence at the 5′ end of the HIV-1 genome conferred resistance to several HIV-1 isolates and considerable inhibitory activity was observed for up to 35 days in vitro [69]. Clinical trials using ribozymes, used as a component of combinatorial gene therapy, are currently active [70].

RNA interference (RNAi) is based on a cellular process in which double-stranded RNA (dsRNA) induces a post-transcriptional degradation of homologous transcripts, and RNAi has been observed in a variety of organisms including fungi, plants, insects, protozoans, and mammals [71]. RNAi is initiated by exposing cells to dsRNA via either transfection or endogenous expression of viral or transposon RNAs. dsRNAs is processed by the RNAse III-like enzyme, called "dicer," into 21–23 nucleotide double-stranded fragments known as short interfering RNAs (siRNAs). These siRNAs form a complex known as the RNAi-induced silencing complex (RISC), which leads to cognate target RNA destruction. Chemically synthesized 21–23 length siRNA nucleotide duplexes with short 3′ overhangs have been synthesized and are capable of mediating RNAi in vivo and in vitro. Several studies have demonstrated that the functional unit of RNAi, the siRNAs, can elicit sequence-specific target downregulation either by introduction of preformed siRNAs via transfection or by endogenous expression of 21–23 base-paired RNAs using polymerase III promoter systems [72]. This enabling of expression of siRNAs in mammalian cells has opened up new possibilities for therapeutic applications of siRNAs.

RNAi can be applied as a potent mechanism for inhibition of HIV-1 infection and represents a potentially powerful tool for anti-HIV therapy [73]. The recent publications demonstrating that HIV replication can be inhibited by using either synthetic or expressed siRNAs, targeting the virus, its receptor, or its coreceptor, have generated a great amount of interest and enthusiasm for further testing of the anti-HIV therapeutic potential of siRNAs. There are at least two different modalities by which siRNA can be used as an antiviral: transfection of the preformed siRNAs or intracellular expression of siRNAs. The latter approach has been utilized in a gene therapy setting, providing anti-HIV-1 RNAs to hematopoietic cells susceptible to HIV-1 infection [70].

Protein-based suppressors

Transdominant mutant proteins act as competitors of cognate HIV-1 proteins and thus suppress normal viral functions. The most experimentally advanced transdominant protein to date is a mutant Rev protein (RevM10) [74]. RevM10 retains two Rev functions: the ability to bind RRE on the viral genome and the ability to form Rev multimers. However, RevM10 is incapable of exerting its regulatory role in transporting unspliced or singly spliced RNAs from the nucleus to the cytoplasm. Hence susceptible cell lines that express RevM10 exhibit long-term (>30 days) resistance to HIV-1 replication and human CD34$^+$ blood progenitor cells, transduced with RevM10, can give rise to T-lymphocytes that exhibit significant resistance to challenge with HIV-1. A RevM10-based gene therapy strategy used in association with allogeneic HSCT has been described [57].

Clinical trials of gene therapy for HIV-1 infection

The first clinical gene therapy study in HIV-1 infected humans examined the potential of RevM10 to prolong the survival of transduced CD4$^+$ T cells in vivo [75]. In this study, the duration of engraftment was limited and transient, but a subsequent study, using a retrovirus delivery vector in lieu of plasmid DNA, achieved improved survival in T cells expressing the RevM10 compared with T cells transduced with a negative control vector [76].

Strategies based on preventing the expression of chemokine coreceptors at the cell surface are particularly attractive primarily because they block the very first step in HIV-1 infection of target cells rather than blocking a viral event that occurs after the establishment of the proviral DNA in the cellular genome. Such chemokine-modified cells should also have survival advantages in vivo in HIV-1-infected individuals, suggesting a potential for selection of resistant T cells during infection with R5 virus [77]. Moreover, a gene-based approach that utilizes a human protein is more likely to induce an immune response against the expressed protein, an undesired outcome that could lead to clearance of transduced cells by the immune system. Because HIV-1 replication can be influenced by a variety of cellular perturbations, there are several other molecular strategies for inhibiting this virus (see Tables 65.4 and 65.5), and these strategies will likely increase as more becomes known about the biology of HIV-1.

Clinical trials of gene therapy using hematopoietic stem cells

Conceptually, HSPCs, transduced with potent anti-HIV-1 genes, should confer anti-HIV-1 protection to all susceptible hematopoietic cell lineages (e.g., CD4$^+$ T cells, monocytes, Langerhans cells, dendritic cells, and astrocytes) for the life of the infected individual. The key question then becomes: how does one develop a practical method to bring HSPC manipulation and transplantation to the target patient population? In the first genetic modification of allogeneic HSPCs, noted above, using RevM10 as the transgene a single recipient of a matched related HSPC was treated after receiving RIC [57]. This patient's course was complicated by grade II acute GVHD and by limited chronic GVHD, but she was alive and in complete remission at 3 years post-HSCT. [Note: this patient subsequently died of thrombotic thrombocytopenic purpura at 3.5 years post-HSCT, at which time her HIV-1 was undetectable on cART (J. Tisdale, personal communication)]. As noted above, ongoing experience suggests that the allogeneic effect itself could have produced an anti-HIV-1 effect, even without genetic manipulation of the stem cells [3]. Studies are in place to determine what role allogeneic HSCT might have in HIV cure strategies [3,4]. The population with AIDS-related hematologic malignancy forms a target group in which therapy with or without artificially induced HIV-1 resistance in donor cells could be tested as a curative strategy for HIV/AIDS.

Gene therapy with autologous HSPCs

Genetic modification of autologous HSPCs has been proposed as a strategy that could engraft HIV-1 resistance and would avoid any problem of GVHD. A large phase II trial of gene-modified autologous cell therapy, in the absence of conditioning therapy, has been reported [78]. This study demonstrated the use of cART interruption in trial design, but in the absence of pre-transplant conditioning there was only a minimal detection of gene-marked PBMCs and only a transient effect on HIV-1 levels. Nevertheless, this pioneering study demonstrated the novel use of cART analytical treatment interruption for testing the anti-HIV-1 efficacy of gene therapy.

The first use of a conditioning regimen in patients receiving autologous gene-modified stem cells was performed in the setting of salvage therapy of AIDS lymphoma [70]. The patients required autologous HSCT, and represent a population in which the potential for engraftment of gene-modified HSPCs could be evaluated. DiGiusto et al. reported this pilot clinical study in which such patients undergoing high-dose chemotherapy and autologous stem-cell transplantation were infused with combined gene-modified and non-modified HSPCs [70]. Four patients were treated with mobilized and selected CD34$^+$ HSPCs transduced with a lentivirus encoding three anti-HIV-1 RNAs, namely TAR, siRNA to tat/rev, and a ribozyme targeting CCR5 [79]. There were no serious adverse events attributable to the research HSPC product, and as shown in Figure 65.2, gene marking was present in all patients for varying periods post-HSCT. This pilot study demonstrated the safety and feasibility of the approach following myeloablative conditioning. Still, the level of marking was less than 0.35% of PBMCs, and studies continue using improved methods of gene delivery to improve this outcome.

A major limitation in use of autologous HSPC-based gene therapy is the requirement for engraftment of adequate numbers of stem cells to overcome the endogenous pool of cells. The use of autologous HSC for delivery of genetically modified stem cells for treatment of HIV/AIDS will require an optimized conditioning regimen, and this is still unknown. One must balance the need for safety with the need to engraft efficiently. For non-cancer populations, dose intensity is usually not ethically warranted, and one must seek an adequate non-myeloablative treatment regimen to ensure stem-cell engraftment. However, it has been suggested that if there is no selective advantage of the gene-modified cells, then non-myeloablative conditioning will ultimately fail [80]. Busulfan is considered non-myeloablative when used alone at <9 mg/kg total dose [81] and, recognizing that myeloablative doses of busulfan are not justified in the non-cancer setting of AIDS, what would be an appropriate non-myeloablative dosing regimen? Clinical trials in the correction of human genetic diseases using gene-modified HSPCs have provided direction with use of busulfan-based regimens [82], and these approaches are now being evaluated in the setting of HIV/AIDS. In this regard, in adenosine deaminase-deficient children, busulfan was first used at 2 mg/kg intravenously for 2 days followed by infusion of CD34$^+$-transduced bone marrow cells [83]. Later, Candotti et al. correlated busulfan dosing with area under the curve (AUC) measurements showing that at doses ranging from 65 to 90 mg/m^2 (equivalent to ~4 mg/kg), the achievement of AUC in the range 4000–4800 mUmin was associated with engraftment [84]. Patients had minimal symptoms but did have transient neutropenia, mild thrombocytopenia, and transient elevated liver enzymes. In another series of patients with X-linked chronic granulomatous disease (CGD), liposomal busulfan at 4 mg/kg/day for 2 days was used, and the absolute neutrophil count recovery occurred after 18–21 days [85]. For the ongoing trials in HIV-1 infected patients without malignancy, the early conditioning regimen of choice has been busulfan in dose escalation study design.

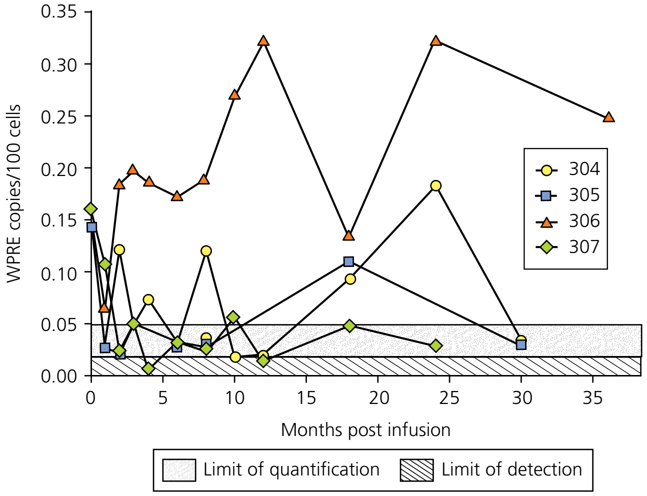

Figure 65.2 Gene marking in peripheral blood following autologous HSCT for AIDS-related lymphoma in four patients (Nos. 304–307). The number of copies of integrated vector per 100 peripheral blood leukocytes is shown over time.

Gene therapy using CD4$^+$ T cells

Use of autologous T-lymphocytes as the target cells for anti-HIV-1 gene modification of CD4$^+$ T cells represents another cell-based strategy for gene therapy [75]. A similar strategy, in which mature CD8$^+$ T cells were modified to kill HIV-infected cells, has also been reported [66]. In either case, mature T-lymphocytes have attributes that make them attractive targets for gene therapy: they are easily obtained from the donor's peripheral blood and they can be expanded to large numbers in vitro. Indeed, a method of cell expansion that involves cell surface stimulation, using antibodies to CD3 and CD28, promotes the expansion of cells to very high numbers [86]. Targeting mature T-lymphocytes for gene therapy has the added advantage that the effect of the therapeutic gene can be rapidly monitored for effects on cell survival, viral load, and other parameters. The transduced cells can also be selected in vitro, using the marker gene included in the vector, before reinfusion in the host, thereby generating a highly enriched population of genetically modified cells. For these reasons, gene therapy approaches that target mature lymphoid populations may be the method of choice for initial evaluation of new gene therapy strategies. Using a lentivirus vector to transfer an antisense RNA target to the HIV-1 envelope, CD4 lymphocytes have been safely infused into AIDS patients who were resistant to antiretroviral therapy [66]. More recently, it has been shown that the CCR5 gene can be edited using zinc finger nuclease (ZFN), and clinical trials are in progress [87].

Future directions of gene therapy for HIV/AIDS

The field of gene therapy is only beginning to demonstrate its potential, and one can only imagine how this will affect the management of patients in the future. Table 65.5 lists some recent discoveries that are likely in the next few years to reach clinical testing. Technology continues to address areas that are key for overcoming current limitations. Improved cellular processing is advancing our ability to manufacture efficiently genetically modified stem-cell products [65], and it is possible that these improvements will make stem-cell therapy available in ways that are affordable to HIV/AIDS patients. ZFN targeted to CCR5 [88], new multiplexed anti-HIV-1 combinations that include host restriction factors [89], and directed expression of neutralizing antibody [90] are new approaches to protection from HIV-1 infection. However,, for autologous therapy to be successful, there will likely need to be a method of selection of the genetically protected cell during HIV-1 infection. There is some expectation that HIV-1 itself could be used for selection [77], but chemical selection using methylguanine methyltransferase (MGMT) has been shown to protect macaques from infection [91].

MGMT-based selection has already been used in humans in cancer treatment, and this approach is moving rapidly to clinical testing in HIV/AIDS.

Conclusion

The application of HSPC technology to the treatment of HIV/AIDS is reaching a point at which clinicians are seeking curative strategies. It remains to be determined how HCT will supplement cART in the management of HIV-1-infected patients. Assuming that cell-based gene therapy will become optimized for safety, specific uses for gene therapy are likely to be identified and the test-of-concept for this approach could be established with a goal of decreasing or destroying the virus reservoir and blocking disease progression.

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CHAPTER 66

Hematopoietic Cell Transplantation for Autoimmune Diseases

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Introduction

An autoimmune disease is characterized by organ damage resulting from an immunologic response to self-antigens. Autoimmunity, on the other hand, is an immune response to self-antigens but without the implication that this has resulted in organ damage. Autoimmunity may be present spontaneously in normal individuals or after an infectious or other disease-related event which results in tissue damage (e.g., infarction). Criteria that support a disease having an autoimmune mechanism are (1) humoral or cellular self-reactivity, (2) disease-related autoantibody or lymphocytic infiltrate in pathologic lesion without other identified cause, and (3) autoantibodies or T cells that cause tissue pathology or cell dysfunction by transplacental transmission, adaptive transfer into animals, or in vitro experiments. An autoimmune disease develops in approximately 3–8% of the population [1]. Autoimmune diseases may involve single organs or multiple systems and may or may not have defined antigenic targets. Most autoimmune diseases are not life threatening and can be effectively treated even if they are not curable. However, some autoimmune diseases may be life threatening or damage critical organs and become refractory to conventional treatments. This, then, would be the candidate population for consideration of an approach requiring autologous or allogeneic hematopoietic cell transplantation (HCT).

Proposed mechanisms for autoimmunity and autoimmune diseases

To understand better how an approach using HCT might be effective for the treatment of an autoimmune disease, an understanding of the pathogenic mechanisms of autoimmune disease is required. There is a marked diversity of the B- and T-cell receptor specificity that results from somatic genome modification in lymphocytes. This allows for an effective immune response to foreign antigens. The unique specificities of these receptors are generated during B- and T-cell differentiation by recombination of the variable (V), diversity (D), and joining (J) genes and then later, in B cells, somatic hypermutation generates further diversity. These random processes of recombination and somatic hypermutation result in a significant percentage of lymphocytes (up to 50%) with B- and T-cell receptors that have an affinity for self-antigens [2].

It has been proposed that there are four cellular strategies to prevent the development of autoimmunity and autoimmune diseases from lymphocytes with self-reactive receptors. First, lymphocytes with high-affinity, self-reactive receptors can be clonally deleted. Clonal deletion occurs in central lymphoid organs during development. Second, a self-reactive receptor can be edited by further V(D)J recombination or somatic hypermutation to display a different receptor that is not self-reactive [3]. Receptor editing induced by self-reactivity can occur in both B and T cells. For B-cell receptor editing, new rearrangements at L chain loci and less commonly at H chain loci are induced. Third, downregulation of cell surface self-reactive receptors and intracellular changes, including those which affect signal pathways, decrease responsiveness to binding of self-reactive receptors, resulting in clonal anergy [4]. Last, extrinsic factors such as cytokine dependence for proliferation and regulatory mechanisms including regulatory T cells may limit the activity of lymphocytes with self-reactive receptors. In B cells with self-reactive receptors, B-cell-activating factor (BAFF) receptor is poorly induced and therefore may be more susceptible to competitive regulation by their requirement for BAFF than other B cells with non-self-reactive receptors.

Some T cells with self-reactive receptors are positively selected in the thymus after T-cell receptor engagement by self-peptide–major histocompatibility complex (MHC) class II complexes. They develop into $CD25^+CD4^+$ $FoxP3^+$ regulatory T cells, which suppress proliferation and interleukin-2 (IL-2) production in autoreactive $CD25–CD4^+$ and $CD8^+$ T cells [5]. Regulatory T cells are IL-2 dependent with constitutively expressed CD25 (IL-2 receptor) and IL-7 independent without CD127 (IL-7 receptor). Natural killer (NK) T cells and other T-cell subsets producing transforming growth factor-β (TGF-β) and IL-10 have also been associated with immune regulation.

The genetics of autoimmune disease

Autoimmune disease can be associated with either single gene defects or susceptibility genes as part of a complex multigenic trait. Four inherited disorders from single gene defects have been described, which have contributed further insights into autoimmune disease mechanisms in humans (Table 66.1) [6–9]. These include defects in (1) autoimmune regulator (AIRE)-dependent negative selection in the thymus, (2) cytotoxic T-lymphocyte antigen-4 (CTLA-4)-dependent inhibition of T-cell function,

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Table 66.1 Single gene defects associated with autoimmunity

Autoimmune disease	Gene defect	Mechanisms of autoimmunity
APS1[a] [6]	*AIRE*	Defective expression of thymic self-antigens
Graves disease, Type I diabetes, others [7]	*CTLA4*	Defect in inhibitory signaling
ALPS[b] [8]	*FAS, FASL*	Defect in apoptosis
IPEX[c] [9]	*FOXP3*	Decreased regulatory T cells

[a] Autoimmune polyglandular syndrome type 1.
[b] Autoimmune lymphoproliferative syndrome.
[c] Immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome.

(3) Fas/FasL (Fas ligand)-dependent apoptosis of B and T cells that recognize self-antigens, and (4) FoxP3-dependent development of regulatory T cells.

The most common autoimmune diseases are considered to result from alleles of susceptibility genes at multiple loci, environmental triggers, and stochastic events. The disease-associated alleles of susceptibility genes have been shown to confer only modest risk for the development of an autoimmune disease and were first identified among the MHC genes [10]. Most correlations of autoimmune diseases were with MHC class II rather than class I molecules. Other susceptibility genes affect, in general, pathways for activation/inhibition of T cells (IL-7R, IL-2R, PDCD-1, PTPN22, CTLA4) or presentation of self-antigens (insulin, PADI4) [11–13]. It is unclear to what extent environmental factors contribute to the risk of developing autoimmune disease. Environmental factors including hydralazine, contaminated rapeseed oil, L-tryptophan, and *Borrelia burgdorferi* infections in association with specific alleles of the MHC loci have been shown to increase the risk of an autoimmune disease.

Family or twin studies may provide further information about the contribution of genetic factors to the onset or presentation of autoimmune diseases. In general, the disease concordance rates among monozygotic twins was ≥3-fold greater than in dizygotic twins for rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), type I diabetes mellitus (DM), and multiple sclerosis (MS) [14,15]. The average concordance rates reported for monozygotic twins ranged from 15 to 30% for DM, MS, SLE, and RA. It has also been reported that there was a higher risk for many autoimmune diseases in other family relatives. Although genetic factors appear to play a role in the development of autoimmune diseases, the relatively low penetrance as noted in the monozygotic twins indicate that non-genetic factors are also important.

Preclinical models of autoimmunity and hematopoietic cell transplantation

There are two potential approaches utilizing HCT to consider for the treatment of autoimmune diseases. The first approach is autologous HCT after high-dose immunosuppressive therapy (HDIT). The therapeutic effects derive from the high-dose cytotoxic immunosuppression. Allogeneic HCT, as the second approach, can be performed after either high-dose or reduced-intensity conditioning regimens. The therapeutic effect of this approach likely derives from the eradication of autoreactive immune effector cells when complete donor hematopoietic chimerism is established. However, remissions of autoimmune diseases have been reported after establishing only mixed hematopoietic chimerism. Therefore, it is likely that the immunomodulation from establishing the donor graft including effects on regulatory mechanisms may have a self-tolerizing effect even if there are persistent host autoreactive immune cells.

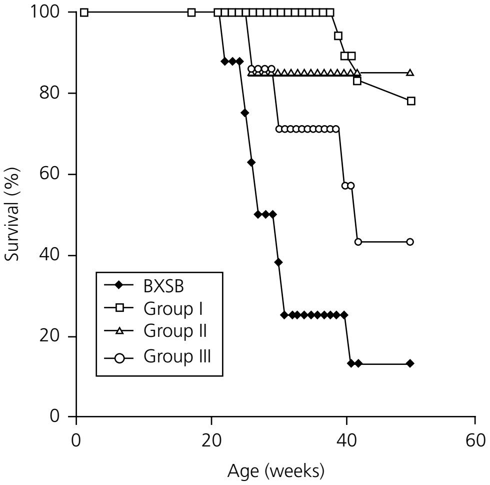

Figure 66.1 Survival curves of BXSB mice (spontaneous lupus-like syndrome) after treatment with 9.5 Gy of TBI (^{137}Cs irradiation; 0.70 Gy/min) and transplantation with T-cell-depleted marrow cells intravenously at 16 weeks of age from BXSB or normal BALB/c donors after the development of the autoimmune disease. The transplant groups were: BALB/c + BXSB → BXSB group (squares; group I; $n = 18$); BALB/c → BXSB group (triangles; group II, $n = 8$); and BXSB → BXSB group (circles; group III; $n = 7$). Untreated BXSB mice served as controls (diamonds; $n = 8$). Compared with untreated controls or group III (pseudoautologous), survival was improved in recipients of the allogeneic marrow grafts. Source: Wang et al. 1999 [16]. Reproduced with permission of the National Academy of Sciences, USA.

There are two types of models for autoimmune disease in which studies of autologous and allogeneic HCT have been conducted: (1) a spontaneously developed autoimmune disease, such as a lupus-like systemic condition in NZB (New Zealand black) mice, or an organ-specific condition, such as diabetes mellitus in NOD (non-obese diabetic) mice, and (2) antigen-induced autoimmune diseases, such as adjuvant arthritis and experimental allergic encephalomyelitis (EAE). In the spontaneous autoimmune disease models, high-dose cytotoxic therapy and syngeneic bone marrow transplantation early in life did not prevent the development of the clinical or pathologic autoimmune process, whereas allogeneic marrow transplants from an autoimmune-resistant strain prevented and, when given early after disease onset, ameliorated the autoimmune manifestations (Figure 66.1) [16,17].

In contrast to the experience in models of spontaneous-onset autoimmune diseases, studies of antigen-induced autoimmune diseases have shown therapeutic responses in recipients after high-dose cytotoxic immunosuppressive treatments and syngeneic or pseudoautologous HCT. Pseudoautologous donors were syngeneic donors with the same disease severity as the recipients at the time of HCT. Experimental allergic encephalomyelitis, an experimental model for MS, may be induced in Buffalo rats and SJL/J mice by active immunization with myelin basic protein (MBP). In this model, high-dose therapy with total body irradiation (TBI) or cyclophosphamide followed by allogeneic, syngeneic, or autologous marrow rescue can prevent or ameliorate disease activity [18,19]. Allogeneic HCT was superior in preventing spontaneous and induced relapses from reimmunization of MBP. In rats, after immunization with a unique peptide fragment of myelin, the CD4$^+$

T-lymphocyte cell initiating EAE is skewed towards overutilization of the V_β8.2 T-cell receptor. When analyzed by reverse transcriptase polymerase chain reaction and in situ hybridization, V_β8.2 T-lymphocytes were not detected in the central nervous system (CNS) after HDIT and syngeneic HCT [20]. Therefore, high-dose therapy was able to eliminate the effector lymphocytes responsible for EAE from the CNS in this model. Similarly, in adjuvant-induced arthritis, the prevention of relapses after HDIT followed by syngeneic HCT and repeat immunization is consistent with a tolerizing effect to the inciting antigens. Increased intensity of the high-dose cytotoxic immunosuppression resulted in more effective induction of remissions and less relapses [21].

Observations in preclinical models of spontaneous-onset autoimmune diseases support the use of allogeneic HCT for those human autoimmune diseases related to single gene disorders with high penetrance (e.g., IPEX syndrome). However, for the other more common human autoimmune diseases, in which environmental or stochastic factors may play a larger role for triggering disease onset, the preclinical data support the investigations of HDIT and autologous HCT.

Outcomes in patients with autoimmune diseases transplanted for another primary disease

Observations in patients with hematologic disorders and a concomitant autoimmune disease gave us our first understanding that the response of human autoimmune diseases to allogeneic HCT could be comparable to the experience in the preclinical models (Table 66.2) [22–36]. Baldwin et al. first reported sustained remissions in patients who were transplanted with severe aplastic anemia (SAA) but also had a diagnosis of RA [22]. Follow-up was short and there were early transplant-related deaths. Subsequent case reports of patients transplanted with a concomitant diagnosis of RA confirmed that remissions could be achieved and be sustained for >20 years [27]. It was also noted that three of the nine patients with RA relapsed after allogeneic HCT. One of the relapses was transient with a subsequent treatment-free remission of 11 years. In one of the two sustained relapses for which data were available, the recipient had complete donor hematopoietic chimerism, but the human leukocyte antigen (HLA)-identical donor was serologically positive for rheumatoid factor but without clinical disease [23].

Sustained remissions and some relapses were also observed in patients with other concomitant autoimmune diseases after allogeneic HCT. Four of five patients with Crohn disease had sustained remission with follow-up for 4.5–15.3 years after allogeneic HCT for leukemia [32]. The one patient who relapsed with Crohn disease had mixed hematopoietic chimerism detected at 3 months after HCT. There were insufficient data from the case reports to develop an understanding of why relapses occurred after allogeneic HCT. In all but one of the cases reporting the use of allogeneic HCT for concomitant autoimmune diseases, the donor was an HLA-identical sibling. Therefore, one possible explanation for recurrence of the autoimmune diseases may be the presence of shared genetic factors between the related donors and recipients. Another possible explanation is the persistence of host immune cells resulting in recurrence of disease activity. These case reports, although small in number, were important in establishing our understanding of the potential role of allogeneic HCT for treatment of patients with severe autoimmune diseases.

High-dose immunosuppressive therapy and autologous hematopoietic cell transplantation for autoimmune diseases: clinical experience

HDIT has been performed most commonly for MS, systemic sclerosis (SSc), SLE, RA, juvenile idiopathic arthritis (JIA), and immune cytopenias [37]. HDIT regimens that have been investigated have had varying intensities. High-dose single-agent cyclophosphamide has been considered a low-intensity regimen [38]. It is highly immunosuppressive but is not myeloablative. Clinical trials of high-dose cyclophosphamide have been conducted with and without the support of autologous HCT. Regimens that included TBI or high-dose busulfan were considered high intensity and required support with autologous HCT.

Patient selection

Patients eligible for clinical trials of HDIT were generally those with severe autoimmune disease (life threatening or threatening the function of critical internal organs) and had failed conventional therapy. Although the criteria for selecting patients depended upon the type of autoimmune disease, it was usually required that patients have a poor prognosis based on the activity and severity of the disease.

Collection and processing of the hematopoietic cell graft

Although some of the earliest adult cases were transplanted with marrow grafts, most of the later patients were transplanted with hematopoietic cell grafts harvested from the peripheral blood [39].

Table 66.2 Outcomes in patients with autoimmune diseases transplanted with an allogeneic hematopoietic cell graft for another primary disease

Autoimmune disease	Hematologic disease	Evaluable patients (total) (*n*)	Remission of autoimmune disease (after HCT) (*n*)	Outcome (alive at last follow-up) (*n*)	Follow-up
Rheumatoid arthritis [22–28]	SAA (*n* = 8), 1 MM	9	7 (2 relapses + 1 transient relapse)	6	2 mo–21 yr
Systemic lupus erythematosus [29]	SAA	1	1[a] (ANA titer +)	1	15 yr
Psoriatic arthritis [30,31]	AML, CML (*n* = 3)	4	3 (1 relapse)	3	1, 3, 5, 5 yr
Ulcerative colitis [30]	AML	1	1	1	4 yr
Crohn disease [32]	CML	5	4[b]	5	4.5–15.3 yr
Multiple sclerosis [33–36]	CML (*n* = 2), LGL leukemia, AML	4	3	3	1, 2, 3, 4 yr

AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; ANA, antinuclear antibody; CML, chronic myelogenous leukemia; LGL, large granular lymphocytes; MM, multiple myeloma; SAA, severe aplastic anemia.

[a] Clinical remission but ANA remained positive.

[b] Relapse occurred in one patient with mixed chimerism.

Marrow was the preferred source of hematopoietic stem cells for many pediatric patients [40]. The optimal dose of peripheral blood CD34$^+$ cells for successful engraftment was $2–5 \times 10^6$/kg in patients with hematologic malignancies, and therefore this was the number targeted for collection in patients with autoimmune diseases. In a multicenter, retrospective analysis, eight of 173 patients (4.6%) failed to mobilize sufficient numbers of CD34$^+$ cells to allow them to proceed to HDIT [39]. Five of these eight patients eventually had adequate numbers of cells harvested by either a supplemental marrow harvest ($n = 2$) or a successful second mobilization ($n = 3$).

Early in the clinical trial experience of HDIT for autoimmune diseases, it was observed that some patients had disease flares during mobilization. In one review, five of 56 patients mobilized with granulocyte colony-stimulating factor (G-CSF) alone had exacerbations of the autoimmune disease (MS, $n = 2$; RA, $n = 3$) [39]. There have been other reports of exacerbations during mobilization of MS and RA patients with G-CSF alone [41,42]. Most exacerbations were transient, but one patient with MS died from the development of severe brainstem dysfunction. As a result of these early flares, most patients have now been mobilized with a combination of cyclophosphamide and G-CSF. In 117 patients mobilized with this combination, no exacerbations were observed. In fact, after mobilization with cyclophosphamide, an improvement was noted in the underlying autoimmune disease. The addition of cyclophosphamide to G-CSF also improved the yield of progenitor cells after mobilization [39]. However, there were three patient deaths in this report from either cyclophosphamide-related toxicity or infections. In another report of 126 patients from a single center who were mobilized with a combination of G-CSF and cyclophosphamide, there was one patient death from infection [43]. Four mild exacerbations of disease and seven infectious events were also observed during mobilization with a cyclophosphamide-based regimen. Another mobilization strategy that has been used in 22 MS patients without disease exacerbations or infections is the combination of prednisone and G-CSF [44]. G-CSF alone has been used to mobilize 34 patients with SSc without any significant disease exacerbations except for a few cases in which there was an increase in telangiectasias or skin tightness [45]. The risk of exacerbations during mobilization using G-CSF alone may be related to the type of autoimmune disease. The use of cyclophosphamide as part of the mobilization regimen should be carefully considered, possibly by the type of autoimmune disease because of the potential risks.

Many of the reported clinical trials used T-cell-depleted autologous grafts as a strategy to prevent recurrence of the autoimmune disease. This strategy was based on observations in preclinical models in which the autoimmune disease could be adoptively transferred with T cells. In most of the clinical trials, CD34 selection was performed and resulted in a 3–4 log depletion of T cells in the autologous graft. Further T- and B-cell depletion was carried out in some clinical trials after the CD34 selection. The importance of T-cell depletion for prevention of progression or relapse was addressed in a retrospective analysis of registry data from the European Group for Blood and Marrow Transplantation (EBMT). There was no significant effect associated with T-cell depletion of the graft [38]. In the most recent analysis of the EBMT registry data, T-cell depletion did not effect 100-day treatment related mortality, 3-year progression-free survival, or 3-year overall survival [37]. Although the risk of infection may be increased with T-cell depletion of the autologous graft, the incidence of infectious disease was low if these patients are managed with the same infection prophylaxis strategies as used for patients after allogeneic HCT [40,46]. The contribution of T-cell depletion of the autologous hematopoietic cell graft to the prevention of progression or relapse remains to be determined in future clinical trials.

High-dose immunosuppressive therapy regimens

The early rationale for HDIT was the systematic depletion of autoreactive immune effector cells. With long-term follow-up of the pilot studies of HDIT with autologous HCT now available, it has been observed that there is a high initial response rate and a significant proportion of patients have achieved sustained remissions [40,45,47–49]. The clinical response early after HDIT likely resulted from the overall reduction of immune effector cells responsible for the autoimmune disease. The sustained response observed after recovery of the lymphocyte counts at 2 years may have resulted from a sustained immunomodulatory effect of the HDIT regimen [46,50,51]. Randomized clinical trials need to be completed to confirm that the clinical responses after HDIT are an improvement over what might have been expected from conventional management strategies.

The intensity of the HDIT regimen appears to be important for the prevention of disease progression. In a report of 473 patients from the EBMT registry, sustained responses were observed in 78% of patients after a regimen with high-intensity conditioning compared with 68% with intermediate- and 30% with low-intensity conditioning regimens ($p = 0.0001$) [38]. Regimens that included busulfan or TBI in combination with cyclophosphamide were placed in the high-intensity conditioning group, and cyclophosphamide alone was placed in the low-intensity conditioning group. All other regimens were considered of intermediate intensity. The analysis of the registry data had some limitations, including variability of the diagnoses, patient selection criteria, and treatments (in addition to having imbalances between diagnosis and type of HDIT regimen), but a dose intensity–response effect on treatment outcomes was evident.

One of the most frequently used HDIT regimens was high-dose single-agent cyclophosphamide (200 mg/kg) followed by autologous HCT for hematopoietic support [38]. A small study compared 100 with 200 mg/kg of cyclophosphamide followed by HCT in patients with RA. Remissions were longer with the higher dose of cyclophosphamide, but all patients relapsed [52]. The high relapse rates after HDIT may be a unique feature of RA in comparison with other autoimmune diseases. The addition of antithymocyte globulin (ATG) to cyclophosphamide may improve the response rate and duration. Since it is not myeloablative, high-dose cyclophosphamide (200 mg/kg) as a single agent without autologous HCT has been reported for the treatment of autoimmune diseases or immune-mediated diseases such as aplastic anemia [53,54]. The median time to recovery of neutrophils counts was 2–3 days longer without the support of an autologous hematopoietic cell graft but the upper limit of the range was 7 days longer. The relapse rate was also high in SLE patients ($n = 14$) after high-dose cyclophosphamide without HCT, but a significant proportion (36%) had durable complete remissions. In this small experience, relapse rate was high even if the infusion of an autologous hematopoietic cell graft was withheld.

High-dose combination chemotherapy with BCNU, etoposide, cytosine arabinoside (Ara-C), and melphalan (BEAM) with or without ATG was considered an intermediate-intensity regimen [38]. For autoimmune diseases, this regimen has most commonly been used for the treatment of MS [55]. It has been well tolerated in this group of patients and no significant data exist for other autoimmune diseases. Regimens with either TBI or busulfan in combination with cyclophosphamide were considered to be high intensity [40,44,45,56–58]. A pilot study of busulfan in combination with cyclophosphamide has been reported for the treatment of MS [58]. The use of TBI in combination with cyclophosphamide has been reported in clinical trials of HDIT for MS, SSc, and JIA. TBI is highly immunosuppressive and was shown to be effective in preclinical models of autoimmune disease [21,59]. Organs can be shielded with lead during TBI to protect them from the potential adverse effects of this form of cytotoxic therapy, which is not possible with systemic chemotherapy. TBI has the potential for killing cycling and non-cycling stem cells and also immune effector cells. Support with an infusion of autologous hematopoietic stem cells is required after both intermediate- and high-intensity HDIT regimens to prevent prolonged pancytopenia or lack of hematopoietic recovery.

From the EBMT registry, treatment-related mortality at day 100 was 5% and 4% in the groups who received the high and the low conditioning intensity regimens, respectively [37]. There was no difference in 100-day treatment-related mortality based on the intensity of the HDIT regimen but there was an association with the type of autoimmune disease and the transplant's center experience. Treatment-related mortality resulted primarily from infections and severe organ toxicity. Several fatal cases and one non-fatal case of Epstein–Barr virus (EBV)-associated post-transplant lymphoproliferative disorder (PTLD) have been reported [57,60]. In patients who have had a high-intensity immunosuppressive regimen, early detection of EBV reactivation and treatment with rituximab may prevent the clinical manifestations of PTLD. The risks of infectious complications and organ toxicities after HDIT are closely related to the type of autoimmune disease being treated. Patients with MS and RA had a lower treatment-related mortality than patients with SSc and SLE who had significant internal organ involvement at the time of transplant. Prolonged or intensive pre-transplant treatment with immunosuppressive agents including corticosteroids, as in the management of patients with severe SLE, likely predispose to infectious complications after HDIT. Better patient selection and modifications to the treatment regimen appear to have reduced the risks of treatment-related mortality in recent years [37]. Long-term complications after high-dose cytotoxic therapy include the risk for secondary myelodysplastic syndrome (MDS) and leukemia [37,45,57]. In one of the reported cases of MDS, evidence of clonal abnormalities was found in cryopreserved pre-transplant hematopoietic cells. The intensity and duration of the pre-transplant chemotherapy are known to be an additional risk factor for the development of secondary MDS and leukemia. Secondary autoimmune diseases (other than the one for which patients were transplanted) have been described late after HDIT and autologous HCT [45,61,62]. Alemtuzumab in the HDIT regimen may increase the risk of this occurring [61,63].

Immune reconstitution after a high-dose immunosuppressive therapy regimen

NK-cell counts recovered by 1 month and this was followed by recovery of B- and $CD8^+$ T-cell counts by 6–12 months after HDIT. There was a slower recovery of $CD4^+$ T-cell counts, which were low-normal by 2 years [46]. Immune recovery at 2 years after HDIT was associated with increasing thymic-derived naïve $CD4^+$ T cells (Figure 66.2). There was an increase in T-cell receptor excision circles (TRECs) (a marker for recent thymic emigrants) in $CD4^+$ T cells at 1 and 2 years after HDIT. There was a steady decrease over time in $CD4^+$ central memory T cells. $CD4^+$ effector memory cells were relatively increased at 6 months after HDIT, likely from homeostatic proliferation, but had recovered to normal levels by 2 years. There were no significant changes in the $CD8^+$ T-cell subsets. There was an increase in $CD4^+$ and $CD8^+$ regulatory T cells, and broader clonal diversity than was present before HDIT [50,51,64,65]. In association with the increased levels of naïve $CD4^+$ T cells, there was hypertrophy of the thymus at 1 and 2 years compared with baseline, especially in the younger patients (less than 43 years of age). This evidence suggested a thymic origin for the recovery of the $CD4^+$ T-cell repertoire after HDIT and autologous HCT. Even though B-cell counts were very low in the first 3 months after HDIT, median serum levels of immunoglobulin G specific for tetanus toxoid, *Haemophilus influenzae* and *Streptococcus pneumoniae*, were normal. The clinical responses to HDIT that have persisted for 5 years or longer may have resulted from these immunomodulatory effects associated with HDIT.

Specific autoimmune diseases and hematopoietic cell transplantation

Clinical trials of HDIT and autologous HCT have been conducted for specific autoimmune diseases, and in some cases the results from long-term follow-up evaluations are available.

Multiple sclerosis

MS is an autoimmune inflammatory disorder of the CNS manifesting as acute focal demyelination and axonal loss with limited remyelination and focal sclerotic changes [66]. Recurrent inflammatory events result in the development of chronic multiple sclerotic plaques in the brain and spinal cord. MS has a lifetime risk of 1 in 400, affects 350,000 people in the United States, and is potentially the most common cause of neurological disability in young adults. Seven of 27 monozygotic pairs (25.9%) and one of 43 dizygotic pairs (2.3%) were concordant for MS, which suggests that there is a genetic predisposition for development of MS with a contribution of environmental factors [15]. Using genome-wide association studies, HLA DRB*1501 has been identified as an MHC risk locus. Other susceptibility loci were identified in the IL-2 receptor and IL-7 receptor which are expressed on regulatory T cells [66]. It is postulated that myelin proteins, such as MBP, proteolipid protein (PLP), and perhaps myelin oligodendrocyte glycoprotein (MOG), are targeted by pathogenic $CD4^+$ T cells in patients with MS. The pathology shows a predominant T-cell response both in perivascular spaces and in the demyelinated lesion. Axonal injury is evident not only in the acute inflammatory and the chronic non-enhancing lesions but also in the normal-appearing white matter.

The clinical manifestations of the disease may include loss of vision from optic neuritis, diplopia, sensory loss and paresthesias, vertigo, fecal and/or urinary incontinence, impotence, intellectual decline, paroxysmal pain, recurrent infections, loss of coordination, or paralysis. Clinical diagnosis depends on two or more attacks involving non-contiguous anatomic regions separated by at least 1 month or progression of symptoms or signs for more than 6 months. About 85% of patients present with relapsing–remitting MS (RRMS), and over 10 years about 50% of these patients will develop secondary progressive disease with cumulative disability. The other 15% of patients have progressive disease from onset (primary). The standard for measuring outcome in studies of MS is the Kurtzke Expanded Disability Status Scale (EDSS) and, more recently, the Multiple Sclerosis Functional Composite (MSFC).

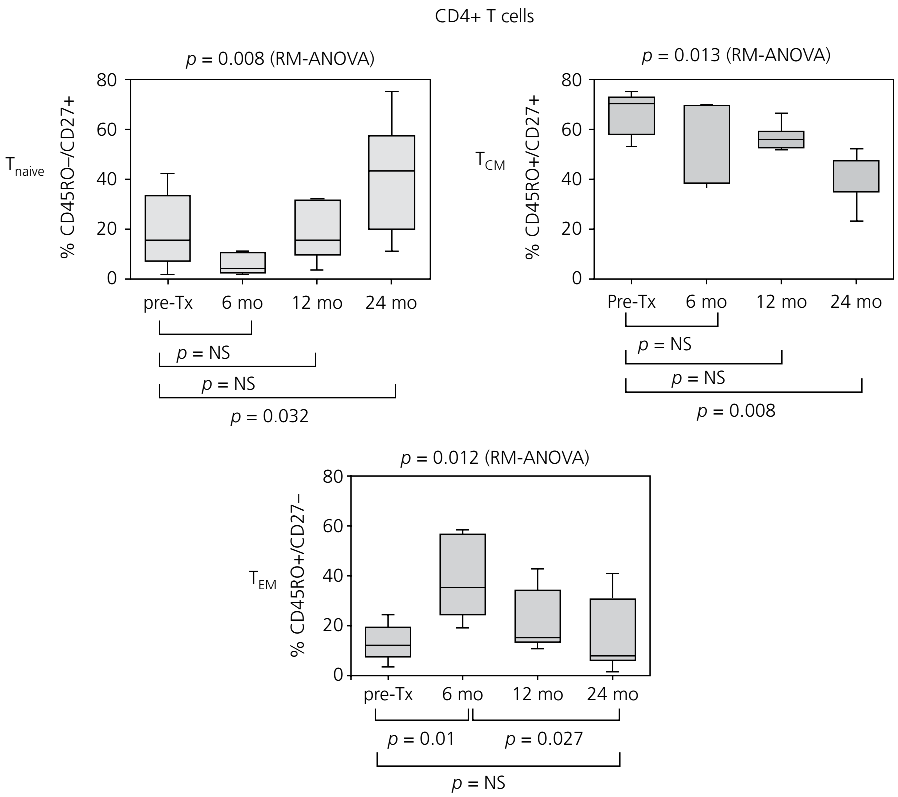

Figure 66.2 Naïve CD4$^+$ T cells increased and central memory CD4$^+$ T cells decreased at 2 years after HDIT and autologous HCT for MS. In a longitudinal analysis of T-cell subsets after HDIT and autologous HCT, a trend towards reduction of naive (CD45RA$^+$/CD45RO$^-$/CD27$^+$) CD4$^+$ T cells was observed at 6 months, consistent with an increase in effector memory (EM) phenotypes resulting from homeostatic proliferation in a lymphopenic environment. Central memory (CM; CD45RA$^-$/CD45RO$^+$/CD27$^+$) CD4$^+$ cells decreased steadily during the follow-up. The proportion of EM (CD45RA$^-$/CD45RO$^+$/CD27$^-$) CD4$^+$ T cells rose significantly at 6 months after HSCT and later declined toward baseline levels after the gradual recovery of the absolute CD4$^+$ T-cell numbers. At the 2-year follow-up, the frequency of naïve CD4$^+$ T cells had increased by 118% as compared with pre-therapy ($p = 0.032$). Correspondingly, CM CD4$^+$ T cells had decreased by 38% at 2 years after therapy ($p = 0.008$). The frequencies of the EM CD4$^+$ T cells did not change significantly at the 2-year follow-up compared with the baseline. These data support the concept that there is significant immunomodulation at 2 years after HDIT and autologous HCT, and this may explain the observed durable clinical remissions in a significant proportion of patients with autoimmune disease. Source: Muraro et al. 2005 [50]. Reproduced with permission of Rockefeller University Press.

There is little influence of MS on survival until at least 15 years after onset. At 20 years after onset, patients with MS had 85% of the expected survival. Prognostic factors for worse outcome have been identified and include increased age, short intervals between relapses, high frequency of relapses after disease onset, and early significant disability. Despite responses to immunomodulating agents, no standard therapy is curative or has been demonstrated to prevent the development of a progressive clinical course. Agents that have been demonstrated to be effective as disease-modifying therapies in RRMS include interferon (IFN) beta-1a, IFN beta-1b, glatiramer acetate (GA), mitoxantrone, natalizumab, fingolimod, teriflunomide, and dimethyl fumarate. Treatment reduced the clinical relapse rates in patients with RRMS by 30–68%, with the newer agents being more effective than IFN or GA. These agents, however, remain inadequate to prevent relapses and progression completely. No effective therapy has yet been identified for primary or secondary progressive MS.

The results of phase II clinical trials of HDIT and autologous HCT for MS have been reported from many North American and European centers (Table 66.3) [44,55–58,67–69]. Although there was variability in the design of each of these clinical trials, they all included patients with advanced MS and the progressive type of the disease. All patients received high-dose combination chemotherapy or TBI and cyclophosphamide. In seven of the eight clinical trials, the hematopoietic cell grafts were mobilized from the peripheral blood, and all but one of the trials included T-cell depletion with CD34 selection. Overall treatment-related mortality in these clinical trials was 2.8% (4/145) and the progression-free survival or rate of neurologic stability reported in the individual clinical trials ranged from 36 to 95% at 2–3 years after treatment. Five patients (3.5%) died after progression of disease and further loss of neurologic function or other complications.

Although no direct comparisons can be made between the clinical trials regarding outcomes, there did not appear to be any advantage to including TBI in the HDIT regimen. In a report by Saccardi et al., progression-free survival at 6 years was 95% [55] (Figure 66.3). In a long-term follow-up report of one of the earliest studies, progression-free survival was 44% at 15 years in patients with active CNS disease at baseline and 10% in patients without active disease at baseline [48]. Although magnetic resonance imaging (MRI) after transplant showed a marked reduction in Gd-enhancing brain lesions in all the groups studied compared with baseline, it is still uncertain if the continued loss of neurologic function observed in patients with progressive MS is the result of a degenerative process or a failure to control completely inflammation related to the autoimmune disease. Randomized clinical trials

Table 66.3 Clinical trials of HDIT and autologous HCT for multiple sclerosis

Study	No. of patients (*n*)	MS type (*n*)	Median EDSS[a] (range)	Mobilization	High-dose therapy	T-cell depletion	Median follow-up (range) (months)	Treatment-/ disease-relatedmortality(*n*)	Clinical result
Fassas [67]	35	SP (19) PP (14) RR (2)	6.0 (4.5–8.0)	G/GM-CSF + CY	BEAM + ATG	CD34 selection	35 (3–67)	1/1[b]	PFS 81% at 5 yr
Openshaw [58]	5	SP	6.5 (5.5–7.5)	G-CSF only	BU/CY	CD34 selection	22 (17–30)	2/0	PFS 40%
Kozak [69]	10	SP	6.5 (6.0–7.5)	G-CSF + CY	BEAM ± ATG	CD34 selection + monoclonals	8 (1–18)	0/0	90% stable or improved
Saiz [68]	14	SP (9) RR (5)	6.0 (4.5–6.5)	G-CSF + CY	BCNU/CY + ATG	CD34 selection	37 (19–55)	0/0	PFS 86%
Saccardi [55]	19	SP (15) RR (4)	6.5 (5.0–6.5)	G-CSF+ CY	BEAM + ATG	None	36 (12–72)	0/0	PFS 95% at 6 yr
Nash [44]	26	SP (17) PP (8) RR (1)	7.0 (5.0–8.0)	G-CSF + prednisone	TBI/CY + ATG	CD34 selection	27 (2–47)	1/1	76% stable or improved
Burt [56]	21	SP (14) PR (6) RR (1)	7.0 (3.0–8.5)	G-CSF + CY	TBI/CY	CD34 selection	24 (12–60)	0/2	62% stable or improved
Samijn [57]	14	SP	6.0 (5.0–6.5)	None (marrow)	TBI/CY + ATG	CD34 selection	36 (7–36)	0/1	PFS 36%
Burt [63]	21	RR	3.1 (2.0–5.5)	G-CSF + CY	CY + Campath or ATF	None	Mean 37 (24–48)	0/0	PFS 100%; disease activity free 62%

ATG, antithymocyte globulin; B, BCNU; BEAM, BCNU, etoposide, cytosine arabinoside (Ara-C), melphalan; BU, busulfan; CY, cyclophosphamide; PFS, progression-free survival; PP, primary progressive; PR, progressive relapsing; RR, relapse-remitting; SP, secondary progressive; TBI, total body irradiation. EDSS, Expanded Disability Status Scale.

[a] In brief, the functional levels of the Expanded Disability Status Scale (EDSS) are graded from 0 to 10 points and include changes in increments of 0.5 points. An EDSS score of 0 indicates a normal neurologic examination in all functional systems (FS). An EDSS score of 10 indicates death from MS. Most of the MS patients who entered these clinical trials of HDIT and autologous HCT had an EDSS score of 5.0–8.0. In general, the function of patients at these different EDSS scores are as follows:

5.0 Ambulant 200 m without aids, difficulty to work full day, FS grade 5.
6.0 Intermittent or unilateral walking aid for 100 m.
7.0 Wheelchair-bound (walking <5 m with assistance). Able to transfer and use wheelchair alone. Sometimes severe pyramidal grade 5.
8.0 Bed- and chair-bound, self-care functions retained (arm function retained), sitting out of bed most of the day.

[b] One patient with progression of MS was started on interferon beta after a relapse, developed a factor VIII inhibitor at 14 months, and died at 28 months after HDIT and autologous HCT.

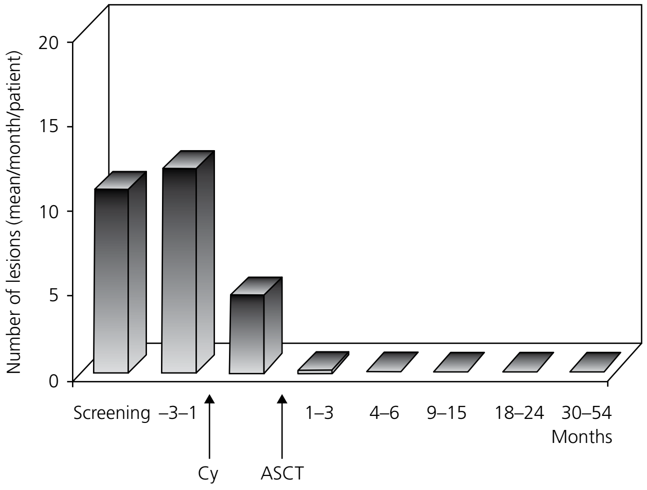

Figure 66.3 Gadolinium-enhancing lesions in the brain after HDIT (BEAM) and autologous HCT: MRI results. The mean numbers of gadolinium-enhancing lesions in the brain per month per patient are reported at baseline during the 3-month period before mobilization (-3–1), after mobilization (Cy), and after transplantation (ASCT) to last follow-up. The duration of gadolinium-enhancement of a new lesion is approximately 4 weeks so the presence of gadolinium-enhancing lesions in the central nervous system indicates recently active disease. After HDIT and autologous HCT there is a marked reduction in enhancing lesions for the duration of the follow-up. Source: Saccardi et al. 2005 [55]. Reproduced with permission of the American Society of Hematology.

need to be completed to confirm if there is a therapeutic benefit of HDIT and autologous HCT in this patient population with advanced MS. More recent studies of HDIT and autologous HCT have focused on patients with RRMS and less advanced neurologic disabilities at baseline [63]. After a mean follow-up of 37 months in a study by Burt et al. [63], progression-free survival was 100% and 16 of 21 patients were free from relapse. Significant improvements were noted in neurological disability and quality of life measurements (Figure 66.4).

Systemic sclerosis

SSc is an uncommon disabling autoimmune disease that is associated with a high mortality. In studies performed in different geographic settings, the prevalence has ranged from 28 to 286 per million, and the incidence has been reported to range from 3.7 to 19.2 per million [70]. SSc is characterized by two major clinical features: (1) a non-inflammatory small-vessel vasculopathy and (2) fibrosis of the skin and multiple internal organs [70]. There is evidence of immune dysregulation with SSc-specific autoantibodies, mononuclear cell infiltrates in several affected organs, and elevated cytokine levels in tissue and serum. The anticentromere antibody is found in the group with limited cutaneous SSc, and the anti-topoisomerase I antibody (Scl-70) is found in 30–40% of subjects with diffuse cutaneous SSc. Antinuclear antibodies occur in 95% of subjects overall. Diffuse cutaneous SSc is a more severe manifestation of disease than limited cutaneous SSc. It has a higher mortality and is associated with substantial morbidity as a consequence of digital ischemia/skin ulcerations from the vasculopathy, both truncal and acral scleroderma, interstitial lung

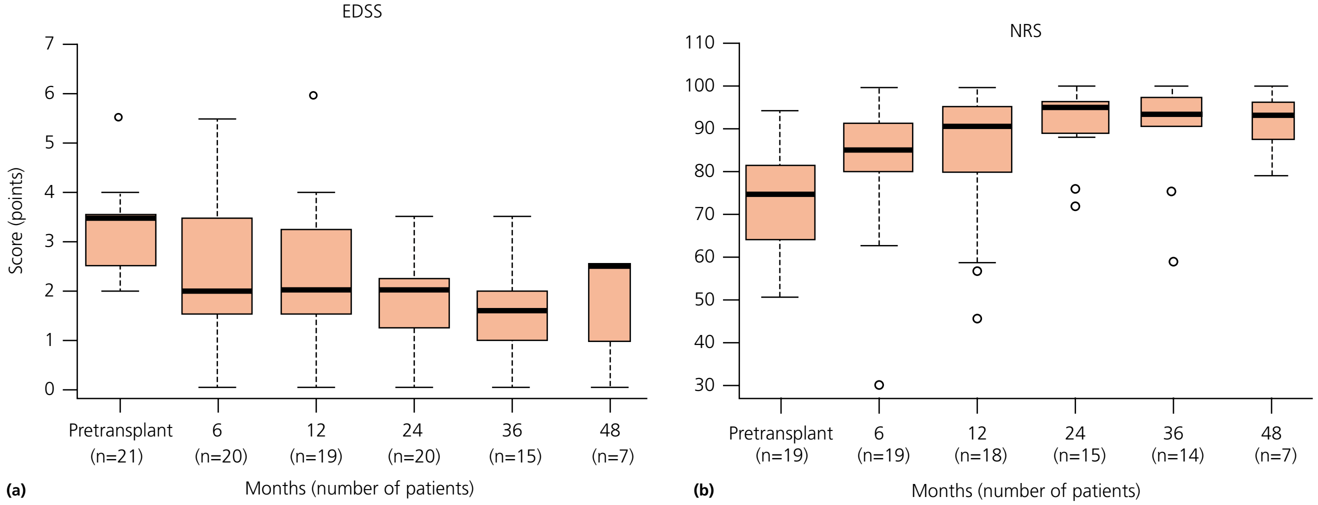

Figure 66.4 Clinical improvement after high-dose immunosuppressive therapy and autologous HCT as measured by the Kurtzke Extended Disability Status Scale (EDSS) and the Scripps Neurological Rating Scale (NRS). (a) The EDSS is a neurologic performance scale that increases with increasing disability at intervals of 0.5 points from 0 (healthy) to 10 (death from neurologic disability). (b) The NRS is a neurological performance scale that increases at intervals of 1 point, with decreasing disability going from 0 points (dead) to 100 points (healthy). Although all patients had EDSS score recorded before transplantation, a few missed one or more of the pre-transplantation tests and a few patients declined a post-transplantation interval follow-up, but did return for the next follow-up visit. Thick lines are medians; boxes are values for upper and lower quartiles. Open circles are outliers, defined as values that are more than 1.5 times the 25th or 75th percentiles. Source: Burt et al. 2009 [63]. Reproduced with permission of Elsevier.

disease, hypertensive renal crisis, diffuse gastrointestinal disease, and myocardial involvement. In one study of the natural history of SSc, survival was <80% at 2 years, <50% at 8.5 years, and <30% at 12 years [71]. Other studies have confirmed the poor survival and poor risk features of SSc, especially when there is internal organ involvement, and that standardized mortality ratios had not changed significantly over the previous 40 years [72–74]. Scoring the skin for the extent of scleroderma improves the prediction of prognosis [75]. The modified Health Assessment Questionnaire Disability Index (mHAQ) is a validated tool for measuring the effect of disease on overall function [76].

Immunosuppressive therapies investigated for severe SSc have been inadequate or ineffective. Cyclophosphamide may be considered a standard of care for individuals with early and severe SSc, particularly those with pulmonary involvement, but the therapeutic benefits are very modest [77]. There are no controlled, prospective clinical trials that demonstrate efficacy of any other conventionally dosed immunomodulatory agents. Important supportive care measures for patients with SSc include angiotensin-converting enzyme inhibitors for management of renal crisis and bosentan or other agents for management of pulmonary hypertension.

Patients selected for clinical trials of HDIT and autologous HCT for SSc had in general a poor prognosis based on the presence of diffuse cutaneous disease and internal organ involvement. In a study of patients (*n* = 26) who had already survived at least 6 months after high-dose cyclophosphamide only and HCT, survival was 96% and event-free survival was 64% at 5 years (Table 66.4) [78]. About half of the patients included in this study had diffuse cutaneous disease but without internal organ involvement.

In a multicenter study of a more intensive HDIT regimen consisting of TBI and cyclophosphamide in 34 patients with diffuse cutaneous disease and internal organ involvement, 17 of 27 (63%) evaluable patients who survived at least 1 year after HDIT had sustained responses (without progression or disease activation) at a median follow-up of 4 years [45]. Patients with sustained responses required no immune-based treatment after HDIT. There was a major improvement in the degree of scleroderma as measured by modified Rodman skin score (mRSS) and in overall function as measured by the mHAQ at final evaluation (Figure 66.5). Skin biopsies confirmed that the improvement in skin score was associated with a decrease in dermal fibrosis. Lung, heart, and kidney function, in general, remained clinically stable. The treatment-related mortality was 23% and the estimated progression-free survival was 64% at 5 years. Better patient selection and modifications to the treatment regimen have reduced treatment-related mortality. In a report from the EBMT on 57 patients in their registry with a median follow-up of 20 months, the cumulative probability of disease progression at 5 years was 48% and the cumulative probability of survival at 5 years was 72% [79].

In a more recent report on 90 patients, relapse-free and overall survival were 70 and 78% at 5 years, respectively [80]. Cardiovascular complications resulted in four of the five treatment-related deaths in this study and treatment-related mortality was 6%. In a randomized clinical trial with short follow-up (*n* = 19), HDIT was associated with improved progression-free survival at 12 months compared with pulse cyclophosphamide (Figure 66.6) [81]. Those patients who failed treatment with pulse cyclophosphamide had similar rates of improvement when crossed over to HDIT. Two randomized clinical trials of HDIT compared with pulse cyclophosphamide (ASTIS and SCOT) have completed enrollment and reports on the results are awaited.

Systemic lupus erythematosus

SLE is an autoimmune disorder that is characterized by the presence of antinuclear antibodies and immune complexes [82]. The incidence and prevalence have been estimated at 5.1 and 52.2 per 100,000, respectively. Numerous organ systems can be involved with SLE, and disease severity may vary from mild to life threatening. Antinuclear antibodies, anti-double-stranded DNA antibodies, and anti-Smith antibodies are present in 98, 70, and 25% of SLE

Table 66.4 Clinical trials of HDIT and autologous HCT for systemic sclerosis

Study	No. of patients (*n*)	Median BL DLCO % (range)	Median BL mRSS (range)	Mobilization	High-dose therapy	T-cell depletion	Median follow-up (range) (months)	Treatment-/ disease-related mortality	Clinical results
Nash [45]	34	61 (40–83)	30 (3–48)	G-CSF	TBI/CY + ATG	CD34 selection	48 (12–96)	8/4	PFS 64% Sustained response (evaluable, *n* = 27) 63% Improvement in skin score (*p* = 0.001) + stable lung function overall
Vonk [78]	28	55 (21–100)	32 (9–51)	G-CSF + CY	CY	CD34 selection	63 (12–90)	2/2[a]	EFS (evaluable, *n* = 26) 64% at 5 years and 57% at 7 years
Burt [81]	19	58 (29–82)	28 (6–48)	G-CSF + CY	CY + ATG	None	12 (all patients)	0/0	PFS 80%

BL, baseline; CY, cyclophosphamide (mobilization: 2 or 4 g/m^2; treatment: 120 mg/kg in combination with TBI or 200 mg/kg if single cytotoxic agent); DLCO, diffusion capacity of the lung for carbon monoxide; EFS, event-free survival; mRSS, modified Rodnan skin score (ranges from 0 to 51; increasing score indicates worsening scleroderma); PFS, progression-free survival.

[a] There was one additional death from lung cancer at 5 years after HDIT.

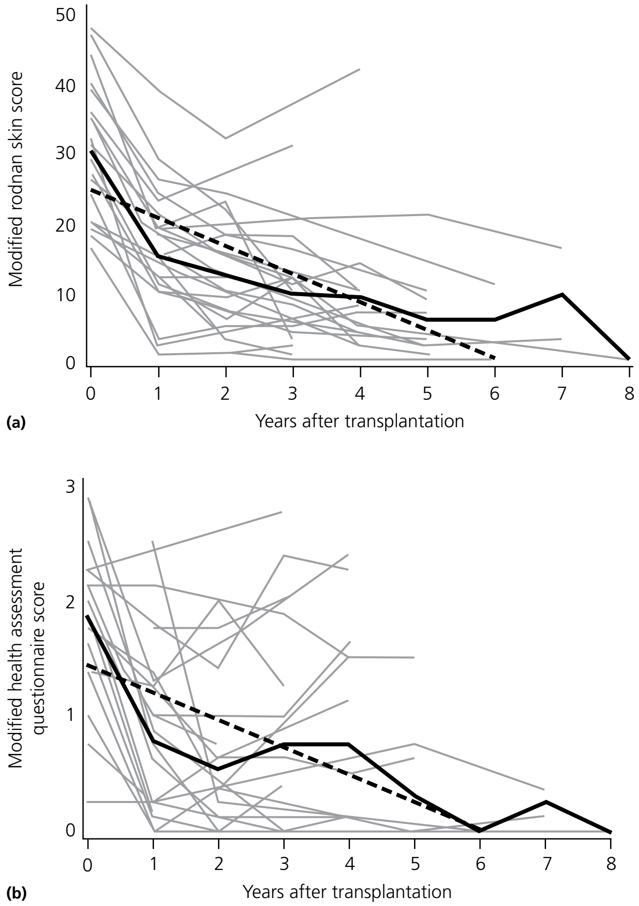

Figure 66.5 Changes in (a) the modified Rodnan skin score (mRSS) and (b) the modified Health Assessment Questionnaire (mHAQ) score after high-dose immunosuppressive therapy (HDIT) and autologous hematopoietic cell transplantation. A determination was made as to whether a parameter value was statistically significantly increasing or decreasing over time using a generalized estimating equation (GEE) model. The bold black dotted line represents an estimate of the modeled linear relationship between the parameter value and time and summarizes the results of the GEE models. The bold black solid line represents the mean value over time for the parameter of interest. The gray solid lines are parameter values for individual patients. The mean mRSS and mHAQ values decreased statistically significantly with time after HDIT as objective markers of less scleroderma and improved function (both $p < 0.001$). Source: Nash et al. 2007 [45]. Reproduced with permission of the American Society of Hematology.

patients, respectively. Overall, the 10-year survival of patients with SLE has been reported as 75–85%, with more than 90% surviving at 5 years [83]. In the group of SLE patients with severe disease who are refractory to standard treatment, survival is expected to be much lower. Standard treatment options are not curative and complete sustained remissions are rare. Antimalarials such as hydroxychloroquine reduce the frequency of disease flares. Low-dose corticosteroids are used for patients in whom disease symptoms have not been controlled by more conservative measures and disease flares are treated with high-dose corticosteroids. Belimumab is a human monoclonal antibody that targets and inhibits B-lymphocyte stimulator (BLyS) resulting in autoreactive B-cell apoptosis and is now approved by the US Food and Drug Administration (FDA) for the treatment of SLE. Agents such as cyclophosphamide, azathioprine, methotrexate, and mycophenolate mofetil have also been found effective for controlling disease activity [84]. The SLE Disease Activity Index (SLEDAI) is a validated tool for following disease activity over time [85].

Clinical trials of HDIT with and without HCT were conducted on patients with SLE who were refractory to standard therapies. In a single-center study (*n* = 50) of HDIT and HCT, patients were mobilized with G-CSF and cyclophosphamide, and the autologous graft was T-cell depleted by CD34 selection [47]. The HDIT regimen included high-dose cyclophosphamide (200 mg/kg) and ATG. The probabilities of overall and disease-free survival at 5 years were 84 and 50%, respectively. There was a significant improvement in the SLEDAI score and renal function stabilized (Figure 66.7). Titers for antinuclear and anti-double-stranded antibodies and complement levels improved after HDIT. Treatment-related mortality was 4% (*n* = 2) and both deaths occurred before HDIT. In comparison, a study of HDIT without HCT in patients with SLE, outcomes after single-agent, high-dose cyclophosphamide (200 mg/kg) (*n* = 14) were similar at a median follow-up of 27 months, with five patients (36%) reported as having durable complete remissions [53]. This approach avoids the reinfusion of cells that has the potential for causing a relapse. However, as might be expected, time to recovery of blood counts is longer, and this may contribute to increased morbidity from the procedure. There was no treatment-related mortality.

The EBMT registry reported the experience with HDIT and autologous HCT for SLE and significant disease responses were observed in 31/50 patients [86]. However, 10 of these 31 patients relapsed and some of the other patients remained on immunomodulatory therapy after HDIT to prevent relapse.

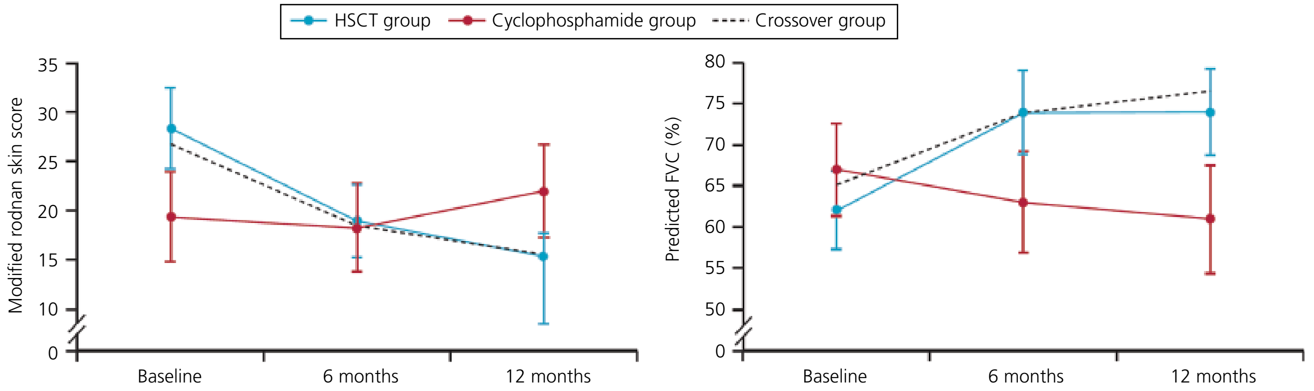

Figure 66.6 Improvement in skin score and lung function after HDIT and autologous HCT in comparison with conventional immunosuppressive therapy. All 10 patients randomly allocated to receive HSCT improved at or before 12 months' follow-up, compared with none of nine allocated to cyclophosphamide (odds ratio 110, 95% CI 14.04–∞; $p = 0.00001$). Eight of nine controls had disease progression (without interval improvement) compared with no patients treated by HSCT ($p = 0.001$), and seven patients switched to HSCT. Compared with baseline, data for 11 patients with follow-up to 2 years after HSCT suggested that improvements in mRSS ($p < 0.001$) and forced vital capacity ($p < 0.3$) persisted. Source: Burt et al. 2011 [81]. Reproduced with permission of Elsevier. (For a color version of this figure, please see the color plate section.)

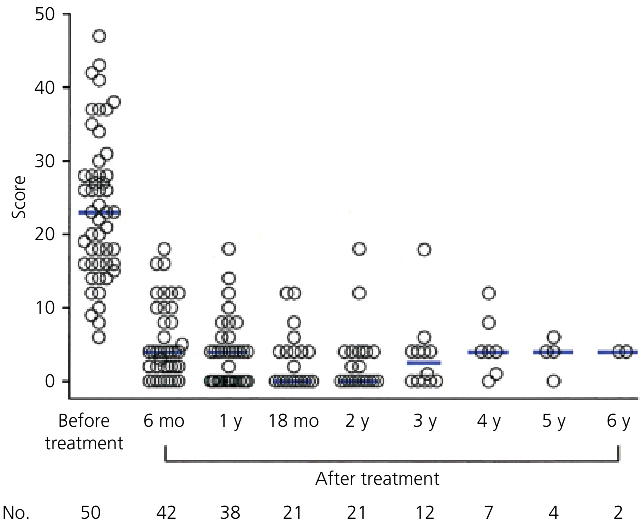

Figure 66.7 SLE disease activity index (SLEDAI) improves after HDIT and autologous HCT. In patients with severe refractory SLE there was a rapid decrease in the SLEDAI (indicating a decrease in disease activity), which was sustained in a substantial proportion of patients for more than 2 years after treatment. Source: Burt et al. 2006 [47]. Reproduced with permission of the American Medical Association.

Treatment-related mortality was 16%. Although the experience is limited, HDIT can induce a high percentage of disease responses in patients with SLE who had otherwise been refractory to standard therapy, and remissions have been durable in a significant proportion of patients.

Rheumatoid arthritis

RA is a chronic multisystem disease characterized by synovial inflammation, cartilage damage, and bone erosions [87]. The criteria for determining response to treatment have been defined by the American College of Rheumatology [88]. The disease affects about 0.8–1% of the population, and the incidence is 25 and 54 per 100,000 persons in men and women, respectively. The pathologic hallmark of RA is synovial inflammation with proliferation of macrophages and fibroblasts. If severe, the inflamed synovium develops into an invasive pannus that destroys cartilage and bone. The disease course can be mild with minimal damage of only a few joints, or severe with many inflamed, damaged joints. Other complications associated with RA are a vasculitis, cervical spine disease, lung nodules or interstitial fibrosis, and cardiac complications, including pericarditis. Risk of progression can be predicted by prognostic factors such as increased number of affected joints, high level of C-reactive protein, presence of rheumatoid factor, and extra-articular features of the disease. The risk of mortality is increased with more severe disease activity or one or more extra-articular disease manifestations [89–91]. There is a mortality of approximately 30% at 5 years for patients with the highest disease activity. There are many anticytokine or immunomodulatory agents approved for RA, including methotrexate, hydroxychloroquine, sulfasalazine, leflunamide, infliximab, adalimumab, etanercept, tocilizumab, abatacept, rituximab, and anakinra [87]. The goal of standard therapy is to relieve the signs and symptoms of the disease since none of these treatments is curative.

Four small clinical trials have been reported of HDIT for patients with RA who had failed standard treatment [52,92–95]. All patients received high-dose cyclophosphamide with or without ATG. The initial reports described early major responses in the majority of the patients. On long-term follow-up, it was noted that all 32 patients accrued to these clinical trials relapsed and required further treatment. However, many of these relapsed patients who had been refractory became responsive to treatment after HDIT. One study showed that after HDIT, there was a decrease in progression of joint damage compared with baseline [96]. No mortality was observed in any of the clinical trials. Seventy-six patients with RA who underwent HDIT and autologous HCT were available for analysis in the EBMT registry, and the outcomes were comparable to the experience reported from the clinical trials [97]. Cyclophosphamide alone was used as the HDIT regimen in 82% and the combination of cyclophosphamide and ATG in 9% of the patients. Major responses were observed in 67% of patients, and there was a significant reduction in the measures of disability. Most patients had restarted immunomodulatory treatment by 6 months after HDIT for persistent or recurrent disease activity. No treatment-related mortality was observed. It is unclear why there is a difference in response of

RA to HDIT compared with other autoimmune diseases. More durable responses might be obtained with more intense HDIT regimens, but this may further increase the risk for treatment-related mortality. Another strategy to decrease relapses or progression may be to add immunomodulatory therapy after HDIT.

Juvenile idiopathic arthritis

JIA is a heterogeneous group of chronic inflammatory diseases involving the joints and extra-articular tissue that begins before 16 years of age [98]. The incidence and prevalence have been estimated as 10 and 65 per 100,000 children, respectively. Patients with unremitting systemic JIA are considered to have the worst prognosis. Severe disease has an effect on bone and joint development, resulting in overgrowth or undergrowth of juxta-articular bone and consequent limb deformities. The macrophage activation syndrome is a potentially life-threatening complication in which there is uncontrolled activation and proliferation of macrophages and T cells, and also a marked increase in serum cytokine levels such as interferon gamma and granulocyte–macrophage colony-stimulating factor (GM-CSF). This complication may occur in 5–8% of patients with systemic JIA, and close monitoring and early treatment are required for the best outcomes. The mortality in this disease is <1% and is mostly in the systemic JIA subtype. One clinical trial of HDIT and autologous HCT in patients with treatment-refractory JIA ($n = 22$) has been reported with a median follow-up of 80 months [40]. Eighteen patients had systemic JIA and four had polyarticular JIA. The HDIT regimen included TBI (400 cGy), cyclophosphamide, and ATG. Early in the clinical trial, two patients developed macrophage activation syndrome less than 1 month after HDIT and died. Precautionary measures added to the treatment may have reduced this risk in later patients. Two other patients died after relapsing and restarting immunosuppressive treatment at more than 1 year after HDIT. Overall and disease-free survival were 82 and 36%, respectively. There were significant improvements in disease activity in the group based on disability and active joint scores. In another study, four of seven patients experienced complete remission for between 5 and 8 years [99]. Two patients progressed within 1 year after transplant and required additional therapy. One patient died within 4 months of starting treatment with disseminated adenovirus infection. In a report from the EBMT registry on 34 JIA patients, 18 (53%) were in complete remission without additional therapy at 12–60 months after HDIT [100]. In a small subset of poor-prognosis JIA patients who fail standard treatment, HDIT and autologous HCT may be of benefit.

Crohn disease

Crohn disease is a relapsing inflammatory disease affecting the gastrointestinal tract with extraintestinal manifestations [101]. In a twin study of Crohn disease in Germany, 35% of the monozygotic pairs had concordance compared with 3% of the dizygotic pairs. There is a genetic association with Crohn disease. Genome-wide association studies have identified 71 susceptibility loci, including NOD2/CARD15. Environmental factors are also considered important for triggering the disease. Associated autoimmune diseases are asthma, type 1 diabetes mellitus, multiple sclerosis, and autoimmune thyroid disease. The goals of treatment are to maintain sustained clinical and endoscopic remissions. Medical therapies include sulfasalazine, prednisone, budesonide, azathioprine, methotrexate, infliximab, adalimumab, and natalizumab. Surgery is not curative and therefore should be considered carefully, but is indicated for management of abscesses, fistulas not responsive to medical therapy, and fibrostenotic strictures with symptoms of small bowel obstruction.

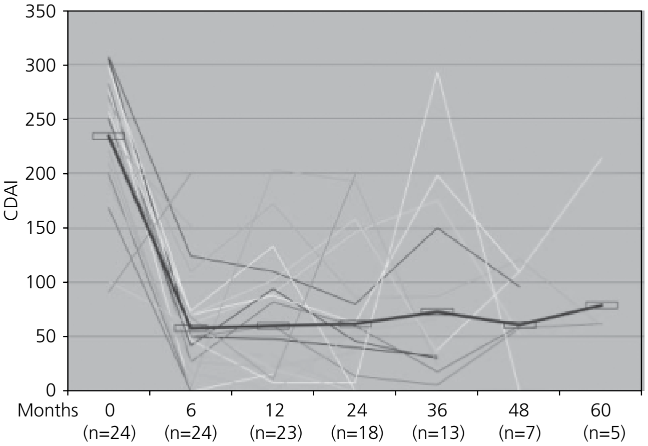

Figure 66.8 Crohn Disease Activity Index (CDAI) pre- and post-HDIT followed by autologous HCT indicates a disease response. The thick line indicates mean CDAI for all patients. There was a significant reduction of Crohn disease activity for as long as 5 years after treatment compared to baseline. Source: Burt et al. 2010 [49]. Reproduced with permission of the American Society of Hematology.

The experience with HDIT followed by autologous HCT has now been reported by several groups. Burt et al. reported the results on 24 patients after high-dose cyclophosphamide and ATG [49]. Eighteen of the 24 patients had follow-up to 5 years after HDIT. Relapse-free survival was 91% at 1 year, 63% at 2 years, 57% at 3 years, 39% at 4 years, and 19% at 5 years (Figure 66.8). The proportion of patients in remission, steroid free, or medication free at any evaluation interval within 5 years after transplant was 70, 80, and 60%, respectively. No treatment-related mortality occurred. In a second study, nine patients with refractory Crohn disease were treated with high-dose cyclophosphamide only [102]. Five of these patients achieved clinical and endoscopic remission but seven of the nine eventually relapsed. After relapse, patients were responsive to low-dose corticosteroids or conventional immunosuppressive therapy. Although responses were substantial, relapses occur with longer follow-up.

Other autoimmune diseases

Investigations of HDIT and autologous HCT have also been conducted in patients with other autoimmune diseases, but experience is more limited. In all these clinical trials, the HDIT regimen was high-dose cyclophosphamide with or without ATG. Six of 14 (43%) patients with refractory chronic thrombocytopenia obtained durable complete responses (platelet count $>100{,}000/\text{mm}^3$), and two patients obtained a partial response at a median of 27 months (range 9–41 months) after HDIT [103]. A clinical trial of HDIT was also performed in patients with recent-onset diabetes mellitus. Fourteen of 15 patients had prolonged periods of insulin independence after HDIT [104]. In a follow-up report, 20 of 23 patients became insulin free. With a median follow-up of 30 months, 12 patients remained insulin free [105]. C-peptide levels increased significantly in both the continuously and the transiently insulin-free groups. The procedure was well tolerated, with minimal adverse events and no treatment-related mortality. The durability of these responses on longer follow-up and the safety of the procedure will determine if this approach could be considered as an acceptable treatment for diabetes mellitus.

Allogeneic hematopoietic cell transplantation for autoimmune diseases: clinical experience

Allogeneic HCT may cure severe autoimmune diseases by replacing the "diseased" autoreactive immune compartment with one that is not autoreactive (although potentially alloreactive). The primary risks of allogeneic HSCT are the morbidity and mortality associated with delayed immune reconstitution and graft-versus-host disease (GVHD). Although the risk of complications and treatment-related mortality is greater than with autologous HCT, there may be a higher potential for sustained remissions of severe autoimmune diseases after allogeneic HCT and therefore improved overall outcome. Survival after allogeneic HCT for non-malignant hematologic disorders or good-risk hematologic malignancies such as chronic myelogenous leukemia in chronic phase has ranged from 85 to 95%. The best outcomes were observed in young patients transplanted from an HLA-matched donor. No clinical trials have yet been conducted of allogeneic HCT for which an autoimmune disease was the primary indication, but a small number of cases have been reported. The largest experience has been in patients with autoimmune cytopenias [106–109]. Of the seven evaluable patients reported from the EBMT registry, five were alive and in remission at a median follow-up of 41 months [106]. One patient with Evans syndrome in the report from the EBMT registry had disease progression and died. The second patient died from treatment-related complications after HCT from an HLA-haploidentical donor. Of the three case reports of Evans syndrome in the literature, one patient relapsed and died and the other two died of transplant-related complications in complete remission [107–109]. There are six cases reported of allogeneic HCT for connective tissue diseases such as SSc, overlap syndrome, and RA [110–114]. Two patients with SSc had high-dose conditioning, one of whom was alive at 5 years in disease remission with resolution of the dermal fibrosis and severe scleroderma. Four patients received reduced-intensity conditioning regimens. All patients were alive at last follow-up and in remission. Three of these patients had stable mixed hematopoietic chimerism and no history of GVHD. The experience from 35 patients with hematologic and non-hematologic autoimmune diseases reported to the EBMT has been summarized [115]. Responses were observed in 78% of patients. Three patients relapsed with the autoimmune disease at a median of 12.3 months after transplant. Treatment-related mortality was 22%. Two deaths occurred from progression of the autoimmune disease. This study was limited by being retrospective and because patients were heterogeneous and data incomplete. Regulatory immune mechanisms associated with the establishment of mixed hematopoietic chimerism may be responsible for inducing remission of the autoimmune disease. Conclusions regarding the therapeutic role of allogeneic HCT for severe autoimmune diseases await the results from prospective clinical trials conducted in a carefully selected group of patients.

Conclusion

The common autoimmune diseases likely result from a genetic predisposition and an environmental trigger. There is a high rate of disease remissions after HDIT and autologous HCT, and a significant proportion are sustained after 4–5 years. The rate and durability of response may depend on the intensity of the HDIT regimen and the type of autoimmune disease being treated. Randomized clinical trials comparing HDIT and autologous HCT with standard therapy or "best clinical practice" are now being conducted in specific autoimmune diseases. The experience with allogeneic HCT as a treatment for autoimmune diseases is still limited but promises to be highly effective. The risks of increased morbidity and mortality from GVHD continue to delay clinical trials of this approach. Carefully selected patients with active autoimmune disease that is life threatening or threatening critical organs and refractory to standard treatment should be considered as candidates for clinical trials of allogeneic HCT or HDIT followed by autologous HCT.

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CHAPTER 67

Hematopoietic Cell Transplantation for Rare Hematologic Malignancies

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Introduction

In this chapter, we review the results of autologous and allogeneic hematopoietic cell transplantation (HCT) for a variety of rare hematologic malignancies that in our opinion would be rarely encountered outside of specialized centers and for which HCT has been performed (Table 67.1). As would be expected with rare disorders, there is paucity of prospective well-designed trials and most published literature is limited to single case reports, small case series, or retrospective analyses that include heterogeneous groups of patients. We have attempted to review the published literature and make recommendations based on the preponderance of evidence. Recognizing the bias towards reporting only positive results that is inherent in single case reports, we have used case series and retrospective analysis of registry data rather than single case reports as the basis for our recommendations whenever possible. It is unlikely that prospective studies to test the role of HCT that contain adequate numbers of patients will ever be performed for the extremely rare entities discussed below. However, there is a need for such trials preferably performed by large cooperative groups for the less uncommon malignancies discussed here.

Rare myeloid malignancies

Langerhans cell histiocytosis

Langerhans cell histiocytosis (LCH) refers to a group of clinical syndromes characterized by clonal Langerhans cell proliferation. These syndromes have historically been designated by different names based on the pattern of organ involvement. These include histiocytosis X, Letterer–Siwe disease, and Hand–Schüller–Christian disease [1]. Although typically a disorder of infants and children, LCH can also occur in adults. Age, pattern of organ system involvement, and organ dysfunction are the most important prognostic factors. In general, children under 2 years of age and adults over 65 years of age do poorly [2–4]. While single-system LCH usually involving the skeleton or skin is an indolent disease, chemotherapy-resistant multisystem LCH (MS-LCH), especially involving organs such as the bone marrow, liver, spleen, and lungs, has an extremely poor prognosis [3, 5].

Various case reports have described successful allogeneic SCT for patients with multisystem LCH [6–10]. One of the first reported that patients who underwent allogeneic HCT for chemotherapy-resistant disease remained in complete remission (CR) 12 years later, showing that long-term disease-free survival (DFS) is possible using this approach [11]. Kudo et al. analyzed the outcome of 15 children with refractory LCH who underwent allogeneic HCT at various institutions in Japan. Ten patients received myeloablative conditioning and reduced-intensity conditioning (RIC) was used in the remaining five. Cord blood was the graft source in 10 patients. The median 10-year overall survival (OS) was 73.3% and was significantly better for patients without risk organ involvement [12]. The French Langerhans Cell Study Group reported results for eight patients with refractory MS-LCH transplanted at six institutions. Three patients underwent autologous HCT and five underwent allogeneic HCT. Of the three patients who underwent autologous HCT, two died of relapse and one was alive and in CR 7 years later. Of the five patients who underwent full-intensity allogeneic HCT, two died of regimen-related toxicity, one died of sepsis 23 months after HCT, and two were alive and in CR 12 years and 21 months, respectively, after HCT, again demonstrating the ability of allogeneic HCT to achieve durable remissions in refractory MS-LCH [13]. The transplant-related mortality of full-intensity allogeneic HCT is high owing to the heavy prior exposure of these patients to chemotherapy and also involvement of vital organs such as the lungs and liver [14]. In an attempt to decrease the treatment-related mortality of allogeneic HCT, Steiner et al. [14] reported the results of reduced-intensity allogeneic HCT using fludarabine-based conditioning in nine pediatric patients with high-risk LCH. Graft sources included matched siblings, matched unrelated donors, mismatched unrelated donors, and haploidentical parental donors. The three patients who underwent haploidentical HCT underwent second transplants for graft failure. After a median follow-up of 390 days, seven of the nine patients were alive without evidence of disease, which is remarkable for this group of heavily treated high-risk patients. The results of multiple reported cases of allogeneic HCT for LCH were summarized by Caselli and Arico [15].

Interestingly, one patient who had engraftment failure with autologous reconstitution after a second allogeneic haploidentical HCT remained in remission 770 days after the first HCT [13], suggesting that autologous HCT may be a viable option for patients who lack suitable donors or are not candidates for allogeneic HCT. Two patients with MS-LCH who underwent successful autologous HCT have been reported. One remained in CR over 4 years and the other at 10 months after HCT [9,16].

Based on the above data and given the poor prognosis of refractory MS-LCH, it seems preferable to perform allogeneic HCT early

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Table 67.1 Rare hematologic malignancies for which HCT has been performed

Langerhans cell histiocytosis
Systemic mastocytosis
Hypereosinophilic syndrome
Acute megakaryoblastic leukemia
Acute erythroleukemia
Chronic myelomonocytic leukemia
Adult T-cell leukemia/lymphoma
Natural killer cell leukemia/lymphoma
Mycosis fungoides
Sézary syndrome
Angioimmunoblastic T-cell lymphoma
Primary central nervous system lymphoma
Blastic plasmacytoid dendritic cell neoplasm
Hepatosplenic T-cell lymphoma (gamma/delta T-cell lymphoma)
T-cell prolymphocytic leukemia
Subcutaneous panniculitic T-cell lymphoma

in the course of disease in patients who have matched donors. A higher relapse rate has been observed with RIC compared to full intensity conditioning [15]. Autologous HCT may be an option for patients who lack matched donors. HCT is a promising treatment modality for treatment of MS-LCH and large clinical trials testing both autologous and allogeneic HCT early in the disease course are warranted.

Systemic mastocytosis

Systemic mastocytosis (SM) is characterized by infiltration of the bone marrow and other organs by neoplastic mast cells. Activating mutations in the *KIT* gene (most commonly D816V) are almost invariably present in these neoplastic mast cells. The World Health Organization (WHO) classification recognizes various clinicopathologic SM syndromes, including indolent SM, SM with associated clonal hematologic non-mast-cell lineage disease (SM-AHNMD), aggressive SM, and mast cell leukemia (MCL), among others [1,17]. Aggressive SM and MCL have a dismal prognosis [17,18]. While indolent SM has a prolonged clinical course, as its name suggests, in the case of SM-AHNMD the clinical course is dictated by the underlying malignancy, which most often is acute myeloid leukemia, myelodysplasia, or a myeloproliferative disorder [19,20]. A specific association exists between SM and t(8;21) acute myeloid leukemia (AML) [20–22].

The HCT experience in SM is very limited. Nakamura et al. transplanted three SM patients from human leukocyte antigen (HLA)-identical siblings using non-myeloablative conditioning with fludarabine and cyclophosphamide (CY). One patient had MCL, another had SM with myelodysplasia (SM-AHNMD), and the third had aggressive SM. All patients achieved complete T-cell engraftment. Although there was clinical and pathologic evidence of a graft-versus-mast cell effect, this did not translate into durable remission and all three patients eventually relapsed. In one patient, there was significant morbidity from mast cell mediator release at day +3, concurrent with flare-ups of acute GVHD [23]. In an analysis of 57 patients with SM who had undergone HCT at various centers worldwide, 3-year OS was 74% for patients with SM-AHNMD, 43% for aggressive SM and 17% for MCL. Survival was lower in patients who received RIC compared to full intensity conditioning [24].

Patients with SM-AHNMD fare poorly compared with patients with the underlying hematologic disease alone [19]. This is especially true of cases where the associated hematologic disorder is t(8;21) AML, an otherwise low-risk AML subset. However, this is not surprising given the fact that *KIT* mutations are associated with a poor prognosis in t(8;21)AML [25,26]. Durable remissions have been described in SM-AML with t(8;21) after allogeneic HCT even when recipient bone marrow mast cells carrying t(8;21) were detected 1 year after allogeneic HCT [22,27]. Prolonged remission after a second myeloablative allogeneic HCT has also been reported in a case of SM with t(8;21) AML that relapsed after an initial non-myeloablative sibling donor HCT [21]. In a reported case of SM associated with myelodysplastic syndrome, mast cell infiltration of bone marrow and liver persisted even after normal hematopoiesis was restored by allogeneic HCT. This patient also developed venous thrombosis in the post-transplant period, which was attributed to mast cell mediator release. [28]. Another patient who was alive 6 years after allogeneic HCT for SM with associated chronic myelomonocytic leukemia has also been reported [20].

Given the extremely grim prognosis of patients with aggressive SM and MCL and some evidence of graft-versus-mast cell effect, it appears reasonable to test the efficacy of allogeneic HCT for these subsets of SM, preferably in the context of a clinical trial. Newer kinase inhibitors such as dasatinib are capable of inhibiting the D816V *KIT* mutation may have a role in conjunction with allogeneic HCT [29]. Given the poor chemosensitivity of mast cells, it is possible that more intense conditioning may also be beneficial [21]. Special approaches may be needed to manage morbidity from mediator release during the peritransplant period in patients with a large mast cell burden [23,28].

For patients with SM and t(8;21), it appears that allogeneic HCT in CR1 is the best option if a suitable donor is available based on the limited data available. Recipient-derived bone marrow mastocytosis may persist after allogeneic HCT and does not appear to have clinical significance [22,27]. The efficacy of HCT for SM associated with other hematologic malignancies is unknown at present. It is unlikely that HCT will have a role in indolent SM, especially with the development of targeted therapies that inhibit D816V *KIT* mutation.

Hypereosinophilic syndrome

Hypereosinophilic syndrome (HES) refers to a heterogeneous group of disorders that are characterized by peripheral blood eosinophilia and a propensity to cause organ damage, especially cardiac dysfunction from endomyocardial fibrosis. Criteria for diagnosis of HES have been defined [1,30,31]. Myeloid and lymphocyte variants of HES are now recognized. With the increased understanding of molecular abnormalities underlying myeloid eosinophilic disorders, the majority of these fall under the separate category created in the WHO classification termed Myeloid and lymphoid neoplasms associated with eosinophilia and abnormalities of *PDGFRA, PDGFRB* and *FGFR1* [1]. A subset of patients almost exclusively male with the myeloid variant carry an interstitial deletion resulting in an *FIP1L1–PDGFRA* fusion and are exquisitely sensitive to imatinib, while the lymphocyte variant is associated with clonal T-cell expansion [31–34]. The major cause of morbidity and mortality in HES is cardiac dysfunction [30]. Chronic eosinophilic leukemia-not otherwise specified (CEL-NOS) is classified under myeloproliferative disorders and is characterized by increased bone marrow blasts, clonal eosinophilia, and lack of *PDGFRA/B* or *FGFR1* rearrangement [1].

The results of allogeneic HCT for HES are limited to case reports. Hence the overall results of allogeneic HCT for this disorder cannot be determined. A number of these reports were summarized by Cooper et al. [35]. The majority of transplants have been performed from HLA identical siblings after full-intensity conditioning. A few cases have undergone reduced-intensity or myeloablative conditioning [35,36]. Disease-free survival after up to 6 years of follow-up and reversal of myelofibrosis have been reported [35,37]. Durable remission after allogeneic HCT for a patient with *PDGFRA* rearrangement who manifested with myeloproliferative disease with eosinophilia, T-lymphoblastic lymphoma, and precursor B-cell acute lymphoblastic leukemia has been described [38]. These reports of long-term remission after allogeneic HCT may be suggestive of the curative potential of allogeneic HCT for this disorder.

An interesting observation is that eosinophilia can recur in some cases even when 100% of bone marrow and peripheral blood cells are of donor origin [35,39,40]. This cannot be considered a failure of HCT and usually resolves spontaneously. It is not clear if these cases had the lymphocyte variant of HES with clonal T-cell expansion. High interleukin-5 levels were detected in two patients, which suggests that abnormal T cells may persist in lymphoid organs (despite full donor marrow engraftment) and lead to eosinophilia, which probably resolves with complete donor engraftment in lymphoid organs [35,40].

Our understanding of the biology of HES is rapidly evolving. Newer classifications based on cytogenetic and molecular abnormalities and better definition of prognostic factors may help predict better those patients who are not imatinib responsive and who are more likely to develop end organ damage. The results of imatinib therapy for *FIP1L1–PDGFRA* rearrangement-positive patients is excellent whereas *PDGFRA/B* rearrangement-negative cases rarely have durable responses to imatinib [31,41]. Prognosis for *PDGFRA/B* rearrangement-negative patients who develop end-organ dysfunction is poor and allogeneic HCT should be strongly considered in suitable patients who have early signs of end-organ damage and have failed prednisone and a second-line agent such as hydroxyurea or interferon-α. Although there are reports of improvement in cardiac function after allogeneic HCT in patients with HES and cardiac dysfunction [42], the transplant-related mortality in this situation is likely to be high.

Acute megakaryoblastic leukemia

Acute megakaryoblastic leukemia (AMKL) accounts for about 1% of AML in adults and about 5–10% of AML in children, with a higher incidence in children with Down syndrome [43–45]. The disease has a bimodal age distribution with peaks in early childhood (age under 3 years) and adulthood [44]. Bone marrow fibrosis is a prominent feature of this disorder, making AMKL the most common cause of "acute myelofibrosis" [1,43]. Acute panmyelosis with myelofibrosis (APMF) is a related entity with distinct pathologic features [1]. AMKL may occur de novo or arise from an underlying myeloproliferative or myelodysplastic syndrome. Although 50% of adult patients achieve a CR with convention AML induction chemotherapy, long-term survival rates are dismal with conventional consolidation chemotherapy [43,46–48]. Hence HCT has been used as consolidation therapy in an attempt to improve the outcome.

Single case reports have been published describing reversal of bone marrow fibrosis and durable remissions after allogeneic HCT [49–52] (Figure 67.1). A retrospective analysis of the results of HCT for de novo AMKL in CR1 has been performed by the European Group for Blood and Marrow Transplantation (EBMT). At 3 years after allogeneic HCT, the results in children and adults were, respectively: leukemia-free survival (LFS) 66 and 46%; overall survival (OS) 82 and 43%; and treatment-related mortality (TRM) 0 and 26%. The results of autologous HCT for children and adults were, respectively: LFS 52 and 27%, OS 61 and 30%; and TRM 3 and 8% [53] (Figure 67.2). Athale et al. reported their single-institution experience treating 41 patients with childhood AMKL who included six patients with secondary AMKL and six with Down syndrome. The 2-year event-free survival (EFS) was 26% for allogeneic HCT compared with 0% for chemotherapy alone. The results of allogeneic HCT were significantly better when performed in remission than with persistent disease (2-year EFS: 46% vs. 0%). In both of the above-mentioned studies, the outcomes were better for children with Down syndrome who had an estimated 2-year EFS of 83% in the latter study [47,53].

Based on the above data, allogeneic HCT from matched sibling or matched unrelated donors should be the consolidation treatment of choice in adults with AMKL who achieve CR. In children, autologous transplant in CR1 is an option in patients who lack a matched sibling donor and there are reports of prolonged survival after this approach. The outcome of autologous transplant in adults is very poor. At present, allogeneic HCT cannot be recommended for patients with resistant disease, who should be treated with investigational approaches. There appears to be no advantage for allogeneic HCT over autologous HCT in patients with Down syndrome and AMKL. Sufficient data are not available to determine efficacy of HCT for APMF.

Acute erythroleukemia

Acute erythroleukemia (AEL) is an uncommon form of AML, accounting for about 4.5% of all AMLs [54]. AEL may occur de novo or secondary to an underlying myelodysplastic or myeloproliferative disorder or after previous chemotherapy [55,56]. AEL has a poor prognosis compared with other AML subtypes, with a median overall survival of only 9 months in one retrospective study [56]. Like AMKL, AEL has a dismal prognosis with conventional chemotherapy [56,57].

The EBMT have reported the results of 103 autologous and 104 HLA-identical sibling allogeneic HCTs performed for de novo AEL in CR1. For allogeneic HCT, the 5-year LFS, relapse incidence and TRM were 57± 5, 21 ± 5, and 27 ± 5%, respectively, and the corresponding figures for autologous HCT were 26 ± 5, 70 ± 6, and 13 ± 4%, respectively. In multivariate analysis, age remained the most important prognostic factor for both allogeneic and autologous HCT [58]. In a smaller study, Killick et al. reported the outcome of 27 patients with AEL, who included eight adults with secondary AEL and five children. Among patients with de novo AEL, 95% achieved CR with induction chemotherapy compared with only 57% of patients with secondary AEL. Eighteen of 22 patients (82%) achieving CR underwent consolidation by HCT (15 allogeneic, three autologous). The median survival of 2.9 years for de novo AEL was not different from that of their matched controls with other forms of AML. The overall relapse rate of only 35% suggests a benefit for HCT consolidation in CR1 [59].

Hence allogeneic HCT is currently the treatment of choice for patients with AEL who are in CR1. Autologous HCT may be an option for patients in CR1 who lack matched donors. The results of allogeneic HCT for refractory and relapsed disease remain unknown at present.

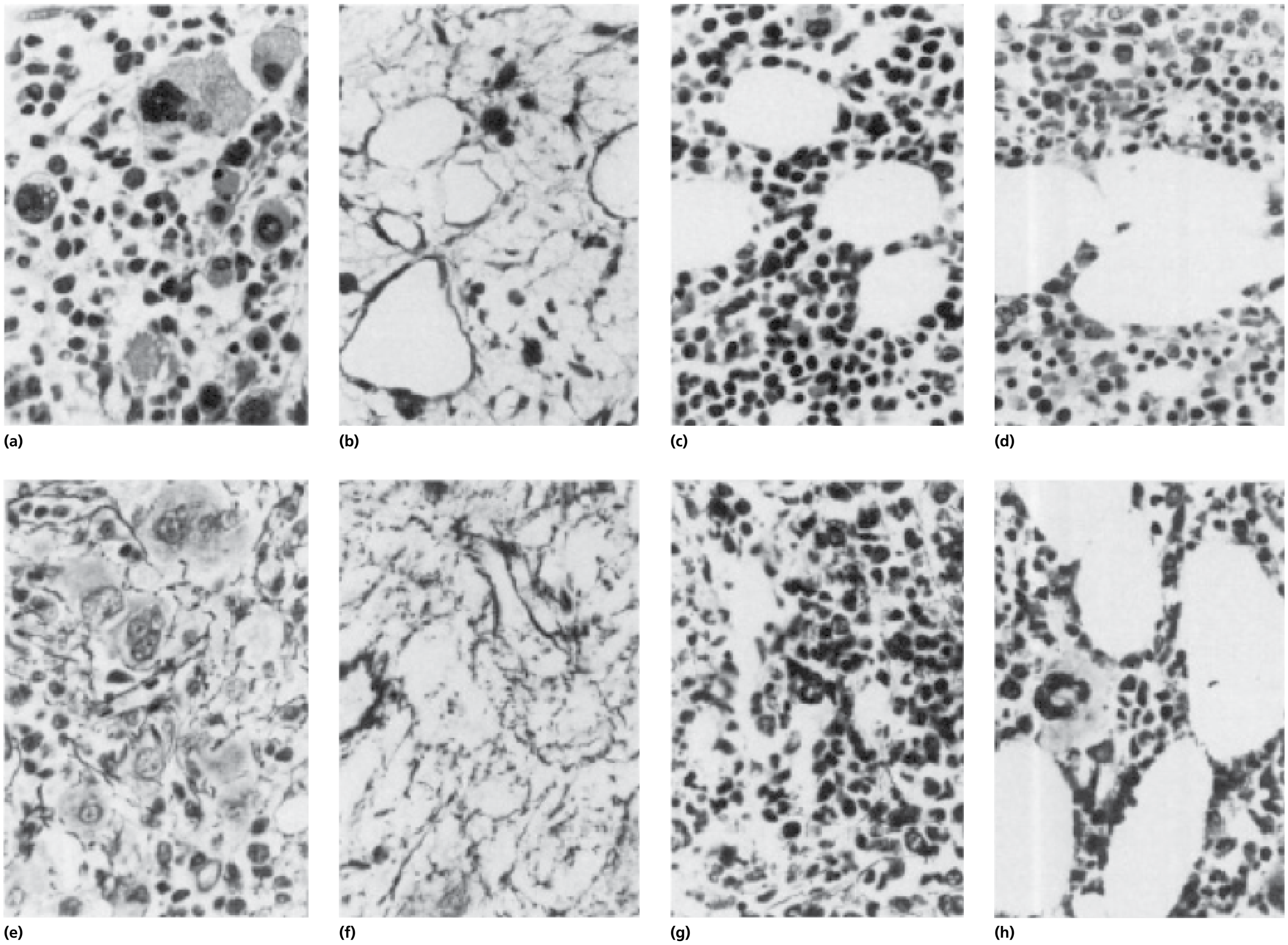

Figure 67.1 (a)–(d) Hematoxylin and eosin stains of bone marrow biopsies from diagnosis, day +15, day +100, and day +360, respectively, after allogeneic HCT in a patient with “acute myelofibrosis.” Progressive restoration of normal hematopoiesis is seen. (e)–(h) Corresponding reticulin stains show total clearing of reticulin fibrosis after allogeneic HCT. Source: Wolf et al. 1982 [51]. Reproduced with permission of the American Society of Hematology.

Chronic myelomonocytic leukemia

Chronic myelomonocytic leukemia (CMML) is clonal stem-cell disorder characterized by peripheral blood monocytosis and shares features with both myelodysplastic and myeloproliferative disorders. The median age at diagnosis is 65–75 years, with a male predominance. Criteria for diagnosis of CMML have been defined in the WHO classification [1]. Since CMML was previously grouped with the myelodysplastic syndromes, patients who underwent allogeneic HCT for CMML were included in reports of HCT results for myelodysplastic syndromes. However, CMML has distinct clinicopathologic features that warrant its consideration as a separate entity.

Although CMML includes a heterogeneous group of patients with varying rates of disease progression, the overall prognosis is poor, with a median survival of only 12 months in one study [60]. CMML is not curable with conventional chemotherapy and the response to targeted therapies is poor except in the small subset of patients who carry a *PDGFRB* fusion oncogene and are sensitive to imatinib [61]. Recognizing this dismal prognosis, allogeneic HCT has been tried as a potential curative option.

In the largest experience reported of patients who have undergone HCT for CMML, Eissa et al. at the Fred Hutchinson Cancer Research Center analyzed the outcome of 85 patients who underwent allogeneic HCT between 1986 and 2008. The median age of patients was 51.7 years and they received a variety of conditioning regimens. Thirty-eight patients underwent matched related donor HCT and 47 matched unrelated donor HCT. Progression-free survival (PFS) was 38% at 10 years and relapse incidence was 27% at 10 years. The significant risk factors for increased mortality were high-risk cytogenetics, high HCT Comorbidity Index and higher age [62] (Figure 67.3). The EBMT reported the outcome of 50 patients who underwent allogeneic HCT for CMML. Forty-three patients received transplants from related donors and seven received unrelated donor transplants. The median age of the patients was 44 years. Conditioning was total body irradiation (TBI) based in 26 patients and chemotherapy based in the remainder. After a median follow-up of 40 months, the 5-year estimated OS, DFS, and probability of relapse were 21, 18, and 49%, respectively [63]. A retrospective analysis of the outcomes of 73 patients with CMML reported to the French Registry (SFGM-TC) between 1992 and 2009 showed a 3-year OS, NRM, EFS and cumulative incidence of relapse of 32%, 36%, 29% and 35% respectively [64]. Allogeneic HCT for CMML has been reviewed by Cheng et al. [65].

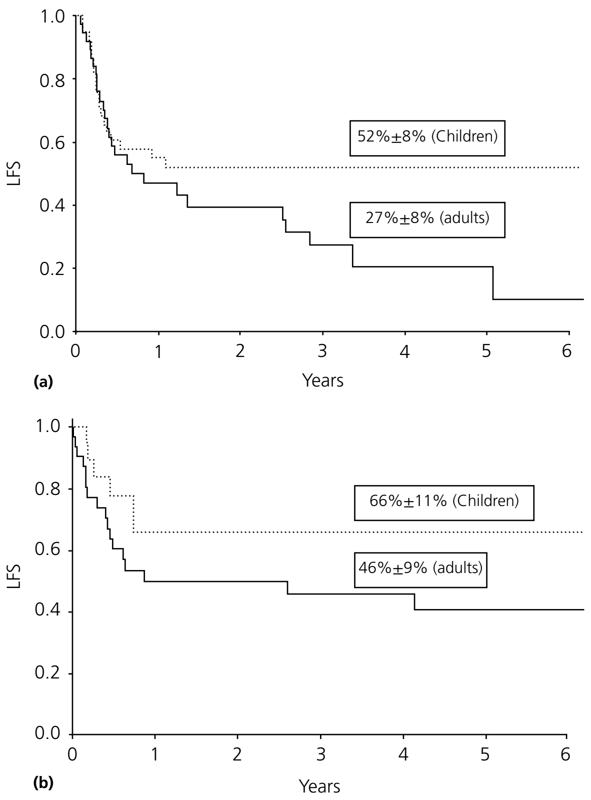

Figure 67.2 (a) Leukemia-free survival (LFS) after autologous HCT in adults and children with de novo AMKL in CR1. (b) LFS after allogeneic HCT in adults and children with de novo AMKL in CR1. Source: data from Garderet et al. 2005 [53].

Allogeneic HCT from matched siblings or matched unrelated donors should be an early consideration for patients with CMML who are suitable candidates. It must be noted that although the median age of patients in the above studies was much lower than the median age at diagnosis of CMML patients as a whole, the TRM for allogeneic HCT is fairly high. Comorbidity is a strong predictor of outcome and must be an important consideration in selecting patients for allogeneic HCT. The majority of reported patients have undergone full-intensity conditioning, which most likely contributed to the high TRM. Hence allogeneic HCT with RIC needs to be explored further as a curative option in CMML.

Rare lymphoid malignancies

Adult T-cell leukemia/lymphoma

Adult T-cell leukemia/lymphoma (ATLL) refers to a specific clinicopathologic syndrome characterized by prominent skin involvement, hypercalcemia, and an acute leukemic or lymphomatous presentation. ATLL is specifically associated with infection with type I human T-cell lymphotropic retrovirus (HTLV-I), which is clonally integrated into the genome of the malignant T cells [1]. Although ATLL is endemic in parts of Japan and the Caribbean, cases also occur in the United States, especially in the south-east [1,66]. Chronic and smoldering forms of the disease do occur and have a more indolent course compared with the acute or lymphomatous types [1,67].

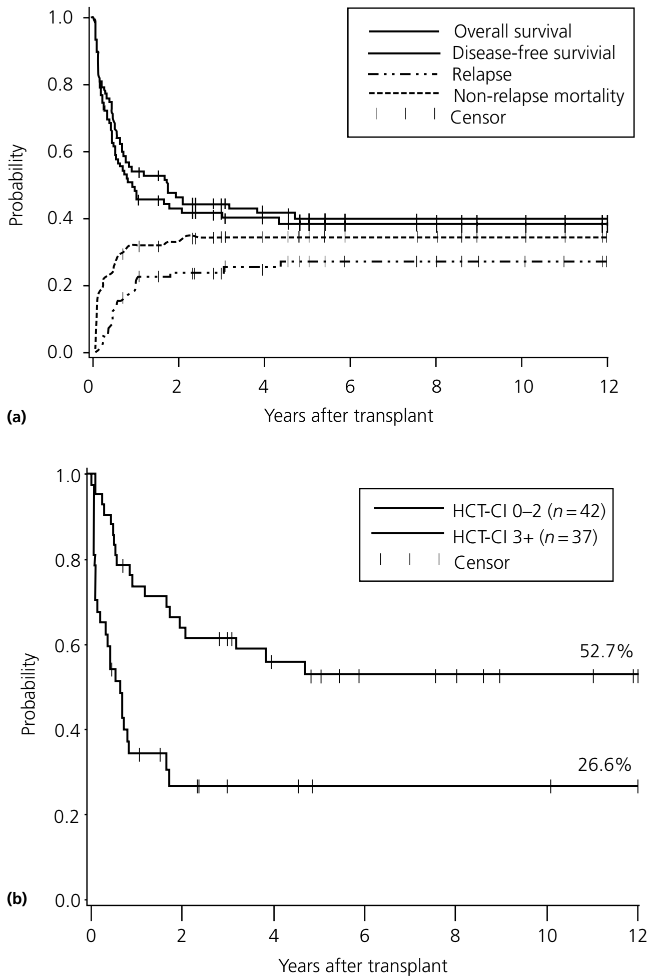

Figure 67.3 (a) Survival, relapse, and NRM for 85 patients with CMML who have undergone allogeneic HCT. (b) Probability of survival according to the hematopoietic cell transplantation-specific comorbidity index. Source: Eissa et al. 2011 [62]. Reproduced with permission of Elsevier.

Not surprisingly, most of the experience in treating ATLL comes from Japan. The results of intensive combination chemotherapy are poor, as are results of autologous HCT [68,69]. Increased use of allogeneic HCT has been implicated in the decreasing trend in mortality observed in Japan [70]. Reported studies of HCT for ATLL were summarized by Jabbour et al. [71]. In one of the initial case series, Fukushima et al. reported results for 40 patients with acute or lymphomatous types of ATLL who underwent allogeneic HCT at seven institutions in Japan [72]. All but one patient received myeloablative conditioning. Twenty-seven patients were transplanted from matched related donors, five from mismatched related donors and eight from matched unrelated donors. Of the 36 donors whose HTLV-1 status was known, nine were HTLV-1 positive. The estimated 3-year overall survival, relapse-free survival, and disease relapse were 45.3, 33.8, and 39.3%, respectively (Figure 67.4). Of 10 patients who relapsed, three were able to achieve remission by reduction in immunosuppression, suggesting a graft-versus-leukemia/lymphoma effect [72]. In the largest retrospective series of allogeneic HCT for ATLL reported to date, among 586 patients who underwent HCT using bone marrow or peripheral blood stem cells at various institutions in Japan, the

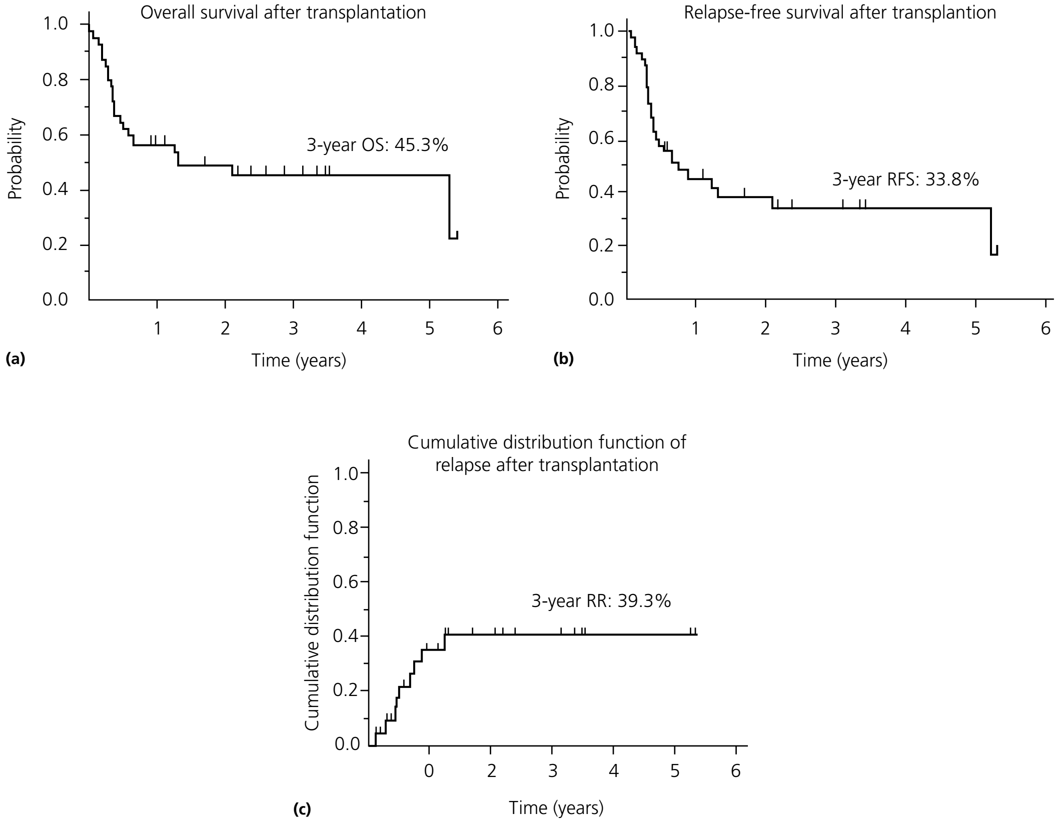

Figure 67.4 (a) Estimated 3-year overall survival of 40 patients who underwent allogeneic HCT for ATLL. (b) Estimated 3-year relapse-free survival. (c) Estimated 3-year relapse risk. Source: Fukushima et al. 2005 [72]. Reproduced with permission of Nature Publishing Group. Copyright 2005.

3-year OS was 36%. The 3-year OS rates were 39 and 34% for patients who underwent myeloablative conditioning and RIC, respectively. In multivariate analysis, factors contributing to poorer OS were older age, male gender, active disease, poor performance status, and use of unrelated donors. No significant difference in OS between myeloablative conditioning and RIC was observed. Among the 174 recipients of cord blood transplants included in this report, the 3-year OS was only 21% [73]. For patients not in CR at time of allogeneic HCT, a higher serum soluble interleukin-2 receptor level was associated with poor outcome [74]. Allogeneic HCT is the only currently available therapy for ATLL that offers any prospect of long-term survival.

Disease relapse/progression and TRM remain major obstacles to improving allogeneic HCT results for ATLL. The Nagasaki Transplant Group recently published the outcome of 35 patients who experienced relapse or disease progression after allogeneic HCT [75]. Complete remissions were achieved in seven patients by either withdrawing immunosuppression or by use of DLI in combination with cytoreductive therapy. DLI-induced remissions were durable and the 3-year OS for these patients was 19.3%. Similar results have been observed in other reports, suggesting that strategies to augment graft-versus-leukemia/lymphoma effect are likely to improve HCT outcome further. Attempts to improve HCT outcome by reducing the conditioning intensity have yielded only limited success. Even with RIC, TRM at 1 year was 29.2% in the large Japanese series compared with 32.7% for myeloablative conditioning [73]. The profound immunosuppression of ATLL patients prior to HCT is most likely the major contributor to the high TRM. For instance, in one report the cytomegalovirus reactivation rate was as high as 83% [71].

Donor-derived ATLL has been reported in a patient who underwent allogeneic HSCT from his HLA-matched brother who was an HTLV-1 carrier [76]. Using a tumor-specific polymerase chain reaction technique, it was later shown that the relapse originated from donor T cells that were already infected in the donor [77]. Thus, the immunosuppression of the recipient appears to have facilitated the transformation of HTLV-1-infected donor T cells to frank T-cell leukemia/lymphoma. The precise incidence of donor-derived relapse is not known and therefore it not clear at present if HTLV-1 seronegative unrelated donors should be preferred over seropositive matched siblings. Use of unrelated donors, however, was associated with poorer overall survival in multivariate analysis.

An interesting aspect of allogeneic HCT for ATLL has been the observation of viral clearance after allogeneic HCT in recipients who have received grafts from seropositive or seronegative donors [78]. This is likely to be mediated by donor-derived cytotoxic T-cell response to viral epitopes resulting in viral clearance.

Natural killer cell neoplasms

Natural killer (NK) cell neoplasms include the extranodal NK/T-cell lymphoma, nasal type, and aggressive NK-cell leukemia subtypes described in the WHO classification. Extranodal NK/T-cell lymphomas have a predilection to arise in the upper aerodigestive tract, but may also involve other sites such as the skin, gastrointestinal tract, and testes. Cases with disseminated disease often have significant comorbidity from cytokine-mediated phenomena such as hepatic dysfunction and hemophagocytic syndrome. Rare cases may have a cytotoxic T-cell phenotype and hence the designation NK/T-cell lymphoma. NK-cell neoplasms are strongly associated with Epstein-Barr virus (EBV), which may have a role in their pathogenesis. There is marked geographic variation in the incidence of NK-cell neoplasms due to their strong predilection for Asians and indigenous populations of Mexico and Central and South America [1,79]. The entity previously termed blastic NK-cell lymphoma ($CD4^+56^+$ hematodermic neoplasm) is now known to be a malignancy of plasmacytoid dendritic cells and therefore is not a true NK-cell neoplasm [1].

In the case of extranodal NK/T-cell lymphoma, only about one-third of patients with localized disease are long-term survivors with the conventional approach of radiation and combination chemotherapy [80]. Locoregional and systemic failures are common and patients with relapsed disease or systemic disease at presentation have a dismal outcome [81,82]. The prognosis is even worse for aggressive NK leukemia, with a median survival of only 2 months in one study [83]. Hence there has been interest in using HCT to improve the poor outcome of NK-cell neoplasms.

Despite not being an extremely rare neoplasm in some geographic areas, systematic prospective trials to evaluate the efficacy of HCT for NK-cell neoplasms have not been performed. Anecdotal reports describe success with autologous and allogeneic HCT even for advanced disseminated disease [84–88]. Au et al. reported their experience with 18 consecutive cases of nasal type NK/T-cell lymphoma treated at two institutions in Hong Kong. Eight cases were treated in CR1, five in CR2, and five with refractory disease. Five patients had extranasal disease before autologous HCT. The most common initial treatment used was combination chemotherapy and radiotherapy. Two patients died of treatment-related causes, nine patients relapsed, and seven remained in CR. All patients with active or disseminated disease at the time of HCT died early of relapse or disease progression. The actuarial survival for the whole cohort was 39% after 6 months. Disease relapse beyond 6 months was not seen [89]. Lee et al. compared the outcome of 47 patients (from three published series) who underwent autologous HCT with that of 107 historical controls matched for prognostic risk group and disease status at HCT. Autologous HCT conferred a significant survival advantage for patients who were transplanted in CR (5-year disease-specific survival of 87.3% for the HCT vs. 67.8% for the non-HCT group) [90]. For patients with localized disease (Stage I/II), autologous HCT should not be performed outside a clinical trial. Autologous HCT should be reserved for patients with advanced disease who are in CR after initial chemotherapy. Improvements in chemotherapy, particularly with the use of combinations including L-asparaginase, have enhanced the ability to achieve CR even in relapsed/refractory disease and have enabled more patients to proceed to HCT. In one study including patients with advanced/relapsed/refractory disease, autologous HCT performed after two cycles of SMILE (dexamethasone, methotrexate, ifosfamide, L-asparaginase, etoposide) chemotherapy appeared superior to allogeneic HCT or continued chemotherapy, but the difference was not statistically significant [91]. Since long-term DFS is possible with asparaginase-containing regimens in a subset of patients even with advanced disease, further investigations to identify candidates who would benefit from autologous HCT are critical. The EBV-DNA level in peripheral blood has been shown to be a marker of disease burden and may prove to be useful in monitoring response to chemotherapy and selecting patients for autologous HCT in CR1 [92].

Murashige et al. reported allogeneic HCT results for 28 patients with NK-cell neoplasms [93]. The majority ($n = 22$) had extranodal NK/T-cell lymphoma. Twelve patients were chemosensitive and 16 were chemorefractory at the time of transplant. Twenty-three patients received grafts from matched related donors and 23 underwent myeloablative conditioning. With a median follow-up of 34 months, the 2-year PFS and OS were 34 and 40%, respectively (Figure 67.5). Patients who did not relapse within 10 months after HCT remained free of disease. In multivariate analysis, stem-cell source (bone marrow versus peripheral blood) and histology (extranodal NK/T-cell lymphoma versus others) were risk factors for PFS (relative risk 3.03 and 3.94, respectively). These results are good considering that 19 of the 28 patients had active disease at the time of HCT [93]. A similar OS rate was seen for 12 patients with NK-cell lymphoma who underwent unrelated donor HCT through the Japan Marrow Donor Program [94] and also in other case series [95,96].

The results and the ability to undergo allogeneic HCT may further improve with advances in initial chemotherapy, particularly with the use of L-asparaginase-containing regimens. In the study by Yamaguchi et al., among advanced/refractor/relapsed patients a CR

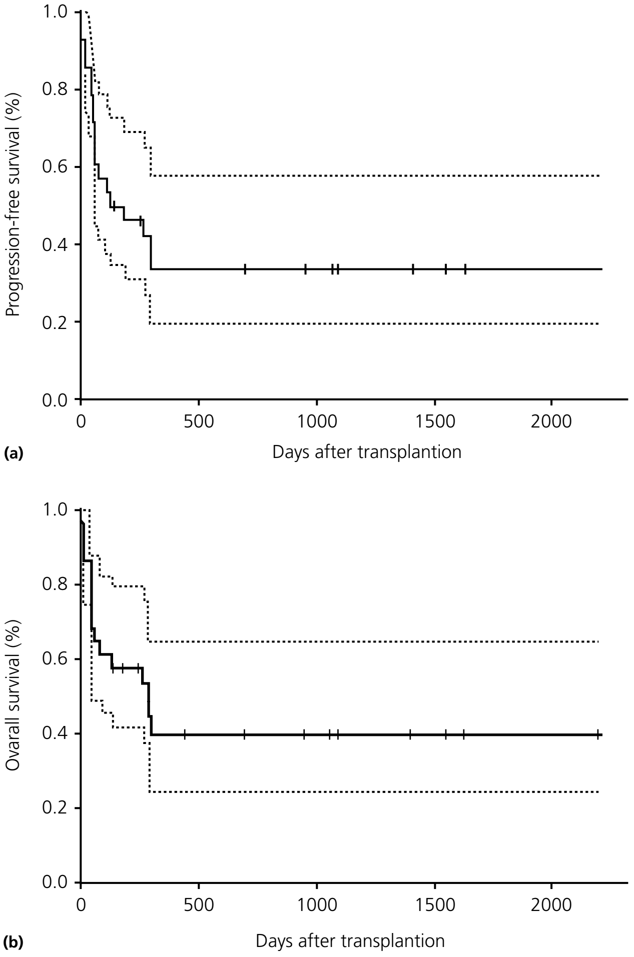

Figure 67.5 (a) Progression-free survival (PFS) and (b) overall survival (OS) of 28 patients with NK-cell neoplasms who underwent allogeneic HCT. PFS was 34% and OS was 40% at 2 years. Median follow-up was 34 months. Broken lines indicate 95% confidence intervals. Source: Murashige et al. 2005 [93]. Reproduced with permission of John Wiley & Sons, Ltd.

rate of 45% was achieved after two cycles. Among 28 patients who completed two cycles of SMILE chemotherapy, 17 were able to undergo allogeneic HCT with a 1-year OS and PFS of 59% [91]. This was not statistically different from the results for patients who underwent autologous HCT or chemotherapy. No conclusions about the efficacy of the RIC approach can be drawn based on available data.

Based on the above evidence, for extranodal NK/- cell lymphoma with advanced stage disease, autologous HCT should be considered as consolidation therapy for patients who enter CR after chemotherapy. Long-term survival has been reported with this approach and TRM is expected to be low. Autologous HCT has no role in refractory nasal-type extranodal NK/T lymphoma or aggressive NK-cell leukemia, and these patients should be strongly considered for allogeneic HCT since this is the only therapy known to result in long-term survival in this group of patients. In the case of systemic NK-cell neoplasms, the major obstacle to allogeneic HCT appears to be the comorbidity from paraneoplastic phenomena such as hepatic dysfunction. Therefore, better strategies for the initial management are needed to realize the full curative potential of allogeneic HCT. Existence of a graft-versus-leukemia (GVL) effect after allogeneic HCT has been suggested [84]. Monitoring EBV-DNA in peripheral blood may allow better selection of patients for HCT and also monitoring of minimal residual disease after HCT.

Mycosis fungoides and Sézary syndrome

Mycosis fungoides (MF), the commonest form of cutaneous T-cell lymphoma, is a neoplasm of $CD4^+$ T-cells that home to the skin. Initial stages of MF are characterized by patch or plaque lesions and typically last for many years. In the late stages, patients develop skin tumors and extracutaneous dissemination to lymph nodes and visceral organs [1,97,98]. Although patients with early stages of MF often respond to current therapies that include retinoids, interferon-α, photopheresis, monoclonal antibodies, and chemotherapy, among others, these therapies have no curative potential and the disease ultimately progresses to its more advanced stages [98]. In one study, the median survival of Stage IV patients was only 13 months from initiation of therapy [99]. Sézary syndrome (SS) is an aggressive variant of MF and is characterized by erythroderma, lymphadenopathy, circulating neoplastic T cells, and a much more aggressive clinical course [1,97,98].

From the limited experience available, it can be concluded that autologous HCT is not capable of inducing durable remissions when performed in the advanced stages of MF in heavily pretreated patients. In a series of 20 patients compiled by Duarte et al., although a CR rate of 90% was achieved with autologous HCT, the 1-year OS was only 50% and time to progression was only 2.3 months [100]. Similarly, lack of benefit for autologous HCT was also evident in a meta-analysis [101]. T-cell depletion of the graft had no effect in improving the dismal outcome of autologous HCT [100,102]. Whether autologous HCT early in disease course, possibly in combination with post-transplant maintenance therapy with currently available active agents, will produce durable remissions remains to be determined.

Given the grim prognosis of patients with advanced stage MF and SS, allogeneic HCT has been attempted to harness a potential GVL effect. Molina et al. first reported the results for a series of eight patients treated at the City of Hope Medical Center with allogeneic HCT [103]. Three patients had tumor stage MF, one had erythrodermic MF, and the remaining three had SS. All patients were heavily pretreated and had failed a median of seven previous therapies. Graft source was from a matched sibling in four patients and a matched unrelated donor in the other four. Conditioning regimens were fractionated total body irradiation (FTBI)/CY (three patients), BU/CY (one patient), and fludarabine/melphalan (four patients). All patients achieved CR and their original T-cell clones disappeared from blood by 60 days after HCT. Although two patients had new T-cell clones detectable in the peripheral blood on follow-up analysis, these did not have any clinical impact and these patients remained disease-free. With a median follow-up of 56 months, six patients remained disease free [103] (Figure 67.6). A subsequent report from the same institution of fludarabine and melphalan (reduced-intensity) conditioned allogeneic HCT showed a 2-year PFS of 45% and a non-relapse mortality (NRM) of 27% among 11 patients [104].

Other larger case series of allogeneic HCT for MF and SS have subsequently been reported. Duarte et al. described a retrospective analysis of 60 patients (MF = 36, SS = 24) who underwent allogeneic HCT at EBMT centers. The 1- and 3-year OS was 66 and 54% and PFS was 42 and 34%, respectively (Figure 67.7). RIC resulted in a significantly lower NRM than myeloablative conditioning (1-year NRM 14% vs. 38%, respectively) without increasing relapse risk. T-cell depletion increased relapse risk. Better PFS was observed in recipients of matched related donor grafts than matched unrelated donor grafts [105]. A recent report on the long term outcome of this cohort showed an OS of 44% and PFS of 30% at 7 years. Relapse/progression occurred in 45%. Median time of relapse was 3.8 months and only 2 relapses occurred beyond 2 years after HCT [106]. Results of allogeneic HCT for CTCL were summarized by Schlaak et al. [107].

Collectively, these reports provide evidence for the feasibility and curative potential of allogeneic HCT even in this group of advanced-stage, high-risk patients. RIC regimens appear to be better tolerated and result in lower NRM. Clear evidence of a potent GVL effect has been demonstrated in MF by the regression of persistent lesions by withdrawal of immunosuppression or DLI [106]. Infection appears to be a major contributor to NRM, no doubt resulting from extensive prior use of therapies with profound immunosuppressive effects. In the light of the substantial impact of MF on quality of life and the inability of current treatments to prevent progression, consideration should be given to allogeneic HCT after failure of two or three initial therapies, at least in patients who are under 60 years of age and have matched sibling donors.

Angioimmunoblastic T-cell lymphoma

Angioimmunoblastic T-cell lymphoma (AITL) is a rare lymphoma accounting for 1–2% of non-Hodgkin lymphomas. AITL is strongly associated with EBV, which can be detected within the B cells in involved tissues in the majority of cases. Systemic symptoms, skin rash, immunodeficiency and autoimmune phenomena such as hemolytic anemia are characteristic features of AITL. Patients with AITL are usually middle-aged or elderly and often present with advanced disease. Although anecdotal reports describe responses to a variety of agents, including immunosuppressants, biologic response modifiers, and chemotherapeutic agents, the overall prognosis for AITL is poor [1,108,109]. In one study that analyzed the outcome of 33 patients treated with chemotherapy, the OS at 5 years was 36%. Notably, two of the long-term survivors in this study had received autologous HCT [110].

Although some patients with AITL have been included in various reports of HCT for T-cell lymphomas, two reports that have analyzed the outcome of autologous HCT for AITL as a separate cohort

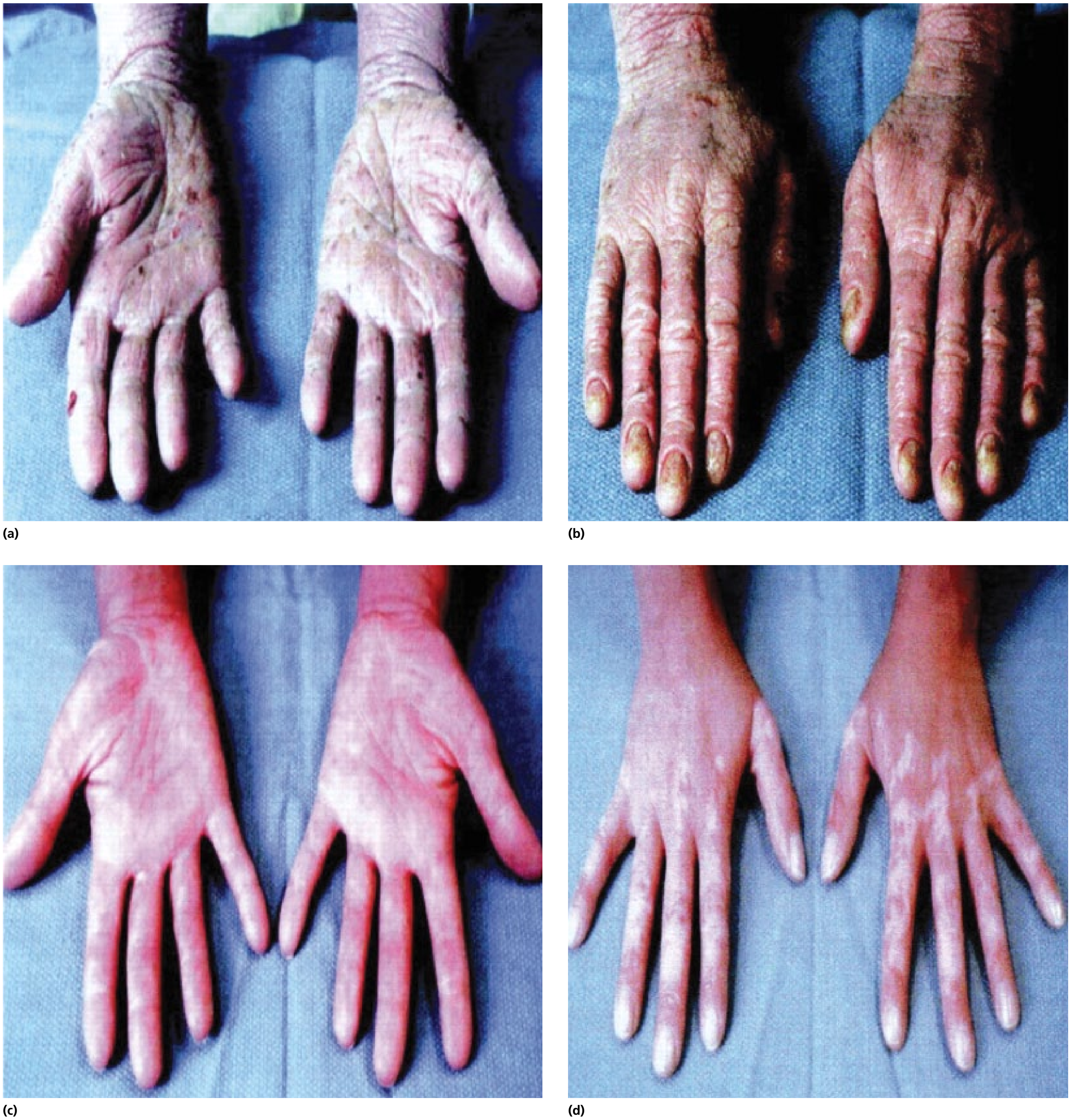

Figure 67.6 (a, b) Hands of a patient with Sézary syndrome shows fissuring, cracking, and nail destruction. (c, d) One year after allogeneic HCT, there is regression of skin involvement and normalization of the nails. Source: Molina et al. 2005 [103] Reproduced with permission of the American Society of Clinical Oncology.

are discussed here. In the first of these reports, from the EBMT, 146 patients with AITL underwent autologous HCT; the majority with BEAM [BCNU, etoposide, Ara-C (cytosine arabinoside), melphalan] conditioning. NRM was 7% at 24 months and actuarial OS was 59% at 48 months. The cumulative incidence of relapse was estimated at 51% at 48 months. Disease status at HCT was the major factor that determined outcome. The PFS rate for patients who underwent HCT in CR was 56% at 48 months as opposed to 23% for patients with chemotherapy-refractory disease [111]. Given the poor outcome with conventional chemotherapy, these results would justify performing autologous HCT in CR1 for AITL.

In another report, the Spanish Lymphoma/Autologous Bone Marrow Transplant Study Group analyzed the outcome of 19 patients who underwent autologous HCT. Again, the median age of 46 years of these patients is low for AITL. Eleven patients were in a first or later CR. Fifteen patients (79%) achieved CR after HCT. The OS and PFS at 3 years were 60 and 55%, respectively, for the whole group (Figure 67.8). Predictably, bone marrow involvement,

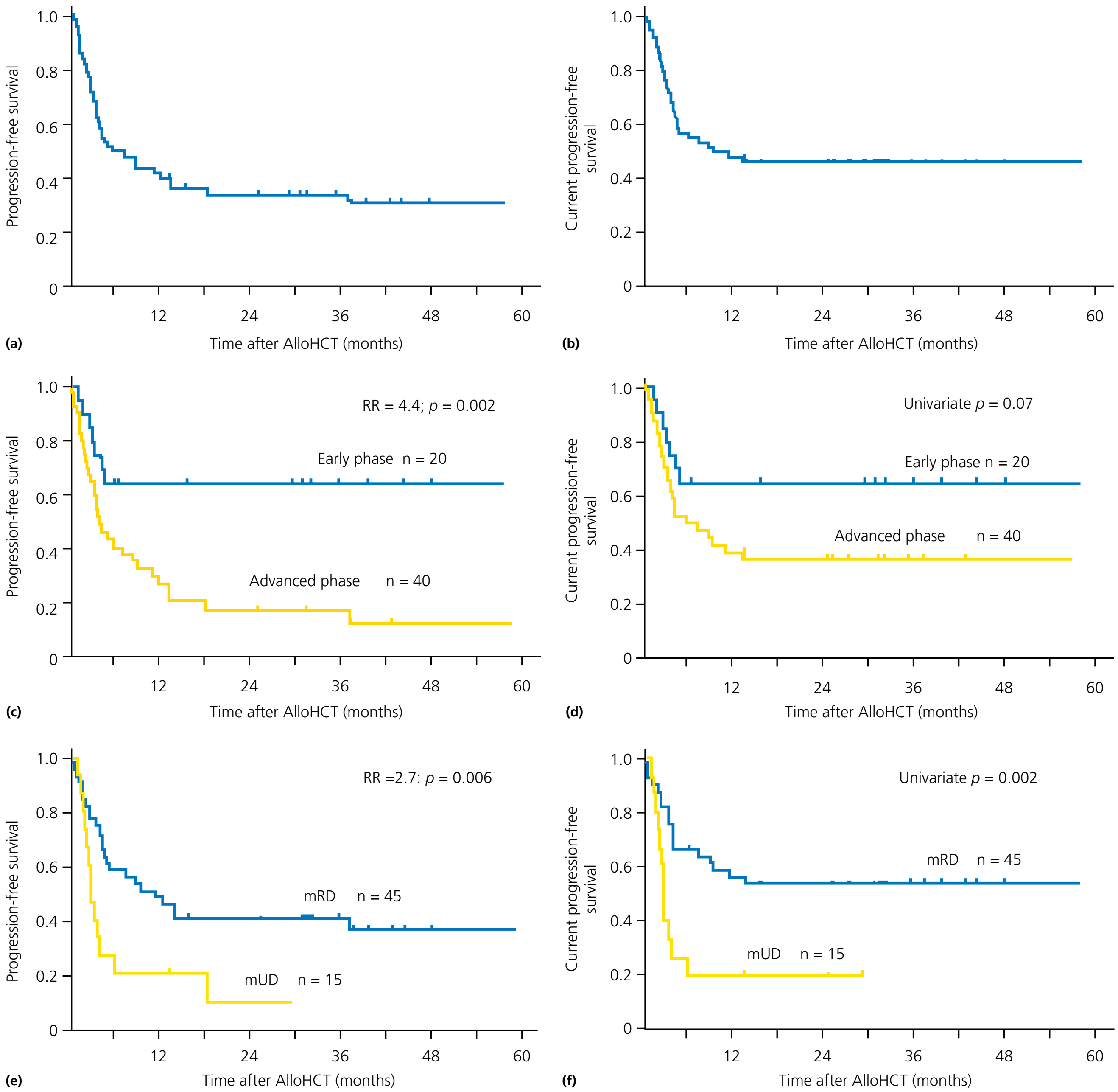

Figure 67.7 Probability of PFS (a, c, e) and current PFS (b, d, f) for 60 patients with mycosis fungoides and Sézary syndrome. Source: data from Duarte et al. 2010 [105].

refractory disease, and more than one factor of the age-adjusted International Prognostic Index (IPI) were associated with poor outcome [112]. Although the above two studies undoubtedly represent a selected group of patients who were able to undergo autologous HCT, the results appear to compare favorably with those of conventional therapy. Patients with AITL are at risk for EBV-positive and also EBV-negative secondary B-cell lymphomas [113,114]. Both EBV-positive and -negative lymphoproliferative disorders can occur after autologous HCT and these appear to respond to rituximab-based therapy [115,116].

The allogeneic HCT experience in AITL has been reported in a retrospective study from EBMT centers. Results for 45 patients with a median age of 48 years were reported. Eleven patients failed autologous HCT. Twenty patients received RIC. NRM was 25% at 1 year and the relapse rate was 20% at 3 years. Relapses were less common in patients with chronic GVHD, suggesting a GVL effect in this disease. PFS and OS were 66 and 64%, respectively, at 3 years and were significantly better in chemotherapy-sensitive patients [117]. The advanced age of patients with AITL and the significant tumor-associated comorbidities are significant hurdles for successful allogeneic HCT. However, allogeneic HCT should be considered in suitable patients who have primary or relapsed refractory disease, since these patients do not benefit from autologous HCT.

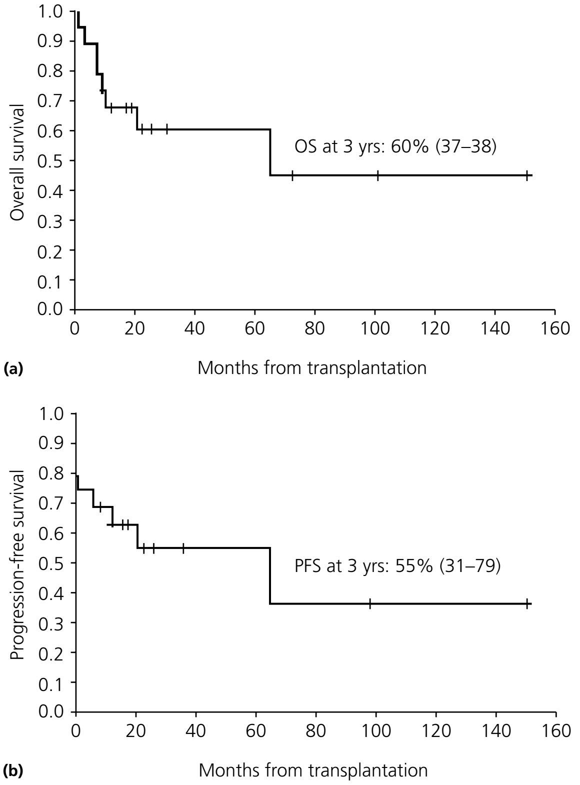

Figure 67.8 (a) Overall survival and (b) progression-free survival of 19 patients who underwent autologous HCT for AITL. Source: data from Rodríguez et al. 2007 [112].

Primary central nervous system lymphoma

Primary central nervous system lymphoma (PCNSL) comprises about 1–2% of NHL cases. Its incidence is rising in both immunocompromised and immunocompetent individuals [118]. PCNSL typically presents as unifocal or multifocal periventricular parenchymal masses. Ocular involvement, either clinical or asymptomatic, may be present in 10–20% of patients at diagnosis. Other systemic involvement is extremely rare at presentation. The median age at diagnosis is 60 years. Over 90% of PCNSLs have a diffuse large B-cell histology and 2–4% of cases are of T-cell origin. Unlike the situation with immunocompromised patients, EBV does not play a role in the pathogenesis of PCNSL in immunocompetent patients [1,119].

Although data from randomized trials are lacking, current therapy for PCNSL involves chemotherapy with high-dose methotrexate alone or in combination followed by whole-brain radiotherapy (WBRT). The long-term outcome of PCNSL is poor. Although 2-year PFS of 57% and OS of 67% were reported in one study [120], the 10-year OS of patients under 60 years of age in another trial was only 32% [121]. Notably, in the latter trial, there were no long-term survivors among patients who were over 60 years of age at diagnosis. Neurotoxicity resulting from WBRT and chemotherapy is a major cause of morbidity in survivors, especially in those above 50 years of age, who comprises the majority of PCNSL patients [122]. Therefore, high-dose chemotherapy with autologous stem-cell support after initial chemotherapy has been tried as a strategy to minimize neurotoxicity by eliminating or reducing WBRT dose and improve the long-term outcome in PCNSL.

The small sizes of reported studies and the marked variations in the initial and high-dose chemotherapy regimens make it difficult to draw firm conclusions regarding the efficacy of the autologous HCT approach as front-line therapy of PCNSL. In general, in these studies chemotherapeutic agents for conditioning were selected based on their ability to cross the blood–brain barrier. Illerhaus et al. reported results for 30 patients younger than 65 years of age with PCNSL who were enrolled in a trial of initial chemotherapy with three cycles of high-dose methotrexate (MTX) followed by a cycle of cytarabine and thioTEPA (*N*-triethylenethiophosphoramide), following which peripheral blood stem cells were collected. High-dose therapy consisted of carmustine and thioTEPA. Fractionated radiotherapy (45 Gy, two doses of 1 Gy/day) was given as consolidation. Twenty-three patients were able to undergo autologous HCT, 15 of whom achieved CR and eight achieved partial remission (PR). All 21 patients who underwent WBRT achieved CR. With a median follow-up of 63 months, the 5-year OS probability was 69% for the whole group and 87% for patients who received HCT. Leukoencephalopathy developed in 17% of patients, all of whom had received WBRT [123]. In another Phase II study, Colombat et al. treated 25 patients below 60 years of age with two cycles of high-dose MTX-based chemotherapy. Seventeen responding patients then received high-dose therapy with BEAM and autologous HCT followed by 30 Gy of WBRT. With a median follow-up of 34 months, the estimated 4-year OS and EFS were 64 and 46% respectively. Although the follow up was short, no leukoencephalopathy had developed in evaluable patients who received the whole planned therapy [124]. In another small study of six patients treated with the same regimen as in the above study, four patients were alive without evidence of disease with a median follow-up of 41.5 months [125]. Other studies have utilized a response-adapted WBRT approach restricting radiation therapy to patients who fail to achieve CR after HCT. Yoon et al. reported on 11 patients who underwent autologous HCT with busulfan, cyclophosphamide, and etoposide conditioning after initial therapy with high-dose MTX and cytarabine. Only two patients who failed to achieve CR after HCT received WBRT. Six patients had relapsed and the median EFS was 15 months, with 2-year EFS and OS of 30.3 and 88.9%, respectively [126]. In another study, among 13 patients treated with MTX and cytarabine /thioTEPA, 11 underwent HCT. Only three of four patients with PR after HCT received WBRT. The 3-year DFS and OS were 76.9% and were similar to those in a previous trial by the same investigators that obligated WBRT. Another multicenter study of this approach showed a 10-year OS of 35% with excellent neurocognitive function in patients who did not receive WBRT [127].

WBRT was completely eliminated from some studies of high-dose therapy for newly diagnosed PCNSL. In the larger study, 28 patients received induction with high-dose MTX and cytarabine followed by high-dose therapy with BEAM in chemosensitive patients. Fourteen patients underwent autologous HCT. With a median follow-up of 28 months, the median EFS was 5.6 months for all patients and 9.3 months for the 14 patients who underwent HCT. Six (43%) of these 14 patients were disease free at the time of the report [128]. In another study, by Alimohamed et al., 21 patients with PCNSL aged 34–69 years underwent autologous HCT with thioTEPA, busulfan, and cyclophosphamide conditioning after initial therapy with high-dose MTX and cytarabine. At a median follow up of 60 months, 11 patients (52%) remained alive and disease free without evidence of neurocognitive dysfunction [129]. Cheng et al. treated seven patients with high-dose MTX induction followed by high-dose therapy with thioTEPA, busulfan, and cyclophosphamide. Two patients in this study received autologous HCT as sole therapy for progressive disease after initial chemotherapy. Five of the seven patients remained disease free at 5–42 months from diagnosis, including the two patients with progressive disease after initial chemotherapy. These authors attributed their good results with these poor-prognosis patients to the use of agents such as thioTEPA and busulfan that have better CNS penetration than the BEAM regimen [130]. No neurotoxicity was observed in all of these studies during the follow-up period.

High-dose chemotherapy with autologous stem-cell support has also been tried as salvage therapy for patients with refractory or relapsed PCNSL or intraocular lymphoma after high-dose MTX. Soussain et al. reported a retrospective analysis of 79 such patients with a median age of 52.4 years who received autologous HCT after high-dose therapy with thioTEPA, busulfan, and cyclophosphamide. With a median follow-up of 56 months, the 5-year OS and EFS probability were 51 and 37.8%, respectively, in the whole population and 62 and 43.7%, respectively, among patients who were chemosensitive to salvage therapy [131]. A single case report described a patient with refractory PCNSL who underwent an allogeneic sibling HCT after non-myeloablative conditioning. This patient remained disease free 30 months after HCT, suggesting that a GVL effect may be operational in the CNS [132]. Results of autologous HCT for PCNSL have recently been reviewed by Reddy and Savani [133].

In summary, high-dose chemotherapy with autologous HCT is feasible as frontline therapy for chemosensitive PCNSL and carries a low treatment-related mortality, at least in patients less than 65 years of age. A reduced dose of WBRT can be given after autologous HCT with low short-term neurotoxicity, although it is not clear if high-dose chemotherapy can allow for complete elimination of WBRT. There is a suggestion that using agents such as thioTEPA and busulfan that have better CNS penetration for high-dose therapy is superior to conventional lymphoma regimens such as BEAM. Autologous HCT may have a role in salvaging patients who are refractory to initial chemotherapy or who have relapsed after chemoradiotherapy, although neurotoxicity appears to be high in this setting.

Blastic plasmacytoid dendritic cell neoplasm

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) (formerly termed blastic NK-cell lymphoma, CD4$^+$/56$^+$ hematodermic neoplasm) is a malignancy of plasmacytoid dendritic cells with a marked predilection for skin involvement. Bone marrow involvement is common [1,134,135].

Given the lack of clarity of the lineage of BPDCN, patients have been treated with chemotherapy regimens for AML as well as acute lymphoblastic leukemia (ALL). Pagano et al. reported a series of 43 patients treated at 28 Italian centers [136]. The median OS was only 8.7 months, demonstrating the dismal treatment outcome for this disease. In this series, patients who were treated with ALL regimens had a significantly higher CR rate and OS than those treated with an AML regimen. Although there are some reports of durable CR after combination chemotherapy, the overall outcome with conventional combination chemotherapy alone is poor . Hence HCT has been attempted with hopes of achieving long-term survival. In the Italian series, six patients underwent allogeneic HCT. These patients had a significantly better OS than non transplanted patients (median OS 22.7 months vs. 7.1 months) [136]. Another case series of 47 patients were reported from France. Ten of these 47 patients underwent HCT (nine allogeneic, one autologous). The mean survival for the patients who underwent HCT was 31.3 months, which was significantly better than patients who did not undergo HCT [137]. Dietrich et al. performed RIC allogeneic HCT from unrelated donors in four patients ranging in age from 56 to 70 years. The two patients transplanted in CR were alive and disease free at 57 and 16 months whereas the two patients transplanted in active disease relapsed at 6 and 18 months after HCT [138]. Yoshimasu et al. reported a case of relapsed BPDCN that was successfully treated with unrelated cord blood allogeneic HCT [139]. Other single case reports of successful allogeneic HCT in children and adults have been published [140,141].

Based on the limited data available, allogeneic HCT offers the best chance for long-term survival if performed in CR1. Relapse rates are high in patients transplanted with active disease and NRM appears fairly high. There are single case reports of durable CR after autologous HCT in patients with widely disseminated disease including bone marrow involvement [142,143]. A report from Japan showed a 4 year OS and PFS of 82% and 73% respectively for 11 patients who underwent autologous HCT in CR1 [142]. Autologous HCT may be an option in selected patients to consolidate a first CR.

Other rare lymphoid malignancies

Hepatosplenic T-cell lymphoma is an aggressive T-cell lymphoma characterized by marked hepatosplenomegaly and almost invariable involvement of the bone marrow. Most cases are of the gamma/delta T-cell type [1]. Various reports have described durable remissions after allogeneic HCT for hepatosplenic T-cell lymphoma even in chemotherapy-refractory cases [144–147].

T-cell prolymphocytic leukemia (T-PLL) is an aggressive neoplasm with a poor response to chemotherapy [1,148]. A recent French registry analysis of 27 T-PLL cases (14 in CR) who had allogeneic HCT showed a 3 year OS and PFS of 36% and 26% respectively. A high NRM (31%) was observed [149]. Kruspe et al. reviewed the literature on allogeneic HCT for T-PLL. Among 13 published case reports, including a case of their own, there were 10 patients who remained in CR for periods ranging from of 2 to 48 months after HCT. Notably, only four of these 13 were in CR at the time of HCT [150].

Subcutaneous panniculitic T-cell lymphoma mostly affects young adults who present with subcutaneous nodules. A hemophagocytic syndrome may occur [1]. In a review of 21 patients treated with a variety of modalities, including five patients who received HCT, the overall survival from diagnosis was only 15 months [151]. There are single case reports of prolonged survival after autologous HCT even in patients who have relapsed disease and other adverse prognostic factors such as elevated lactate dehydrogenase and bone marrow involvement [152–156]. Allogeneic sibling HCT has been reported in a patient with progressive disease who remained in CR 31 months after allogeneic HCT [157].

HCT should be considered early in the treatment plan for the three extremely rare neoplasms discussed in this section. For hepatosplenic lymphoma and T-prolymphocytic leukemia, allogeneic HCT upfront appears to be the treatment of choice, preferably after inducing remission with chemotherapy or antibody-based therapy. There is currently no evidence to suggest a benefit for autologous HCT for these two disorders. In the case of panniculitic T-cell lymphoma, it appears reasonable to perform autologous HCT to consolidate an initial CR or at chemosensitive relapse. Allogeneic HCT could be reserved for primary refractory disease or relapsed cases that are chemorefractory and for patients who have poor prognostic factors such as extensive bone marrow involvement. Although no conclusions about the overall effectiveness of HCT can be drawn from the reported cases, at present HCT is the only therapy known to produce durable remissions in these aggressive lymphoid malignancies.

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CHAPTER 68

Adoptive T-cell Therapy for Viral Disease in the Setting of Hematopoietic Cell Transplantation

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Introduction

Following hematopoietic stem cell transplantation (HSCT), reconstitution of the recipient immune system occurs with donor-derived cells. Murine and human studies have clearly shown that the presence of a virus-specific T-cell immune response is required to control viral infections [1–3] and viral reactivation or infection is therefore an important cause of morbidity and mortality until this process is complete. A number of factors influence the risk of viral infections, including the degree of tissue mismatch between donor and recipient, the degree of immunosuppression, and the immune status of the donor. For example, the use of antithymocyte globulin (ATG) or alemtuzumab in conditioning regimens has been associated with a significant increase in the incidence of viral infections [4]. As absence of a virus-specific cellular immune response is clearly associated with viral reactivation and disease in these patients [3,5,6], cellular immunotherapy with virus antigen-specific T cells [cytotoxic T-lymphocytes (CTLs)] to restore viral-specific immunity is an attractive option. The first study that evaluated adoptive transfer of virus-specific T cells was published in 1992 [7], and since then this strategy has been tested for a number of viruses, with many studies showing encouraging response rates. Recent manufacturing advances have greatly simplified production so that several approaches are now being evaluated in late phase or licensing studies that may allow adoptive transfer of virus-specific CTLs to become standard of care post-HSCT.

Common viral infections post-transplant

The number of viruses associated with disease after HSCT is increasing owing to increased surveillance with more reagents becoming available for detection by polymerase chain reaction (PCR) and the discovery of new viruses [8]. Some new viruses such as metapneumovirus are clearly associated with disease whereas the contribution of other viruses to post-transplant morbidity is less clear. Transplant recipients are at risk of both reactivation of latent viruses such as cytomegalovirus (CMV), BK virus, herpes simplex virus (HSV), varicella zoster virus (VZV), human herpesvirus 6 (HHV6) and Epstein–Barr virus (EBV) and new infections with respiratory viruses such as adenovirus, parainfluenza, and influenza. Antiviral pharmacologic agents are available for some of these viruses but their use is limited by significant toxicities and the outgrowth of drug-resistant mutants. Moreover, infections often recur when the agents are discontinued.

Cytomegalovirus

CMV is a latent beta-herpesvirus that usually causes an asymptomatic infection in immunocompetent individuals. Around 65% of healthy adults are seropositive and in these individuals CMV can replicate in epithelial cells, fibroblasts, and monocytes. CMV-specific $CD4^+$ and $CD8^+$ lymphocytes play an important role in immune protection from both primary infection and subsequent reactivations, so seropositive individuals or seronegative recipients of seropositive grafts have a significant risk of reactivation and infection post-transplant [9].

Epstein–Barr virus

EBV is a gamma-herpesvirus that infects more than 95% of the world's population. Primary infection usually produces a mild self-limiting disease, which is followed by latent infection in B cells and productive replication in B cells and mucosal epithelium. In immunocompromised hosts, outgrowth of B cells expressing Type 3 latency may lead to the development of post-transplant lymphoproliferative disorder (PTLD) [10]. The overall incidence of PTLD after HSCT is less than 1%, but the incidence is increased in recipients with an underlying diagnosis of immunodeficiency and for recipients of stem cells from unrelated or human leukocyte antigen (HLA)-mismatched donors who receive grafts that are selectively depleted of T cells to prevent graft-versus-host disease (GVHD). PTLD is highly susceptible to control by virus-specific T cells [11].

Adenovirus

Adenovirus is a non-enveloped lytic DNA virus. Humans are susceptible to infection with 51 serotypes forming six distinct adenovirus species (A–F), which differ in their tissue specificity and virulence. Although acute infection is rarely fatal in healthy adults, it is a significant cause of morbidity and mortality in immunocompromised individuals, in whom it may produce pneumonia, hemorrhagic cystitis, nephritis, colitis, hepatitis, and encephalitis. Transplant recipients may develop new infections but may also reactivate previous infections with this virus. Adenovirus has a particularly high incidence after pediatric HSCT. Several reports have shown that clearance of adenovirus infection is associated with detection of adenovirus-specific T cells [6,12] and recovery is significantly delayed in recipients of matched unrelated donor and haploidentical transplants who receive intensive immunosuppression such as with alemtuzumab [13].

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Varicella zoster

VZV, a member of the herpesvirus family, causes chickenpox with a primary infection and shingles upon viral reactivation. Once the primary infection has resolved, the virus becomes latent in the dorsal root ganglia. Reactivation of VZV can occur in approximately 30% of patients after HSCT and correlates with the degree of immunosuppression [8].

BK virus

Human polyomavirus type I, commonly called BK virus (BKV), infects up to 90% of humans worldwide before the age of 10 years. Shedding of BK in the urine is frequently seen in transplant recipients as in healthy individuals and some of these recipients will develop BKV-associated hemorrhagic cystitis [8].

HHV6

Most individuals are infected with HHV6 in childhood and the virus remains latent in lymphocytes and monocytes [14]. HHV6 reactivation is common during the early period after allogeneic HSCT transplant, with viremia occurring in approximately 40–60% of patients, and some patients develop symptomatic disease such as encephalitis. Recovery of interferon-gamma (IFN-γ)-producing HHV6-specific T-cells may be associated with ability to control the reactivation [15].

Herpes simplex virus

HSV is a double-stranded, enveloped DNA virus that becomes latent in sensory ganglia after primary infection. Reactivation in the peri- and post-transplant setting is decreased by prophylaxis with acyclovir but it can still be associated with significant morbidity [8].

Respiratory viruses

HSCT patients are susceptible to community-acquired and nosocomial spread of respiratory viruses such as respiratory syncytial virus (RSV), influenza, and parainfluenza [8]. These infections can have a high mortality when they develop in the early post-transplant period and would be amenable to immunotherapy approaches.

Prerequisites for T-cell therapy

Since recovery of virus-specific T cells is clearly associated with protection from viral infection [16], adoptive immunotherapy to decrease the time to immune reconstitution or to treat viral reactivations and infections is an attractive approach. Donors who are seropositive for a particular virus will have circulating T cells specific for this virus, with the frequency ranging from very low levels to up to 1% of total T cells for the latent herpes viruses CMV and EBV. If the donor has a high frequency of virus-specific T cells, they may be directly selected from an apheresis product, although this option is generally only viable for viruses where the frequency of virus-specific precursors is high. In all cases, however, virus-specific T cells can be generated in vitro from seropositive donors by repeated stimulation with antigen-presenting cells (APCs) that express viral antigens. Under these conditions, alloreactive T cells should not survive.

Choice of antigen

To generate virus-specific T cells *ex vivo* requires a defined immunogenic antigen and an antigen-presenting cell that can effectively present the antigen to T cells with appropriate costimulatory signals. It is therefore necessary to know, for a given virus, which viral antigens are immunodominant and induce protective T cells. Also, it is crucial to identify the antigens that are most important for viral persistence and will therefore be the best targets for immunotherapy. For some viruses such as CMV and EBV, the immunodominant antigens expressed at different stages of infection have been well defined [5], but for other viruses such as adenovirus the appropriate target antigens had to be identified to design adoptive immunotherapy studies [17]. Antigen characterization has been simplified by the availability of bioinformatic tools that allow the effective mapping of viral epitopes recognized by both CD4$^+$ and CD8$^+$ T cells.

Antigen presentation

The defined immunogenic antigens must be presented on an autologous APC that expresses major histocompatibility complex (MHC) antigens to present virus antigen-derived peptides in addition to costimulatory molecules sufficient to induce T-cell activation and expansion. The choice of APC also depends on the type of antigen to be used and the proposed method of delivery. Some approaches use whole virions as antigen whereas others use specific viral genes, proteins, or peptides and the APCs used include fibroblasts, dendritic cells (DCs), monocytes, and B cells. Specific examples include the following:

1. *Whole virus or viral lysate to expand virus-specific T cells.* The use of the entire viral antigen (e.g., CMV) enables a broad CTL response to be generated and CMV lysate or antigen has been used to infect fibroblasts or pulse DCs [18,19]. Similarly, intial studies used EBV-infected lymphoblastoid cell lines (LCLs) to reactivate EBV-specific CTLs, which produced a broad EBV-specific response [20]. However, the use of CMV lysate containing infectious CMV- or EBV-transformed LCLs makes it difficult to develop the approach for late-phase trials.
2. *Whole proteins*, such as the EBNA-1 to expand EBNA-1-specific T cells for EBV-associated PTLD [21].
3. *Gene-modified APCs.* An adenoviral vector encoding CMV pp65 has been used to genetically modify various APCs including DCs, monocytes, and LCLs to expand T cells specific for CMV pp65 [22,23]. This approach has the added advantage of also enabling the expansion of T cells recognizing the adenoviral virion proteins hexon and penton expressed by the adenoviral vector. In studies in which the adenoviral vector is used to tranduce LCLs, the responding T cells are also exposed to latent and early lytic cycle EBV proteins expressed by the LCLs [22,24]. More recently, to avoid the use of viral vectors, plasmids expressing viral antigens derived from CMV, EBV, and adenovirus have been used to transfect DCs to stimulate multi-virus-specific T cells [25].
4. *Peptides and peptide mixtures.* Several groups have explored the use of peptides such as the A2-restricted CMV pp65 epitope NLV to stimulate virus-specific T cells [26]. Concerns with this approach are that targeting a single epitope will result in escape mutants and also that it is restricted to specific HLA types. Recently, the availability of overlapping peptide pools or pepmixes where overlapping peptide libraries represent the entire protein sequences of the target viral antigens has provided a convenient source of antigen that has been validated in preclinical studies [27–30] and is being tested in several clinical trials.

Expansion strategies

"Classic" ex vivo expansion

In the "classic" expansion approach, APCs expressing the relevant antigens are generated and then used in multiple stimulations (usually weekly) to activate and expand a virus-specific T-cell product which is enriched for the T cells targeting the viral antigens expressed by the APC (Figure 68.1a). The advantage of this strategy is that the prolonged ex vivo culture enriches for virus-specific cells

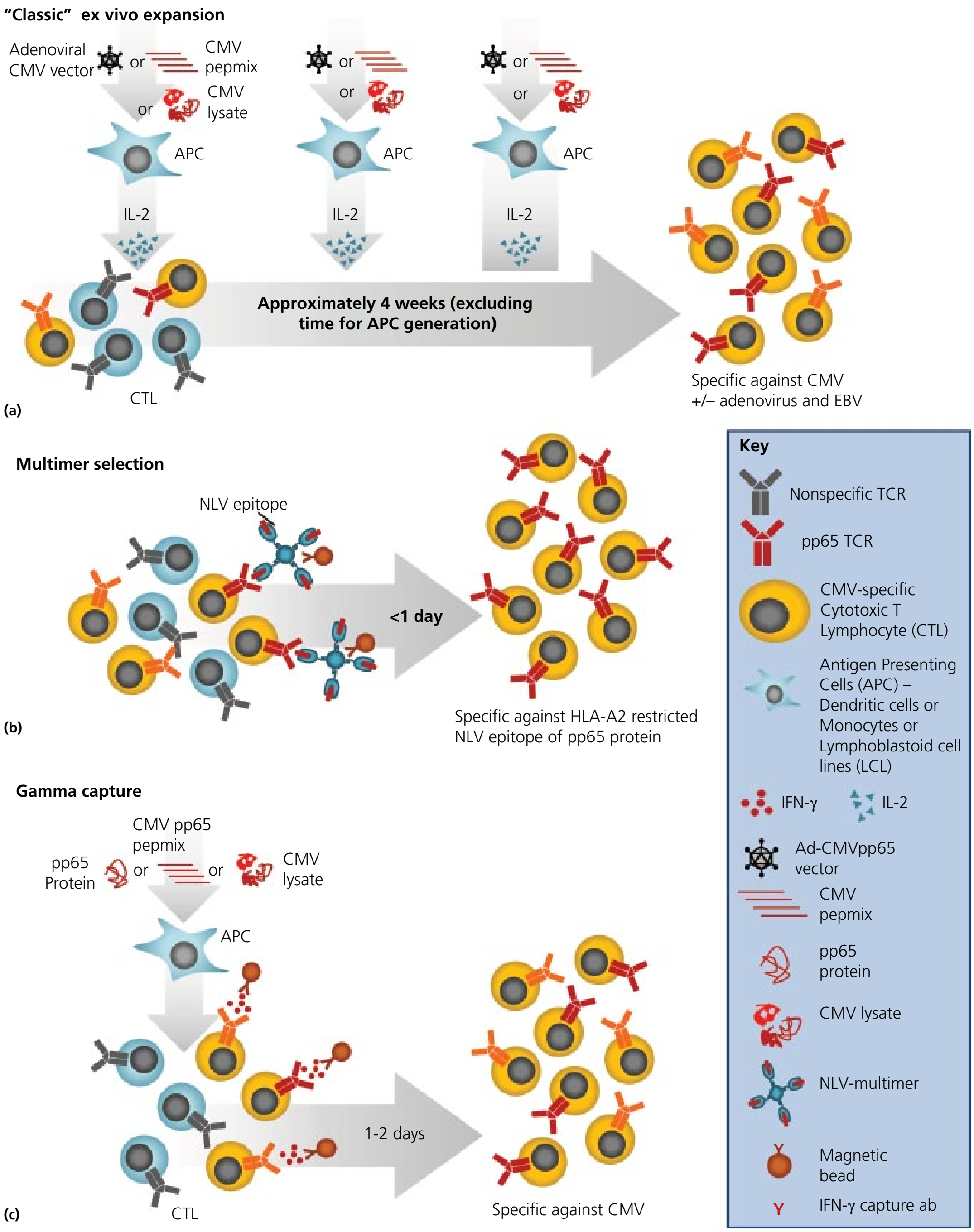
"Classic" ex vivo expansion
Adenoviral CMV vector
or
CMV pepmix
or
CMV lysate
APC
IL-2
Approximately 4 weeks (excluding time for APC generation)
CTL
Specific against CMV +/– adenovirus and EBV
(a)
Multimer selection
NLV epitope
<1 day
Specific against HLA-A2 restricted NLV epitope of pp65 protein
(b)
Gamma capture
CMV pp65 pepmix
pp65 Protein
or
or
CMV lysate
APC
1-2 days
CTL
Specific against CMV
(c)
Key
Nonspecific TCR
pp65 TCR
CMV-specific Cytotoxic T Lymphocyte (CTL)
Antigen Presenting Cells (APC) – Dendritic cells or Monocytes or Lymphoblastoid cell lines (LCL)
IFN-γ
IL-2
Ad-CMVpp65 vector
CMV pepmix
pp65 protein
CMV lysate
NLV-multimer
Magnetic bead
IFN-γ capture ab

Figure 68.1 GMP compliant approaches for the generation of CMV-specific T cells. (a) "Classic" ex vivo expansion method of CMV-CTL manufacture where antigen-presenting cells (APCs) are prepared using either dendritic cells or monocytes or EBV-transformed B lymphoblastoid cell lines (LCLs). The APCs are then either pulsed with CMV peptides or protein or infected with CMV lysate or transduced with an adenoviral vector expressing the CMV pp65 transgene. The APCs are then irradiated and used to stimulate peripheral blood mononuclear cells (PBMCs) from the same donor. Approximately 9–12 days later, and weekly thereafter, the T cells are restimulated with the APCs usually in the presence of IL-2. At the end of the CTL manufacturing process (approximately 4 weeks), the CTLs are frozen and released. Functional testing is performed prior to infusion into the patient. (b) This shows how CMV-specific T cells can be selected using multimer selection. In the example shown, T cells recognizing the HLA A2-restricted CMV pp65 epitope NLV are selected directly from the donor PBMCs by first incubating with the HLA–peptide tetramer and then selecting the tetramer-positive T cells with magnetic beads. This process generally takes less then 1 day of processing. (c) This shows an alternative method of rapidly generating a CMV-specific T cell line using the gamma capture technology. Donor PBMCs are stimulated with either recombinant CMV protein or CMV lysate or GMP grade-selected HLA-restricted CMV peptides or overlapping peptides spanning entire CMV antigens such as pp65. After overnight stimulation, IFN-γ-secreting cells are selected using a Cytokine Secretion System and a CliniMACS immunomagnetic separation device (both from Miltenyi Biotech) which "capture" the IFN-γ-secreting CMV-specific T cells. CMV-specific T cells selected using this methodology can either be infused immediately after the isolation procedure or cryopreserved for future use. The manufacturing time for this procedure is 1–2 days. (For a color version of this figure, please see the color plate section.)

while depleting alloreactive T cells. Moreover, under most culture conditions, the resulting product is broadly specific, containing both CD8$^+$ and CD4$^+$ T cells, which is considered advantageous since the presence of antigen-specific CD4-helper T cells is important for survival of cytotoxic CD8-positive T cells in vivo. The disadvantage is the long time required for manufacture. For example, EBV-specific T cells generated using LCLs as APCs require approximately 6 weeks for the manufacture of the LCLs generated by infecting donor peripheral blood mononuclear cells (PBMCs) with a laboratory strain of EBV, then 4–6 weeks to stimulate and expand EBV-specific CTLs by co-culture with irradiated LCLs.

Rapid CTL selection or expansion

In an attempt to make CTL manufacture simpler and more broadly applicable, several groups have developed methods for rapid virus-specific T-cell generation. All of these more rapid manufacturing approaches rely on the ability of small numbers of antigen-specific T cells to undergo substantial expansion in vivo as soon as they encounter antigen, thus providing protection against the virus being targeted. A potential caveat is that if the virus being targeted is not latent (e.g., adenovirus), the T cells may not be effective in the prophylaxis setting when antigen may not be present in vivo at the time of adoptive T-cell transfer. Moreover, the potential for GVHD may be increased since the shorter culture period may result in an increased chance for residual alloreactive T-cell populations to be present in the product. Rapid approaches that have been used clinically are highlighted below [31–35].

Multimer selection

One potential strategy for viruses such as CMV where seropositive donors may have a high frequency of virus-reactive T cells is to select directly T cells reactive with an immunogenic peptide using magnetically labeled peptide multimers (Figure 68.1b). The first report of this approach used incubation with HLA–peptide tetramers containing peptides from CMV pp65 or IE-1 followed by selection with magnetic beads to isolate CMV peptide-specific CD8$^+$ T cells from the donor's peripheral blood [35]. Although the investigators infused very small numbers of T cells, CMV-specific activity could be detected in the recipient's blood within 10 days. More recently, Schmitt et al. used a streptamer (a multimer conjugated to a marker that allows isolation and can then be dissociated) technique to select CMV-specific T cells from the transplant donors. They transferred CMV-specific CD8$^+$ effector T cells to HSCT recipient patients with recurrent CMV reactivations and saw reconstitution of CMV immunity and clearance of elevated CMV viral load [33]. These results are encouraging and multimer selection is being tested in a phase III randomized trial in the United Kingdom to treat CMV reactivation (NCT01077908). However, caveats include the large volume of donor blood required for manufacturing, the limitation that donors need to express HLA alleles for which viral peptides are available and the requirement that sufficient circulating T cells specific for the peptide must be detected in the donor. There is also a risk of viral evasion since the administered cells have restricted specificity [36].

IFN-γ capture

Another approach for rapid selection of virus-reactive T cells is the IFN-γ capture technology that involves selecting T cells that respond to a *single* stimulation by APCs expressing viral antigens (Figure 68.1c). In this approach, only the T cells secreting IFN-γ in response to the viral stimulus are selected. This strategy has been used for the selection of CMV-, EBV-, and adenovirus-specific T-cell populations (Table 68.1). Advantages of this strategy include the rapid manufacturing process, which permits the urgent treatment of a sick patient with viral infection/disease. Further, the approach is not restricted to certain HLA types and will select polyclonal T cells recognizing multiple epitopes, which reduces the risk for immune evasion. However, the limitations are again the large volumes of donor blood required for the T-cell manufacture and also the potential for the selection of alloreactive IFN-γ-secreting T cells.

Single stimulation and expansion with APCs pulsed with peptides or transfected with plasmid-derived viral antigens

Several groups are exploring approaches that avoid the need for apheresis by using a single stimulation of donor PBMCs using APCs pulsed with peptides or plasmids followed by a short ex vivo culture of 7–14 days in optimized cytokines to expand virus-specific T cells. The advantages of such strategies are that they not only use a small volume blood draw but also avoid the use of live viruses in APCs. Dendritic cells nucleofected with DNA plasmids encoding immunogenic EBV (LMP2, EBNA1, BZLF1), adenovirus (hexon, penton), and cytomegalovirus (pp65 and IE1) have been used as APCs to generate multivirus CTL lines after expansion with the cytokines IL-4 and IL-7 [25]. The use of overlapping peptide pools as a source of antigen to expand T cells from PBMCs is being increasingly evaluated since it further simplifies the process and shortens the culture time [27,29,30]. Clinical trials using these approaches are under way [37].

Clinical results using immunotherapy targeting single viruses

The majority of T-cell studies have focused on CMV, EBV, and adenovirus, and trials of donor-derived CTLs specific for single viruses are summarized in Table 68.1. Although methodologies are being developed for other viral pathogens such as BK virus [27,29], HHV-6 [38], and VZV [28], clinical trial results have not yet been reported.

EBV

T-cell therapies aimed at reconstituting EBV-specific T-cell immunity have been used for around 20 years [20,39–41]. In the first reported studies, unmanipulated donor lymphocyte infusions (DLIs) were administered to HSCT recipients with established disease [42,43]. The approach was effective since most EBV-seropositive individuals have a high frequency of EBV-specific T-cell precursors and the infusion of unmanipulated donor lymphocytes resulted in restoration of the EBV-specific immunity and resolution of EBV–PTLD in many cases [42–44]. The Sloan Kettering group recently reported a long-term response rate of more than 70% of HSCT patients with established PTLD [45]. However this approach is limited by the presence of alloreactive T cells in the DLI product and the resultant potential for GVHD [43–45].

To overcome this problem of alloreactivity, EBV-specific T cells generated using repeated stimulations with LCLs as APCs were manufactured for high-risk recipients receiving T-cell-depleted transplants from mismatched or unrelated donors. We published results for 114 patients who received EBV-specific CTLs as prophylaxis ($n = 101$) or treatment ($n = 3$) for PTLD [39]. The approach had relatively limited toxicity with no development of de novo GVHD after EBV-specific T-cell therapy, and the main adverse

Table 68.1 Clinical trials using transplant donor-derived single virus-specific CTLs for prophylaxis or treatment of viral infections

Virus	No. of patients	Transplant patient population	Type of CTL	Acute GVHD	Antiviral effects	Ref.
EBV	113	*Prophylaxis:* T-depleted HSCT from unrelated or mismatched family donor or diagnoses with high risk *Therapy*: patients with PTLD	LCL-activated	8 had a recurrence of previous acute GVHD post-CTLs	*Prophylaxis:* None of 101 developed PTLD *Treatment:* Induced CR in 11/13 patients	Rooney et al. 1995 [20] Heslop et al. 1996 [41] Rooney et al. 1998 [40] Heslop et al. 2010 [39]
EBV	14	HSCT recipients with biopsy proven PTLD	LCL-activated	None	CRs in 10 patients 4 with progressive disease	Doubrovina et al. 2012 [45]
EBV	6	Recipients of T-depleted HSCT or of ATG/OKT3 conditioning with elevated EBV viral load	LCL-activated	None reported	Decreased EBV DNA levels in 5 patients; 1 patient died of PTLD	Gustafsson et al. 2000 [69]
EBV	1	T-depleted HSCT recipient with PTLD	LCL-activated	1 patient reactivated acute skin GVHD	Patient attained CR	Lucas et al. 1998 [44]
EBV	1	Mismatched HSCT recipient with PTLD	LCL-activated	None	Patient failed to respond	Imashuku et al. 1998 [70]
EBV	4	Haploidentical HSCT recipients – 3 with recurrent PTLD post rituximab and 1 with elevated viral load	LCL-activated	None	CRs in 3 patients with recurrent PTLD post-rituximab; decrease in EBV DNA in patient without overt PTLD	Comoli et al. 2007 [46]
EBV	6	HSCT (5 haploidentical) recipients with biopsy-proven PTLD not responding to standard therapy		None	CRs in 3 patients; no response in 3 others	Moosmann et al. 2010 [48]
EBV	10	HSCT recipients with refractory EBV viremia and/or PTLD	Cells responding to EBV nuclear antigen 1 (EBNA-1) protein or overlapping peptides using IFN-γ capture	1 transient skin GVHD	CR in 7/10 patients	Icheva et al. 2013 [21]
CMV	14	Matched sibling HSCT recipients at risk of CMV reactivation	$CD8^+$ CMV-specific T-cell clones, stimulated by autologous fibroblasts infected with the AD169 strain of CMV	3 patients developed grade I or II GVHD post-infusion	Reconstitution of CMV-specific immunity in all patients. No patients developed CMV viremia or CMV disease	Riddell et al. 1992 [7] Walter et al. 1995 [18]
CMV	8	HSCT recipients with persistence or recurrence of CMV DNA in the peripheral blood after 4 weeks of antiviral chemotherapy	CMV-specific CTL lines, generated using CMV lysate and antigen	None	5 cleared after 1 dose of CTL; 1 pt cleared after a second dose; 1 did not clear, 1 not evaluable	Einsele et al. 2002 [50]
CMV	16	HSCT patients with CMV reactivation	CMV-specific CTLs generated using dendritic cells pulsed with CMV antigens derived from a CMV-infected human lung fibroblast cell line	3 developed grade I skin GVHD that responded to topical steroids in all cases	Reconstitution of immunity to CMV. In 8 cases antiviral drugs were not required. Subsequent reactivation occurred in only two patients	Peggs et al. 2003 [19]
CMV	25	Haploidentical HSCT recipients at risk of CMV reactivation	CD4 clones of CMV-specific T cells derived by stimulation with CMV antigen	1 case	7 patients underwent CMV reactivation and 5 developed CMV disease, which was fatal in 2	Perruccio et al. 2005 [49]
CMV	9	Patients expressing HLA-A*0101, *0201, HLA-B*0702, *0801, or *3502 with CMV reactivation	CMV-specific CD8 T cells staining with HLA–peptide tetramers and selected with magnetic beads	1 grade I 1 grade II	8/9 patients cleared CMV viremia	Cobbold et al. 2005 [35]
CMV	9	Allogeneic HSCT recipients at risk of CMV reactivation	CTLs stimulated with dendritic cells pulsed with the $CD8^+$ HLA-A2 restricted epitope NLVPMVATV derived from pp65	3 developed grade III GVHD, which was fatal in one	Increase in NLV-tetramer binding T cells in 6 recipients. 2 patients developed reactivation which cleared without therapy. No patients developed CMV disease	Micklethwaite et al. 2007 [26]
CMV	30	Allogeneic HSCT recipients at risk of CMV reactivation	CMV-specific CTLs generated using DCs pulsed with CMV lysate	None in 19 Grade I in 7 Grade II in 1 Grade III in 3	Reconstitution of CMV immunity and prevention of late reactivations	Peggs et al. 2009 [71]
CMV	16	Allogeneic HSCT recipients with CMV disease or viremia refractory to antiviral chemotherapy	Ex vivo stimulation with pp65 protein, followed by isolation of IFN-γ-producing cells	1 possible (GI bleeding)	Partial or complete response in 14/16 patients	Feuchtinger et al. 2010 [51]

CMV	18	Allogeneic HSCT recipients at risk of or with early CMV reactivation	CMV-specific T cells, directly isolated on the basis of IFN-γ secretion in response to recombinant pp65 or pool of overlapping peptides from CMV pp65	7 with GVHD (5 grade I skin only)	Reconstitution of CMV immunity *Prophylaxis:* None of the 7 patients required antiviral drug therapy in the next 6 months *Preemptive:* 2 of 11 patients required no antiviral drugs and 9 required antiviral therapy to clear viral load while CMV immunity recovered	Peggs et al. 2011 [31]
CMV	2	HSCT recipients with recurrent high CMV antigenemia	Selection of CMV pp65-specific T cells with streptamer technology	None	2/2 with viral clearance	Schmitt et al. 2011 [33]
CMV	7	HSCT recipients with CMV reactivation for >2 weeks	Stimulation with peptide mixes derived from full-length pp65 and IE1	None	5 /7 had increase in CMV activity in peripheral blood with CR or PR of viral load	Bao et al. 2012 [72]
CMV	6	Allogeneic HSCT recipients with persisting CMV viremia for >2 weeks while receiving pharmacotherapy or early relapse (within 2 weeks) after therapy	Stimulation with HLA-A*0201-restricted and/or HLA-B*0702-restricted CMV pp65 peptides (NLV/TPR), selection by IFN-γ capture, then expanded for 7–14 days	None	6/6 cleared CMV viremia	Meij et al. 2012 [52]
CMV	13	Allogeneic recipients with persistent CMV viremia, refractory to at least 2 weeks of ganciclovir or foscarnet	T-cells were stimulated by autologous monocyte-derived DCs loaded overlapping peptides from CMV pp65. The culture period was 21–35 days	None	12/13 cleared viremia by 2–8 weeks post-infusion; one failed to respond	Koehne et al. 2012 [37]
ADV	9	HSCT recipients with persistent ADV after 2 weeks of therapy	IFN-γ capture after short in vitro stimulation with ADV antigen	1 grade II GVHD skin	Adoptive transfer of ADV-specific immunity was successful in 5/6 evaluable patients and 4 cleared adenovirus infection	Feuchtinger et al. 2006 [54]
JCV	1	Matched sibling recipient with JCV-associated PML	Stimulation with peptides derived from VP1 and large T viral proteins	None	JCV-DNA was cleared in the cerebrospinal fluid	Balduzzi et al. 2011 [55]

EBV, Epstein–Barr virus; CMV, cytomegalovirus; HSCT, hematopoietic stem-cell transplantation; GVHD, graft-versus-host disease; DCs, dendritic cells; CR, complete remission; PTLD, post-transplant lymphoproliferative disease; ADV, adenovirus; JCV, polyomavirus JC; PML, progressive multifocal leukoencephalopathy.

effect was reversible inflammatory reactions at disease sites in four patients with PTLD [39]. Of the patients who received the CTLs as prophylaxis, up to a 4-log expansion of EBV-specific T cells was observed in vivo and none developed PTLD compared with an 11.5% incidence in controls. Thirteen patients received EBV-specific CTLs for active disease and 11 achieved durable remissions. One of the non-responders developed an escape mutant which deleted immunodominant epitopes in the patient's tumor [36]. Important information regarding the persistence of the infused CTLs was also obtained from this study since the first 26 patients received T cells that had been genetically modified with a retroviral vector encoding the neomycin resistance gene. Tracking of this marker gene using quantitative PCR confirmed that the infused T cells persisted for up to 9 years [39].

In another study, Doubrovina et al. treated 14 patients with active PTLD with donor-derived EBV-specific T cells and showed complete responses in 10 [45]. In three of the non-responders, the CTLs recognized the EBV–LCLs transformed with the B-95 laboratory strain of EBV used to generate them, but did not recognize the tumor cells or spontaneous LCLs outgrowing from the patients' blood. This result illustrates that antigenic differences between EBV strains causing PTLD and the laboratory B95-8 strain used to generate LCLs for manufacturing is a potential problem. In the fourth patient, the donor was only a 7/10 match with the recipient and the donor CTL line was skewed in its response to EBV antigens by the HLA antigen A1101, which was present only in the donor [45]. This observation along with the immune escape observed by our group [36] confirms the hazards of infusing T cells with a restricted specificity.

The results of these and other studies (Table 68.1) confirmed that donor-derived EBV-specific CTL therapy is safe and effective when used either as prophylaxis or as treatment for PTLD after HSCT. However, although the manufacturing methodology is reproducible, and can be exported to other institutions, a limitation is the 2–3-month time frame for the generation of EBV-specific CTLs (Figure 68.1a). Although many of these studies using EBV-specific T cells were conducted during the pre-rituximab era, there are still an appreciable number of patients who have rituximab-resistant disease that is responsive to EBV-specific T cells [46]. Hence other, more rapid, manufacturing strategies have been evaluated for this disease. Multimer selection (Figure 68.1b) has been used to treat EBV PTLD in a recipient who developed PTLD after a cord blood transplant. In this case, HLA A2-restricted T cells specific for epitopes in two EBV antigens were selected from a haploidentical donor, infused, and expanded greatly, inducing a clinical response [47]. EBV PTLD recurred at 12 months after transplantation and a second infusion also induced a response.

Other groups have evaluated the IFN-γ capture technology to select donor-derived EBV-specific T cells to treat patients with PTLD after HSCT. Moosmann et al. incubated donor mononuclear cells with 23 class I and II peptides derived from 11 EBV antigens and selected the T cells that secreted IFN-γ in response to these peptides [48]. Three patients treated early in their disease course responded to the T-cell therapy, but three patients with more advanced disease failed to respond [48]. Icheva et al. used APC pulsed either with whole EBNA-1 protein or with EBNA-1 overlapping peptide pools to stimulate EBNA1-specific T cells, which were then selected by IFN-γ capture (Figure 68.1c). The selected EBNA1-specific T cells were effective in seven out of 10 patients treated for PTLD [21]. Moreover, in both of these studies, no significant toxicities were seen and only one patient developed grade I GVHD of the skin, which was transient [21]. These results are certainly encouraging, although it is still unclear whether there is increased incidence of failure due to immune escape when only targeting a limited number of EBV antigens and/or epitopes. Further, it is currently unclear which EBV antigens will most likely induce T cells that will be protective in vivo.

CMV

The administration of CMV-specific T cells after HSCT was first explored by the groups of Riddell and Walter [7,18], who infused donor-derived CMV-specific $CD8^+$ clones in a dose-escalation study to recipients of matched sibling donor grafts. CMV-specific $CD8^+$ T-cell responses were detected in the blood of all patients following infusion and no adverse events were reported [7,18]. No patient in this study developed CMV disease, thereby demonstrating the importance of CMV-specific $CD8^+$ T cells in preventing CMV reactivation after HSCT [18]. An interesting observation in this study was that the magnitude of the CMV-specific T-cell responses declined in patients who lacked detectable $CD4^+$ CMV-specific T cells in vivo, implying that as in several animal models, $CD4^+$ T cells are required for long-term persistence of adoptively transferred $CD8^+$ T cell clones. This study was an important proof of principle demonstrating the potential of adoptive immunotherapy to reconstitute antiviral immunity, but the use of donor fibroblasts as APCs and the AD169 strain of CMV, and also the prolonged manufacturing process for clones, precluded broader application.

Numerous groups have built on these initial studies of CMV-specific T-cell therapy for patients after HSCT. Peggs et al. used DCs pulsed with CMV antigen derived from a CMV-infected human lung fibroblast cell line to stimulate and expand polyclonal CMV-specific $CD4^+$ and $CD8^+$ T cells [19]. The use of the entire CMV antigen permitted the expansion of a CTL product with broad specificity. Patients were infused at the time of first PCR reactivity. There were no infusion-related toxicities and none of the 16 patients developed CMV disease, although two patients did develop CMV reactivation and were successfully treated with ganciclovir [19]. In this study, only three patients developed grade I skin GVHD, and all three responded to topical steroids. It is important to note, however, that these patients had early withdrawal of their GVHD prophylaxis around the time of T-cell infusion.

Perruccio et al. administered $CD4^+$ CMV-specific T-cell clones rather than polyclonal CTLs to 25 recipients of haploidentical donor grafts [49]. Since clearance of CMV is mediated by $CD8^+$ T cells, it was thought that the infused $CD4^+$ cells might have provided T-cell help to stimulate and expand endogenous CMV-specific $CD8^+$ T cells. T-cell clones were expanded after infecting donor PBMCs with CMV antigens and infused as a single infusion at a dose range of 10^5–10^6 cells/kg. Infusion of CMV-specific $CD4^+$ clones was effective in preventing CMV reactivation in 16 patients. However, seven patients reactivated CMV and five developed CMV disease, two of whom died, suggesting that the use of a broad product containing both $CD4^+$ and $CD8^+$ T cells may produce greater therapeutic benefit.

Based on the successful application of CMV-specific T cells as prophylaxis, Einsele et al. investigated the therapeutic utility of adoptively transferring CMV-specific CTLs to patients who had persisting or relapsing CMV infection despite antiviral pharmacotherapy [50]. CMV-specific T cells were initiated by pulsing donor-derived PBMCs with CMV lysate and expanded using autologous irradiated feeder cells and CMV lysate in the presence of interleukin-2 (IL-2). Eight patients received CMV-specific T cells at a dose of 1×10^7 cells/m^2 and there were no adverse events [50]. Of the seven evaluable patients, six cleared viremia long term after one or two doses of T cells coincident with an increase in both CD4$^+$ and CD8$^+$ CMV-specific T cells [50]. The advantage of this approach is that it relies on the monocytes infected with the CMV lysate for antigen presentation and thereby avoids the use of DCs. However, there still remains the potential risk for infection when using CMV lysate.

In an attempt to move away from the use of live CMV virus or viral antigens, several groups have explored approaches using CMV protein or peptides to expand T cells [26,30]. Initially immunodominant peptides restricted by common HLA alleles were used, although the recent availability of overlapping peptides has obviated the problem with HLA restriction. Several strategies have been used, including two studies in which DCs pulsed with single peptides or peptide mixes were used to expand CMV-specific T cells [26,27], and three groups used IFN-γ capture to select the T cells responding to the relevant peptides [31,51,52]. Feuchtinger et al. infused CMV-specific T cells isolated by IFN-γ capture after stimulation with pp65 protein into 18 patients after allogeneic HSCT with CMV disease or viremia and reported responses in 83% [51]. Peggs et al. [31] infused cytomegalovirus-specific T cells, isolated from donor apheresis after stimulation with recombinant CMV pp65 protein or overlapping peptides derived from the CMV pp65 antigen, to 25 recipients of related-donor HSCT and reported reconstitution of CMV immunity. Meij et al. infused T cells stimulated with two immunodominant CMV pp65 peptides (NLV which is HLA-A*0201-restricted and TPR which is HLA-B*0702-restricted) to six recipients with CMV reactivation who all achieved complete responses [52].

The use of HLA tetramers avoids the use of an APC to select donor-derived CMV-specific CD8$^+$ T cells [35]. In a study evaluating this approach for CMV, nine patients received a median dose of 8.6×10^3 CMV tetramer-staining CTLs/kg. CMV-specific CTLs were detectable at increasing levels in the peripheral blood within 10 days of infusion and 8/9 patients cleared the viremia, including one patient with drug-resistant disease [35]. More recently, Schmitt et al. used the streptamer technique and treated two patients with pp65-specific T cells, showing viral clearance in both [33]. This is therefore a promising approach but again is limited to small numbers of donors who have sufficient circulating T cells reactive with a known HLA-restricted CMV peptide epitope.

Overall, the response rates in studies of donor-derived CMV-specific CTLs to treat CMV reactivation or disease have been encouraging, ranging from 80 to 100%. In addition, when used in the prophylaxis setting, apart from the five patients who received CD4$^+$ clones, no other patients were reported to develop CMV disease (Table 68.1).

Adenovirus

There are published reports on the use of T cells solely targeting adenovirus, although a case report documented the successful use of DLI in a patient with hemorrhagic cystitis due to adenovirus infection [53]. More recently, Feuchtinger et al. reported a study that selected adenovirus-reactive T cells by simulation of donor PBMCs with adenoviral antigen followed by IFN-γ capture [54]. Nine pediatric patients with systemic adenoviral infection after HSCT received the resulting product in doses ranging from 1.2×10^3 to 50×10^3 T cells/kg. Although the infused cell number was very low, four of the nine patients cleared the virus [54].

JC virus

The polyomavirus JC (JCV) is associated with the usually fatal disease progressive multifocal leukoencephalopathy (PML). Balduzzi et al. generated JCV-specific T cells by stimulation of donor PBMCs with overlapping 15-mer peptides derived from the VP1 and large T viral antigens and administered these cells to a patient diagnosed with JCV-associated PML after HSCT [55]. After T-cell infusion, JCV-specific T-cells were detected in the peripheral blood coinciding with clearance of JCV–DNA in the cerebrospinal fluid and a dramatic clinical improvement. No adverse events attributable to CTL infusion were reported. Although this is a single case, it supports the use of JCV-specific T-cell immunotherapy to restore T-cell immunity to this virus and control disease in patients after HSCT.

Clinical results using immunotherapies targeting multiple viruses

Since it is not infrequent for patients after HSCT to develop multiple viral infections, several groups have explored the use of T cells targeting more than one virus to administer to patients after HSCT either as treatment or prophylaxis. These trials are summarized in Table 68.2.

Targeting CMV, EBV, and adenovirus

In the first reported study using multivirus-specific T cells, the group at Baylor College of Medicine developed a strategy to generate trivirus-specific donor-derived T-cell lines recognizing CMV, EBV, and adenovirus [22]. A chimeric adenovirus-CMV pp65 vector (Ad5f35-pp65) was used to modify monocytes and EBV LCLs that served as APCs to generate a single culture of CTLs specific for EBV, CMV, and adenovirus. A total of 26 patients received the trivirus-specific CTL products either as prophylaxis or as treatment in doses ranging from 1×10^7 to 1×10^8 cells/m^2. None developed more than grade II GVHD or other toxicities over the 3 months of safety monitoring after infusion, and there was immune reconstitution to CMV and EBV 4 weeks after CTL infusion as measured by IFN-γ ELISPOT (Enzyme-Linked ImmunoSpot) assay. Immune responses to adenovirus were only detected in patients with a reactivation or infection. Twenty viral reactivations were observed in patients before or immediately after CTL infusion. Nine of the 11 patients with CMV reactivation cleared CMV viremia without pharmacotherapy, with a corresponding rise in CMV-specific CTLs in peripheral blood [22,56]. An additional patient cleared with a combination of CTLs and ganciclovir while the final patient failed to respond to any treatment modality. Each of the six patients with high EBV loads cleared their virus, as did five of six patients with adenoviral infection [22]. This study demonstrated that multivirus-specific CTLs derived from the peripheral blood of seropositive donors were able to expand in vivo and mediate activity against multiple viruses from a single product. More recently, rapid multivirus CTLs generated using DCs nucleofected with DNA plasmids were administered to 10 allogeneic HSCT recipients with a response rate of 80%, including two patients with EBV lymphoma and three with double viral reactivations [57].

Adenovirus/EBV

Another trial by this group targeted recipients who received HSCT from CMV-seronegative donors. In this study, a chimeric adenovirus-specific vector alone (Ad5f35) was to modify monocytes and EBV LCLs, to generate a single culture of PBMCs that gave rise to CTLs specific for EBV and adenovirus [58]. Fourteen patients received bivirus-specific CTL after HSCT with no infusion-related toxicity and no patient developed more than grade II GVHD. All three patients with high EBV loads cleared their virus, as did two of three patients with adenoviral infections/disease, suggesting that this strategy is also efficacious and safe when used in the prophylaxis or treatment setting [58].

Table 68.2 Clinical trials using transplant donor-derived multi virus-specific CTLs for prophylaxis or treatment of viral infections

Virus	No. of patients	Transplant patient population	Type of CTL	GVHD	Antiviral effects	Ref.
CMV, EBV, and adenovirus	23 on study 3 emergency use or compassionate	Allogeneic HSCT recipients at risk of or with CMV, ADV, or EBV reactivation or disease	Activated by monocytes and LCLs transduced with adenoviral vector encoding CMV pp65	2 patients developed skin rashes that responded to topical steroids	6/6 with EBV cleared infection 5/6 with adenovirus cleared infection 10/11 with CMV cleared infection. 1 patient with CMV had disease that progressed despite CTLs and pharmacotherapy	Leen et al. 2006 [22] Bollard et al. 2011 [56]
CMV, EBV, and adenovirus	10	Allogeneic HSCT recipients with CMV, ADV, or EBV reactivation or disease	Activated by DCs nucleofected with DNA plasmids encoding viral antigens for EBV (LMP2, EBNA1, BZLF1), adenovirus (hexon, penton), and CMV (pp65 and IE1)	1 with skin rash possibly GVHD, possibly BK infection	CR in 2/3 EBV reactivation (including 2 with EBV-PTLD) CR in 4/5 CMV CR in 5/5 adenovirus	Gerdemann et al. 2013 [57]
EBV and adenovirus	12 on study 2 compassionate	Allogeneic HSCT recipients at risk of or with ADV or EBV reactivation or disease	Activated by monocytes and LCLs transduced with adenoviral vector	3 patients developed skin rashes that responded to topical steroids	11 patients treated as prophylaxis remain negative 2/3 patients with adenovirus cleared the infection	Leen et al. 2009 [58]
CMV and adenovirus	40	Allogeneic HSCT recipients at risk of CMV reactivation	Activated by adenoviral vector encoding CMV pp65	Grade II–IV acute GVHD occurred in 24% of the CTL cohort vs. 18% of the controls	Fewer CMV reactivations than controls and only 1 patient required CMV-specific pharmacotherapyImmune reconstitution to CMV in CTL recipients	Micklethwaite et al. 2008 [23] Blyth et al. 2013 [59]
CMV and EBV	3	Haploidentical HSCT recipients with EBV or CMV reactivation or disease	Induced by DCs pulsed with EBV-LMP2, CMV pp65 and CMV IE peptides	1 grade I	*Treatment:* Cleared in 2/2 *Prophylaxis:* No infections in 1	Dong et al. 2010 [60]

EBV, Epstein–Barr virus; CMV, cytomegalovirus; ADV, adenovirus; DCs, dendritic cells; HSCT, hematopoietic stem-cell transplantation; GVHD, graft-versus-host disease; CR, complete remission.

CMV/adenovirus

Using the same chimeric adenovirus–CMV pp65 vector described above, Micklethwaite et al. transduced donor-derived DCs which were used to stimulate autologous PBMCs to stimulate and expand CMV- and adenovirus-specific T cells [23]. In a recent update they reported that CMV/adenovirus-specific T cells were administered to 40 adult patients after HSCT as prophylaxis any time after day 28 post-transplant [59]. No infusion-related adverse events were observed and they saw no significant difference in the incidence of acute or chronic GVHD or in the rate of CMV reactivation compared with concomitant controls who did not receive CTLs. However, they did observe a significant reduction in the percentage of patients who required CMV-directed antiviral therapy and in the total number of treatment days per patient in the cohort receiving CTLs [59].

CMV and EBV

Dong et al. reported three pediatric patients who received donor-derived T cells directed towards CMV and EBV after haploidentical donor transplantation [60]. T cells were generated using DCs pulsed with peptides directed towards LMP2, pp65, and IE1 epitopes. Two patients developed CMV viremia less than 32 days after HSCT and cleared the virus with tapering immune suppression and T-cell infusion. (60]. The third patient was treated prophylactically and remained virus free more than 14 months after transplantation.

Targeting EBV, adenovirus, CMV, BK virus, and HHV6

Recently, the group at Baylor College of Medicine reported a study where rapidly manufactured multivirus specific T cell single-cultures were administered to 11 patients after HSCT. The multivirus specific T cells recognized 12 immunogenic antigens from five viruses (EBV, CMV, Adenovirus, BK virus and HHV6). Eight of the 11 patients had up to four active virus infections at the time of T cell infusion. These rapidly generated (10 days) T cell products were safe in allogeneic HSCT recipients and produced sustained virological and clinical response rates of 94% [75].

Risk of alloreactivity

In summary, these studies show that virus-specific T cells targeting one to three viruses have efficacy after HSCT to reconstitute immunity and clear viral reactivations and disease. Although the potential for de novo alloreactivity is a concern for all these approaches, the GVHD incidence did not appear to be increased over that which would be expected in the patient population (Tables 68.1 and 68.2). There was a particular concern in the mismatched setting as in vitro studies have shown that that a majority of virus-specific CTL lines possess cross-reactivity against allogeneic HLA molecules [61]. However, such alloreactivity was not observed among 153 recipients who received virus-specific CTLs, including 73 patients for whom there was an HLA mismatch with the donor [62]. The concern for alloreactivity, however, has meant that most studies have excluded patients with GVHD of grade II or greater.

CTLs for HSCT patients with virus-naïve donors

All of the donor-specific T-cell strategies discussed so far have utilized products derived from donors who are seropositive for the virus of interest. Adoptive transfer of virus-specific CTLs is currently not an option when the donor lacks viral immunity for the infecting virus or the recipient has received umbilical cord blood transplantation. Preclinical studies have evaluated circumventing this problem by transfer of artificial receptors specific for viral antigens [63,64]. but this strategy has not been tested in the clinic. There is also considerable interest in modifying culture conditions to reactivate CTLs from

Table 68.3 Clinical trials using third party virus-specific CTLs

Viruses targeted	No. of patients	Transplant patient population	Type of CTL	Serious adverse events possibly attributable to CTLs	Results	Ref.
EBV	2	HSCT recipients with PTLD	Closely matched allogeneic EBV-specific CTL	None reported	2 attained CR	Haque et al. 2007 [66]
EBV	5	HSCT including cord recipients with biopsy-proven PTLD	Closely matched allogeneic EBV-specific CTL	None reported	4 attained CR: 1 had progressive disease	Barker et al. 2010 [73] Doubrovina et al. 2012 [45]
EBV	1	Cord recipient with PTLD resistant to rituximab	Haploidentical GLC-peptide separated CTLs	None reported	1 attained CR Recurrence 9 months later responded to second infusion	Uhlin et al. 2010 [47]
CMV	2	Allogeneic HSCT recipients with CMV disease or viremia refractory to antiviral chemotherapy	Ex vivo stimulation with pp65 protein, followed by isolation of IFN-γ-producing cells	None reported	1 CR 1 failed to respond	Feuchtinger et al. 2010 [51]
Adenovirus	1	Unrelated HSCT recipient with intractable adenovirus infection	Gamma capture selected adenovirus-specific T cells from haploidentical donor	Liver GVHD (no third-party cells on biopsy)	1 CR	Qasim et al. 2011 [68]
EBV, CMV, and adenovirus	50	HSCT recipients with persistent CMV, EBV, or adenovirus despite standard therapy	Closely matched allogeneic trivirus-specific CTLs	Reactivation GVHD in 6 De novo GVHD in 2	Cumulative rate of complete or partial responses at 6 weeks post-infusion was 74% (73.9% for CMV, 66.7% for EBV, 77.8% for adenovirus)	Leen et al. 2013 [67]
EBV, CMV, and adenovirus	6	HSCT recipients with persistent CMV, EBV, or adenovirus despite standard therapy	Multimer selected T cells specific for CMV, EBV, and adenovirus	None reported	CMV: 3/4 responses Adenovirus: no response EBV: CR after second infusion	Uhlin et al. 2010 [47] Uhlin et al. 2012 [74]

HSCT, hematopoietic stem-cell transplantation; PTLD, post-transplant lymphoproliferative disease; GVHD, graft-versus-host disease; CR, complete remission; PR, partial remission.

virus naive donors [65] and a recent report demonstrates the feasibility of this approach clinically [76]. Finally several studies have evaluated whether third-party CTLs sharing HLA antigens with the HSCT recipient have activity against viral antigens, and studies evaluating this approach are summarized in Table 68.3.

Third-party EBV CTLs

One option that could provide an "off-the-shelf" product to all recipients is to use banked virus-specific T-cell lines generated from virus immune individuals with common HLA polymorphisms. Haque et al. used banked polyclonal EBV CTL lines to treat EBV PTLD after HSCT or solid organ transplantation in a phase II multicenter trial of 33 patients and reported an overall response rate of 52% at 6 months [66]. Investigators at the Memorial Sloan Kettering Cancer Center reported complete responses in four of five PTLD patients who received third-party EBV CTL lines [45]. In a multicenter study evaluating the use of third-party multivirus-specific CTLs to treat patients with refractory viral infections, the overall cumulative incidence of first complete/partial remission in 50 patients by day 42 after infusion was 74% (73.9% for CMV, 66.7% for EBV, and 77.8% for adenovirus) [67]. Although the response rates are slightly less than with donor-derived CTLs, this approach nonetheless shows considerable promise. Most studies have not shown an increased risk of GVHD, although there is one report of bystander-induced liver GVHD after third-party adenovirus CTLs [68]. If CTL banks are further developed, it will be important to establish criteria for HLA matching that would provide broad coverage, perhaps with the use of homozygous donors. A product targeting one virus may also have broader specificity than a multivirus line due to decreased antigenic competition during manufacture.

Cord blood-derived CTLs

With the increasing use of cord blood (CB) grafts, there are appreciable numbers of patients who are recipients of virus-naïve donor grafts. CMV reactivation usually occurs from endogenous virus, and seropositive recipients with seronegative donors remain the highest risk group for developing CMV. The development of multivirus-specific T cells from recipients of CB grafts requires the priming of naïve T cells rather than the simple expansion of preexisting memory T cells from seropositive donors. Using a protocol stimulating CB-derived T cells with autologous CB-derived DCs and EBV LCLs transduced with the Ad5f35CMV pp65 vector in the presence of IL-7, -12 and -15, multivirus-specific T cells can be primed in vitro from the 20% fraction of a CB unit [65]. So far, six patients have received CTLs as prophylaxis or treatment after CB transplant without toxicity [76]. No infusion-related toxicities/GVHD have been observed and, despite receiving only 80% of the CB unit, all patients engrafted neutrophils within 30 days. Early evidence of efficacy has also been demonstrated with clearance of EBV, CMV, and adenovirus in two patients with the other four patients remaining virus free. Although the study is ongoing, the results suggest that transfer of naïve T-cell (CB)-derived virus-specific T cells to patients after CB transplant may be safe and facilitate long-term reconstitution of virus-specific T cells in vivo.

Conclusions and future directions

Adoptive transfer of virus-specific CTLs can reconstitute viral immunity and treat viral infections that have failed to respond to conventional therapies. The overall response rate in HSCT recipients with active disease receiving donor-derived virus-specific CTLs from their transplant donors is around 80% (Tables 68.1 and 68.2) and around 70% for those receiving third-party cells (Table 68.3). In most cases the responses are durable so this strategy has many advantages compared with pharmacologic therapies. The broader application, however, has been limited by the complexity of some of the CTL manufacturing methodologies and the lengthy production time. Over the past few years, several groups have developed more rapid CTL generation protocols that appear to have equivalent activity and would allow definitive testing in late-phase trials.

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CHAPTER 69

Adoptive T-cell Therapy for Malignancy in the Setting of Hematopoietic Cell Transplantation

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Introduction

A variety of cellular therapies have been incorporated into the setting of hematopoietic stem-cell transplantation (HSCT). These include the infusion of polyclonal or antigen-specific T cells, lymphokine-activated killer cells, natural killer (NK) cells, dendritic cells (DC), and macrophages. The infusion of allogeneic T cells to enhance antitumor effects, referred to as donor lymphocyte infusions, is described in Chapter 70. The use of adoptive T-cell therapy to control infections and treat Epstein–Barr virus (EBV)-related tumors and other tumors induced by viruses is described in Chapter 68. In this chapter, we describe the background, rationale, and current clinical use and experimental approaches for adoptive T-cell therapies for the treatment of cancer in the setting of HSCT. Finally, we discuss the potential evolving with new cellular and molecular technologies that will likely influence the future directions, efficacy, and applicability of T-cell therapy.

History and rationale for adoptive T-cell transfer therapy

Background

The concept of adoptive cellular therapy for tumor allografts was first reported for rodents 60 years ago by Mitchison [1]. The cloning of T-cell growth factors made possible the first ex vivo expansion of tumor-specific T cells for adoptive immunotherapy in mouse syngeneic tumor models. There are several excellent reviews of the rationale and experimental basis for adoptive T-cell therapy of tumors [2,3]. In the earliest clinical trials, patients were given adoptive transfers of autologous, allogeneic, and xenogeneic lymphocytes for a variety of tumors. The results of these early trials were not promising, which is not surprising since they were carried out before the principles of T-cell biology and tumor antigens were understood. The field of adoptive cellular therapy during the first 25 years was reviewed by Rosenberg and Terry in 1977 [4].

Rationale

The seminal finding by Weiden et al. [5] that HSCT using sygeneic donors was less effective at preventing relapse of leukemia than the use of sibling donors provided a strong rationale for adoptive T-cell therapy. The rationale derived from that study is based upon the interpretation that the genetic differences provide alloimmune targets on tumor cells that passenger T cells from the syngeneic donor T cells are incapable of recognizing. Given the durable antitumor effects of T cells on human tumors, one could propose active immunization to boost T-cell numbers and function. However, there is now a broad consensus that therapeutic vaccines do not have the potency of approaches that are based on T-cell adoptive transfer. This is particularly true in the setting of hematopoietic cell transplantation (HCT) with the accompanying immunosuppression with quantitative and qualitative defects in T cells that can persist for months and years after the procedure [6], presenting a formidable obstacle to successful vaccine-based immunity.

Principles of T-cell transfer

Some considerations of basic immunologic principles as they apply to T-cell transfer in the setting of HSCT are presented in Table 69.1. Rational use of adoptive T-cell therapy is predicated upon an understanding of the relevant principles of cellular and molecular immunology and cancer cell biology. The reasons for the disappointing efficacy of many previous forms of adoptive cellular therapy are now clear based on lessons from advances in the basic sciences.

The typical host conditioning in HSCT presents challenges for active (vaccine) immunotherapy due to the depletion of lymphocytes. In contrast, host conditioning that renders the host lymphopenic creates an attractive setting for T-cell transfer because the engraftment and expansion of adoptively transferred T cells are facilitated under these conditions. There are many factors that lead to homeostatic expansion [7], and the expansion is accompanied by the acquisition of enhanced effector function [8].

During steady-state lymphopoiesis, the total lymphocyte count remains stable. However administration of cytokines from the common gamma chain family such as interleukins IL-2, IL-7, and IL-15 can increase total T-cell numbers above baseline levels. However, T cells, unlike B cells, are regulated at the clonal level [9], so that the adoptive transfer of large numbers of T cells with a single T-cell receptor (TCR) specificity may not result in long-term and high-level persistence of the adoptively transferred clones.

The optimal composition of adoptively transferred T cells remains controversial. Furthermore, it is likely that the best cell products vary depending on the tumor and goals of the adoptive

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Table 69.1 Strategies to augment efficacy of adoptive T-cell therapy

Adjuvants to promote maintenance of Th1 cell function
Antibody infusions to enhance 4-1BB, CD27, or ICOS signals
Check point blockade: CTLA-4, PD-1, HVEM, LAG3, TIM3
Choosing the best cells: Tcm, Tn, Th17
Cytokine administration, for example, IL-7, IL-12, IL-15, IL-18, or IL-21
Cytokine antagonists, for example, TGF-β blockade or inhibition
CD4⁺ Th cell infusion to sustain CTL function
Enhance replicative capacity of infused cells
Host lymphodepletion
Host Treg-cell depletion or inhibition
Improving T-cell receptor affinity
Non-myeloablative stem-cell transplantation to facilitate allogeneic T-cell transfers
Tumor bed manipulation, for example, chemotherapy, chemokine expression in tumor bed to promote CTL trafficking cryoablation, phosphodiesterase-5 inhibition
Vaccine therapy to boost CAR T cells

therapy. For many years, it was thought that effector T cells were preferred. However, there is now evidence indicating that infusion of naïve T cells [10], central memory T cells [11], Th17 cells [12], and T stem cells [13] may all have certain advantages. In cases where the eradication of large tumors is desired, the optimal T cell is probably different to that when the goal is long-term persistence and immunosurveillance.

It is possible that previous clinical adoptive T-cell therapy clinical trials have been unsuccessful because the prolonged ex vivo culture process resulted in a population of cells that had reached, or were near, replicative senescence. Preservation of telomere length and replicative capacity correlates with the engraftment efficiency and antitumor efficacy of adoptively transferred T-cell lines in patients with melanoma [14]. CD28 stimulation maintains telomere length in T cells [15], and cultures that optimize costimulation might improve the engraftment and persistence of adoptively transferred T cells. Studies indicate that IL-15 induces a sustained level of telomerase activity and this minimizes telomere loss in memory $CD8^+$ T cells after a substantial number of cell divisions [16]. Constitutive expression of hTERT can extend the replicative potential of human T cells [17].

Genetic engineering of T cells has the potential to augment function through various cell autonomous mechanisms, as discussed above. In addition, T cells can be engineered for resistance to cell extrinsic forms of immunosuppression such as mediated by transforming growth factor beta (TGF-β) and regulatory T (Treg) cells [18,19]. Therefore, as with other forms of immunotherapy, it is probable that the ultimate clinical application of adoptive T-cell transfer will employ combinatorial approaches.

Strategies for T-cell culture and engineering

Rationale

For many years, the approach used by most investigators was based on the transfer of large numbers of effector T cells. This was necessary because the infused T cells were essentially non-replicative, and functioned essentially as drugs rather than as persisting memory T cells. Based on recent results in trials with engineered T cells [20,21], we now propose that in most cases, the infusion of small numbers of cells may be suffice, and that the most of the T-cell expansion can occur in the host rather than ex vivo in cell culture. One exception to this new approach may be in the setting of transiently engineered cells such as mRNA transfected T cells that require large numbers of cells to be infused on multiple occasions [22,23].

Approaches for T-cell culture

Studies indicate that, on a per cell basis, the adoptive transfer of T cells with extensive replicative capacity had improved engraftment and antitumor effects rather than transfer of terminally differentiated effector cells that have a more potent cytotoxic effector function [14]. This paradox is likely explained by the ability of central memory T cells (T_{CM} cells) to self-renew and differentiate into effector T cells in vivo, whereas terminal effector memory T cells (T_{EM} cells) have lost this plasticity. A controversy has arisen in the field regarding how best to achieve this goal [24]. One approach is to isolate T_{CM} cells with the desired specificity in vitro by sorting or other means of physical separation, engineer the desired specificity, and expand and then infuse the T_{CM} cells [11]. We have proposed that manipulation of bulk T-cell culture conditions can enrich and maintain T_{CM} cells, and thereby obviate the need for cell sorting procedures. Cell culture conditions that augment CD28 and CD137 (4-1BB) costimulation in vitro promote the maintenance of T_{CM} cells in vitro [25,26] and in vivo [27]. The use of memory stem cells, that is, T cells programmed for the most extensive self-renewal, has significant potential [13,28].

Two basic approaches are being tested for clinical adoptive T-cell therapy. The first approach has been to isolate and activate in vitro antigen-specific T cells from peripheral blood or tumor specimens, and then to use repetitive stimulation to expand clonally in vitro the antigen-specific T cells by various approaches. In the second approach, polyclonal ex vivo activation of the T cells is done based on three assumptions: first, tumor-specific T cells are present in the patient; second, the tumor-specific T cells have been primed in the patient; and third, the in vivo function of the tumor-specific T cells in the patient is impaired. In the second approach, the cells are activated in a polyclonal fashion by various means in vitro, and are then reinfused into the patient with the expectation that they will respond directly to the tumor or to tumor antigens presented by antigen-presenting cells (APCs). The first approach guarantees antigen specificity but is costly and labor intensive; the second approach is technically more rapid and feasible. In practical terms, only the second approach has been sufficiently robust to support randomized clinical trials [29,30], and therefore has the potential for regulatory approval.

The most appropriate methods of ex vivo T cell culture mimic the physiologic processes whereby DCs generate a constellation of antigen-specific and costimulatory signals in the T cells. However, although useful for therapeutic vaccination, owing to practical considerations such as substantial manufacturing costs and the logistics of maintaining independent culture systems, DCs are not useful as APCs for large-scale adoptive T-cell therapy trials. The optimal cell culture system varies depending on the T-cell subset that is propagated (Figure 69.1). General approaches have been to produce artificial APCs, either by coating beads with CD3-specific antibody or peptide–major histocompatibility complex (MHC) complexes, or by transfecting cells that lack endogenous MHC molecules with MHC molecules and costimulatory molecules.

Approaches for T-cell engineering

Advances in basic science have presented a plethora of approaches to engineer lymphocytes at the genomic, RNA, epigenetic, and protein levels, with the goal of pharmacologically enhancing the immune system [31]. An overview of permanent and transient strategies for engineering T cells is shown in Figure 69.2 and Table 69.2. For T cell-based therapies, chromosome-integrating vectors that are derived from gamma retroviruses or lentiviruses

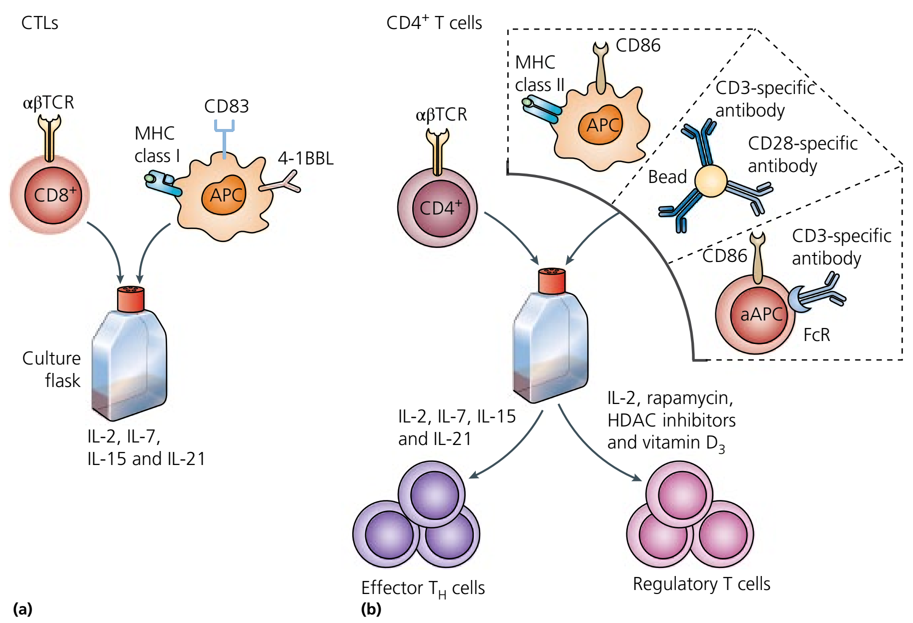
CTLs
αβTCR
CD8+
CD83
MHC class I
APC
4-1BBL
Culture flask
IL-2, IL-7, IL-15 and IL-21
(a)
CD4+ T cells
MHC class II
CD86
APC
αβTCR
CD4+
CD3-specific antibody
CD28-specific antibody
Bead
CD86
CD3-specific antibody
aAPC
FcR
IL-2, IL-7, IL-15 and IL-21
IL-2, rapamycin, HDAC inhibitors and vitamin D3
Effector TH cells
Regulatory T cells
(b)

Figure 69.1 Cell culture approaches for adoptive transfer of lymphocyte subsets. Culture conditions required for distinct lymphocyte subsets vary, depending on the activation and costimulatory requirements. CTLs express αβ TCRs and are stimulated by APCs that express MHC class I. CTLs require 4-1BB stimulation for expansion, and have been expanded by the addition of feeder cells or artificial APCs (aAPCs) that express the costimulatory ligands. CD4 T cells are stimulated by APCs that express peptide-loaded MHC class II. The dominant costimulatory molecule for CD4 cells is CD28. Effector CD4 cells can be stimulated by beads or cell-based aAPCs in the presence of various cytokines. Regulatory CD4 T cells (Treg cells) require culture in IL-2, and the addition of number of reagents to the culture may enhance the suppressive functions of ex vivo expanded Treg cells. (For a color version of this figure, please see the color plate section.)

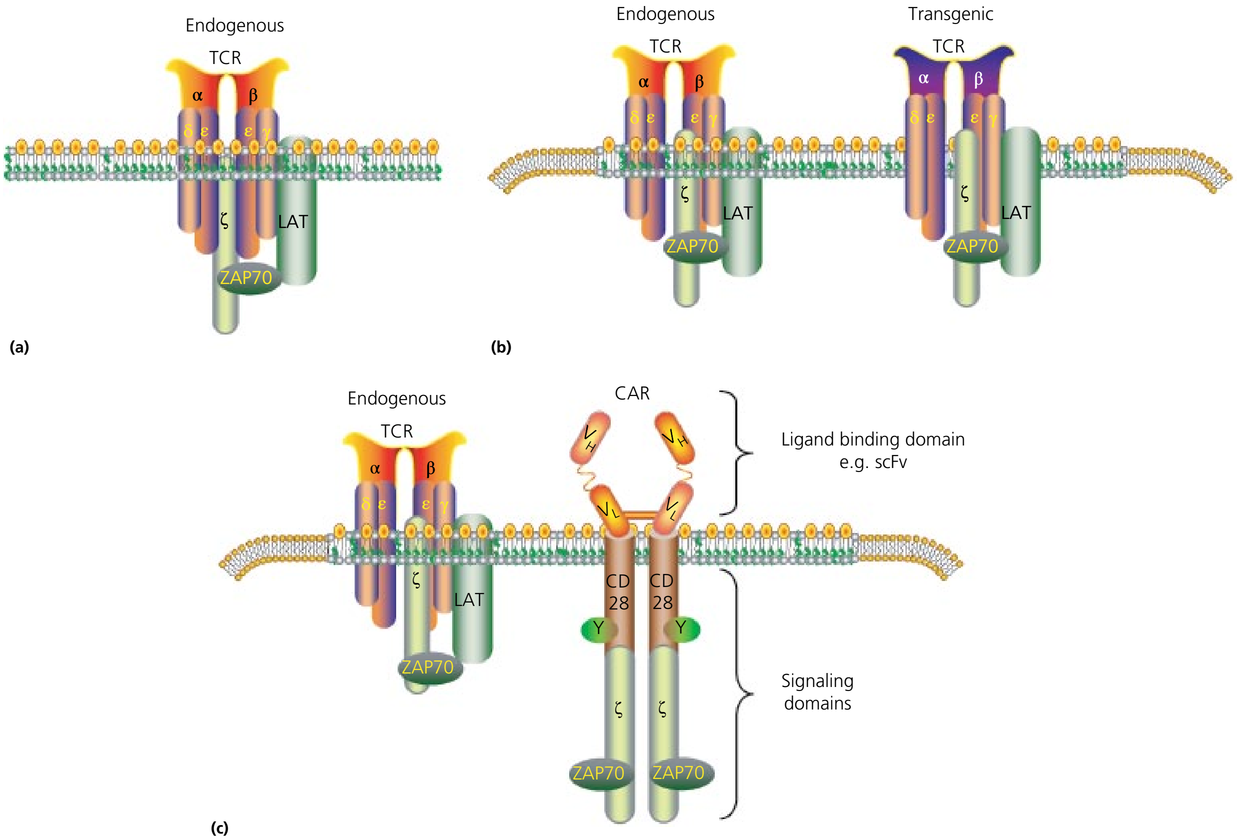
Endogenous
TCR
α
β
δ ε
ε γ
ζ
LAT
ZAP70
(a)
Endogenous
TCR
α
β
δ ε
ε γ
ζ
LAT
ZAP70
Transgenic
TCR
α
β
δ ε
ε γ
ζ
LAT
ZAP70
(b)
Endogenous
TCR
α
β
δ ε
ε γ
ζ
LAT
ZAP70
CAR
VH
VH
VL
VL
Ligand binding domain e.g. scFv
CD 28
CD 28
Y
Y
ζ
ζ
ZAP70
ZAP70
Signaling domains
(c)

Figure 69.2 T cells can be engineered to have retargeted specificity for tumors. (a) Endogenous T cells express a TCR with a single specificity. Bispecific T cells are created by the introduction of genes that encode proteins that recognize antigens expressed by target tumor cells. (b) These genes can encode natural TCRs that function in the same MHC-restricted manner as endogenous TCRs but have tumor-antigen specificity. (c) Alternatively, these genes can encode chimeric tumor antigen–specific receptors (CARs) that target surface antigens in an MHC-independent fashion. T bodies express an extracellular ligand generally derived from an antibody and intracellular signaling modules derived from T-cell signaling proteins. (For a color version of this figure, please see the color plate section.)

Table 69.2 Approaches for engineering lymphocytes

Approach	Advantages	Disadvantages	Use in the clinic	Ref.
Virus-based approaches				
Gammaretrovirus vectors	Extensive safety profile with 2 decades of clinical use	Insertional oncogenesis documented after infusion of engineered hematopoietic stem cells Poor efficiency for modifying other subsets of lymphocytes such as NK cells Deliver smaller DNA sequences than lentiviral vectors Susceptible to silencing, particularly in HSCs Require induction of cell division for integration	Extensive clinical use	[32]
Lentivirus vectors	High transduction efficiencies Transduce quiescent T cells	Increased production costs compared with gammaretroviral vectors Potential genotoxicity owing to LTR sequences and HIV accessory genes; third-generation self-inactivating vectors have improved safety following removal of these sequences	Early-stage clinical trials with engineered T cells using VSV-G pseudotyped lentiviral vectors	[33]
Spumavirus vectors or foamy virus vectors	Non-pathogenic in humans following zoonotic infection Favorable integration pattern compared with gammaretrovirus-based and perhaps lentivirus-based vectors	Clinical-grade vector manufacturing not yet available	Not yet reached the clinic; foamy virus-mediated insertion of CD18 into HSCs corrected congenital CD18 deficiency in dogs	[129]
Ad5/F35 vectors	Recombinant Ad5/35 vectors, which use CD46 as an entry receptor, efficiently infect human T cells and permit high level transgene expression for about 1 week Potential to introduce plasmids and antigens into T cells to generate T cells that can function as APCs	Lymphocytes express low levels of the Ad5 receptor, so are refractory to infection; vector tropism can be modified through manipulation of Ad5 fiber protein	Ongoing clinical trial with modified $CD4^+$ T cells for HIV infection (ClinicalTrials.gov NCT00842634).	[130]
Lymphotropic herpesvirses (HHV6 and HHV7)	Infect nearly all humans during childhood, with low pathogenicity Large payload capacity Transferred genes expressed efficiently in T cells	Clinical-grade vectors not yet produced.	Proposed for use in gene transfer and vaccine therapy	[131]
Non-virus-based approaches				
Random integration with electroporation	Efficient short-term expression of transgenes; long-term expression achieved in rare cells	For long-term expression, need to select and expand the rare T cells with integrated DNA in culture, leading to replicative senescence	Clinical trial with a CD20-specific CAR successful and resulted in engineered T cells with suboptimal engraftment and antitumor effects	[68]
mRNA transfection	Efficient expression in T cells Less expensive than virus-based vectors	Short persistence of transgene may require multiple T-cell infusions	Clinical trial with mesothelin CAR ongoing (ClinicalTrials.gov NCT01355965)	[22]
Transposon- based integration	A larger payload than retrovirus vectors High-level, persistent transgene expression Reduced cost compared with virus-based vectors	Tests needed to determine the safety and genotoxicity relative to lentivirus vectors	Clinical trial with CD19 CAR ongoing (ClinicalTrials.gov NCT00968760)	[87]
Genetic editing	Can selectively increase or decrease potency of T cells	Early in development but zinc finger nucleases and crispr/cas appear promising	Clinical trial with ZFN modified CD4 cells in HIV/AIDS	[145]

have been most useful for long-term gene expression because of their ability to integrate into the host genome, a feature that can result in permanent expression of the transgene, and for their low intrinsic immunogenicity [32,33].

For some applications, genomic alteration of adoptively transferred lymphocytes may not be required to achieve substantial therapeutic effects. RNA-based electroporation of lymphocytes using in vitro-transcribed mRNA mediates transient expression of proteins for approximately 1 week, and redirected T cells transduced with RNA encoding TCRs or chimeric antigen receptors (CARs) have the expected gains of function [22]. Clinical trials using mRNA-transduced DCs have been safely conducted [34] and trials using mRNA-electroporated T and NK lymphocytes are ongoing at several centers.

Clinical approaches in the setting of HSCT

Tumor-infiltrating lymphocytes

The adoptive transfer of tumor-infiltrating lymphocytes (TILs), expanded from resected melanoma specimens and selected for reactivity with tumor-associated peptides displayed by MHC molecules on cancer cells, can mediate durable tumor regression in a subset of patients with advanced metastatic melanoma [35]. A major advance was the recognition that host immunoablation could enhance the engraftment and efficacy of adoptively transferred T cells in melanoma [36] and lymphoma [37]. In non-controlled sequential trials, TIL therapy in the setting of autologous HSCT with high-dose chemotherapy and total body irradiation appears superior to TILs given with less intense host immunosuppression [35]. Technical issues with producing tumor-specific T cells currently present a formidable barrier to conducting randomized clinical trials using TILs.

Cytotoxic T-lymphocyte (CTL) therapy

MHC class I restricted CTLs are effective for viral infections and malignancies; see Chapter 68 for the use of CTLs for viral disorders. A dearth of tumor-associated antigens that are common to human tumors of a given type initially stymied investigators, preventing them from generating tumor-specific CTL populations. However, at present there is a plethora of suitable CTL targets for many

Table 69.3 Potential antigenic targets for CTL therapy

Antigen class	Example [Ref.]
1. Tissue-specific differentiation antigens that are not tumor-specific but may be selected as targets if injury to the normal tissue is tolerable, such as mesothelin in pancreatic cancer and prostate-specific antigen in prostate cancer	[132,133]
2. Cancer-testes (germ cell) antigens, such as NY-ESO-1 and the MAGE family, that are detected in many tumors, such as myeloma and melanoma, but not in normal adult tissues with the exception of the testes	[134,135]
3. Overexpressed self-proteins, particularly those associated with driver mutations, such as c-erbB2 in breast cancer	[136]
4. Mutational antigens that are tumor specific, such as $\text{BRAF}_{\text{V600E}}$ mutations and BCR-ABL translocations	[137]
5. Viral antigens that are tumor specific, such as EBV in Hodgkin disease, human papillomavirus in cervical cancer, and polyomavirus in Merkel cancer	See Chapter XX
6. Minor histocompatibility antigen (mHA)-specific T cells (allogeneic T cells)	[138]
7. Target tumor suppressor genes such as WT1	[139]

tumors [38]. The antigenic targets for T-cell therapy can be classed into six major categories (Table 69.3). All six classes of antigens shown in Table 69.3 have been targeted with acceptable safety with therapeutic vaccines; however, it is likely that only tumor-specific targets will have an acceptable therapeutic index with adoptive transfer of potent CTLs that have high-affinity TCRs. It is possible that conditional suicide systems expressed in self-reactive CTLs may increase the therapeutic index of CTLs in the setting of HSCT [39].

Improved CTL cell culture technology [40] permitted the first clinical tests of adoptive transfer of CTLs, and the approach seems to have substantial activity in patients with melanoma, although so far there has been limited efficacy outside of the setting of melanoma. For example, CTLs derived from peripheral blood lymphocytes (PBLs) were used to treat patients with refractory, metastatic melanoma [41], and the in vivo efficacy of the infused T-cell population was indicated by the destruction of normal melanocytes and outgrowth of a MART-1$^-$ tumor, demonstrating the selection of a tumor variant with loss of MART-1 expression [42]. Potent antileukemic effects have been observed in patients relapsing after allogeneic HSCT when minor histocompatibility antigen (mHA)-mismatched donor T cells are infused [43]. Perhaps the most worrisome issue revealed with CTL transfers is the emergence of antigen escape variants, which seems to be more common in human tumors than mouse syngeneic tumor models [44].

Combination approaches using vaccines and adoptive T-cell transfer

In mice, adoptive T-cell therapy enhances the effects of therapeutic vaccines [45], and this combined approach in the setting of lymphopenia results in a further enhancement of tumor immunity [46,47]. In humans with myeloma, idiotype vaccination of sibling donors with the unique tumor-specific immunoglobulin produced by the patient myeloma cells followed by adoptive transfer in the setting of allogeneic stem-cell transplantation can result in the induction of potent antitumor immunity [48].

Several phase I clinical trials using adoptive transfer of autologous activated T cells in patients transplanted for hematologic malignancies have been reported. In the first trial [37], patients with relapsed or chemotherapy-refractory non-Hodgkin lymphoma (NHL) were given a CD34$^+$ HSC transplant followed by infusion of autologous T cells expanded ex vivo with CD3- and CD28-specific antibodies. Infusion of the autologous costimulated T cells resulted in a rapid reconstitution of lymphocyte numbers. Importantly, the expanded cells were functionally superior to those obtained directly from the patients, as determined by their ability to produce interferon-gamma (IFN-γ) when stimulated with tumor cells in vitro. In a second randomized trial, the feasibility of pre-transplant immunization and adoptive transfer of vaccine-primed T cells in the setting of autologous transplantation for multiple myeloma was tested [29]. Patients were vaccinated with Prevnar, the heptavalent pneumococcal conjugate vaccine (PCV), and 2 weeks later T cells were harvested and expanded in vitro using CD3- and CD28-specific antibodies. Patients received a standard autologous peripheral blood HSC transplant after melphalan conditioning, the ex vivo expanded autologous T cells either 14 or 100 days post-transplant, and two doses of PCV, beginning 1 month after transplant. Prompt T-cell recovery was observed in patients who received ex vivo expanded autologous T cells 14 days after the HSC transplant, whereas those who received the T cells 100 days after the transplant remained substantially lymphopenic. Early adoptive transfer of PCV-primed T cells resulted in the induction of potent immunity, as only those individuals who received PCV-primed T cells soon after the transplant developed and maintained protective levels of pneumococcal-specific antibodies, as well as vaccine-specific CD4$^+$ T-cell responses. Similar trials have shown that the combination of adoptive transfer and vaccination can induce potent humoral and cellular response to human telomerase reverse transcriptase (hTERT) and influenza in patients with advanced myeloma autologous HSCT [30,49]. In the setting of autologous HSCT for pediatric neuroblastoma, adoptive transfer of T cells given on day 2 were superior to infusions given on days 12 or 90 after stem-cell infusion when TCR repertoire diversity and the humoral response to a pneumococcal vaccine were assessed [50].

The primary toxicity of combined T-cell transfer and vaccine strategies has been an autologous graft-versus-host disease (GVHD)-like syndrome with rash and colitis in about 25% of patients [51]. These data demonstrate that a combination of vaccine and adoptive T-cell therapy, consisting of a single pre-transplant vaccine and an early post-transplant infusion of in vivo antigen-primed, ex vivo-costimulated autologous T cells followed by post-transplant booster immunizations, improved the severe immunodeficiency associated with high-dose chemotherapy, and led to clinically relevant immunity in adults within 1 month of HSC transplantation, in contrast to the 1 year that is required to reconstitute immunity following a standard transplant procedure [6]. These studies provide a useful foundation for the design of future strategies using combinations of tumor vaccines and vaccine-primed T cells in lymphodepleted patients with various malignancies.

Synthetic biology with engineered T cells

Rationale

The high level of tolerance to most tumor antigens combined with immunosuppressive microenvironments supports the conclusion that augmentation of immunity with adoptive transfer of T cells may not be sufficient in most patients with advanced malignancy. An emerging discipline is synthetic biology, which combines elements of engineering, chemistry, computer science, and molecular biology to assemble cellular and biological tools necessary to improve the natural function [52]. The principles of gene transfer combined with adoptive cellular therapy are poised to overcome these limitations.

Genetic modification of T cells to engineer improved antitumor effects and enhanced immune reconstitution of immunosuppressed patients is an attractive strategy in many settings [53]. A potential safety concern when infusing individuals with engineered T cells is one that arose with genetically engineered HSCs [54], when viral insertional mutagenesis was shown to cause cellular transformation. However, in patients with congenital and acquired immunodeficiency, genetically modified T cells have been shown to persist after adoptive transfer in humans for more than a decade without adverse effects [55,56], indicating that the approach to genetically modify mature human T cells is fundamentally safe.

Strategies

To overcome tolerance to tumors that results from deficiencies in the TCR repertoire, two approaches are being developed. In the first approach, TCR genes are isolated from tumor-reactive T-cell clones, assembled in gene transfer vectors, and introduced into T cells from a patient of the appropriate MHC type to confer tumor specificity. In the second approach, genetic modification of T cells is accomplished with CARs containing sequences that encode antibody-based recognition domains linked to signaling sequences. An advantage of CARs is that since they are specific for cell surface molecules, this overcomes the constraint of TCR recognition of peptide antigens presented by only certain MHC molecules, and avoids tumor escape through impairments in antigen presentation or HLA expression. Genetic modification of T cells is not limited to conferring new antigen reactivity to recipient T cells, but can also be used to insert genes that improve the efficacy of the T cells that are transduced. Such genes include those encoding molecules involved in costimulation [57], the prevention of apoptosis [58], the remodeling of the tumor microenvironment [59], the induction of homeostatic proliferation [60], and CARs encoding chemokine receptors that promote T-cell homing [61].

Clinical trials with CAR T cells

The design of CARs in clinical trials can be roughly classified into three generations. First-generation CARs encode antibody-based external receptor structures and cytosolic domains that encode signal transduction modules comprised of the immunoreceptor tyrosine-based activation motif such as TCRζ or FcRγ [62]. Second-generation CARs also include a costimulatory signaling domain such as CD28 or 4-1BB [63,64], and third-generation CARs include three or more cytosolic domains [65].

First-generation CARs

The first CAR trials were conducted in patients with HIV. Two phase I and one phase II trial testing a first-generation CD4ζ CAR were reported [66]. The trials demonstrated modest antiviral efficacy; however, the CD4ζ CARs T cells have persisted at high frequency in most patients. Encouragingly, retroviral integration site analysis showed no evidence of persistent clonal expansion or enrichment of integration sites near oncogenes or tumor suppressor genes [56].

Several phase I clinical trials were conducted in patients with cancer. A trial testing T cells expressing a CAR specific for a folate-binding protein that is present on ovarian carcinoma cells indicated that the approach was safe, but poor expression and persistence of the transgene encoding the CAR were observed in vivo [67]. Similarly, a pilot test in children with neuroblastoma treated with autologous T cells retargeted for a tumor-associated adhesion (CD171) molecule indicated that the approach is safe but was limited by poor persistence of the T cells [68]. Lamers et al. tested T cells expressing a CAR receptor specific for carbonic anhydrase IX, an antigen present on the surface of clear cell renal cell carcinoma [69]. They observed an unexpected serious hepatic toxicity in several patients within 1 week of T-cell infusion, which was due to carbonic anhydrase IX expression in the biliary tract. This study indicates that CAR T cells can traffic to and exert effector function at sites of antigen expression. Furthermore, it indicates that the targets of chimeric antigen receptors must be carefully chosen to avoid unwanted adverse effects, or that additional safety features, such as suicide switches [70], need to be incorporated into the vectors driving the expression of the chimeric receptor. In several instances, the engineered cells persisted for several weeks before elimination by host immune responses, indicating that a technical challenge for this approach is to prevent a host immune response from eliminating the adoptively transferred cells.

One of the lessons from the trials testing first-generation CARs T cells was that the infused product could be immunogenic. Both B-cell [67,69] and T-cell responses [71,72] have been identified in CAR trials. In the trial by Lamers et al., the plasma from these patients neutralized target cell recognition by the CAR T cells. In addition, they have shown [72] that 8/9 evaluable patients also developed cellular immunity against their carbonic anhydrase IX-specific CAR, and that patients who developed a B-cell response against the CAR also exhibited a cellular response against CAR T cells, but not necessarily the other way around.

Another lesson from the trials testing first-generation CARs in cancer trials was that the persistence and expansion were poor. This is likely the result of CAR design in part, and the use of suboptimal cell culture approaches that favored terminally differentiated T_{EM} CAR products rather than T_{CM}. The best persistence of CARs reported was in the pediatric neuroblastoma trial (ClinicalTrials.gov NCT00085930), where CARs could be detected at very low levels (0.0001–0.001%) at up to 4 years after infusion [73]. In contrast, in the CD4ζ CAR trial (ClinicalTrials.gov NCT01013415), the frequency of CAR T cells in blood was several orders of magnitude higher (0.6–6%) in patients 5 years after infusion [56].

A final lesson from the first-generation CAR trials in cancer patients was that efficacy was disappointing. The best clinical results were reported in patients after infusion of a GD2-specific CAR, with two of 11 patients having long-term remissions [73].

Second- and third-generation CARs

For ethical reasons, it was necessary initially to evaluate first-generation CARs to exclude safety concerns such as the induction of a T-cell lymphoproliferative syndrome. Based on principles of T-cell activation [74], it would be predicted that that the first-generation CARs would become anergic unless the tumor target provided costimulation [19]. In addition, resting T cells cannot be activated with CAR containing a TCRζ or FcRγ signaling moiety in the absence of costimulation [75]. In 1998, two laboratories showed that the CD28 signaling domain provided costimulation when engineered into the CAR design, in cis with the TCRζ domain [63,76]. It was later shown that members of the tumor necrosis factor receptor family such as CD27, 4-1BB (CD137), and OX40 (CD134) also provided costimulation [77–79]. These second- and third-generation CARs are now in a number of early-stage clinical trials at academic medical centers.

CAR trials targeting B-cell malignancies

Other than normal B cells, CD19 is not present on normal tissues including pluripotent blood stem cells, and is not shed as a soluble form into the circulation, which makes CD19, or CD20 as evidenced by rituximab, a relatively safe target presenting a minimal risk of autoimmune disease or irreversible myelotoxicity. Many trials are currently ongoing to test second- and third-generation CARs (reviewed in [80,81]). Promising results have been obtained targeting the B-cell lineage-restricted CD19 molecule that is expressed on B-cell leukemias and lymphomas with CD19-specific CAR T cells [20,82–84]. At the University of Pennsylvania, durable remissions beyond 2 years have been observed in the initial cohort of patients with refractory and relapsed B-cell chronic lymphocytic leukemia (CLL) after the infusion of autologous T cells transduced to express a CD19-specific CAR that contained a 4-1BB costimulatory domain [20,21]. In these studies, the infusion of low doses of T cells led to 2–4 log10 in vivo expansion and persistence of CAR T cells, subsequent tumor lysis, and a persistent aplasia of normal CD19$^+$ B cells [20]. Significant antitumor activity, depletion of normal B cells, and side effects related to tumor lysis and cytokine release have also been reported in patients with CLL and lymphoma by groups at the National Institutes of Health, Memorial Sloan Kettering Cancer Center, and Baylor College of Medicine in trials in which autologous T cells were modified to express CD19 CARs that contain a CD28 costimulatory domain [82–85]. In addition to activity in CLL and mantle cell lymphoma [20,65], CD19:4-1BB CARs have potent activity in pediatric acute lymphoblastic leukemia (ALL) with efficient trafficking to bone marrow and cerebral spinal fluid [86]. It is currently unknown whether CARs with a CD28 or 4-1BB signaling domain are preferable. A clinical trial led by Brentjens and colleagues infusing an equivalent number of CD19-specific CARs containing either a CD28 or 4-1BB domain is ongoing to address this issue (ClinicalTrials.gov NCT01044069).

The above trials employed efficient retroviral or lentiviral transduction to introduce CARs into T cells. Whether one vector is superior remains unknown. An ongoing trial (ClinicalTrials.gov NCT00968760) is testing CD19 CARs that are expressed using the *sleeping beauty* transposon system [87]. There are many other questions outstanding about the use of CARs for B-cell malignancies. In the area of CAR design, in addition to the open questions on optimal costimulatory domains, the optimal characteristics of the scFv (affinity and epitope), the hinge length, and transmembrane domain remain to be defined. In addition, there are major questions regarding clinical trial design, such as whether to provide cytokine support to the patient after CAR infusion and whether host conditioning chemotherapy is necessary.

Toxicity with CAR T cells

As with all cancer therapies that have efficacy, there is an emerging set of toxicities with CAR T cells. Many types of adverse events have been reported following infusion of human autologous or allogeneic lymphocytes. The toxicities can be classified as those due to extrinsic factors present in the culture process, those due to accompanying cytokines that can be co-infused with the cells, and those due to the cells themselves. The spectrum of the third form of adverse effects is still being defined, and for the moment seems to be related to whether the cell product is genetically engineered. For cell products that have not been genetically engineered, the adverse effects are limited and are similar to those observed with therapeutic vaccines. Retinitis, iritis, hepatitis, autoimmune thyroiditis with hypothyroidism, and vitiligo occur following autologous T-cell infusions [36,42,69,88]. Respiratory obstruction has been reported following CTL infusion for EBV-related lymphomas [89]. This is probably due to a T-cell-induced inflammatory response that results in tumor edema and necrosis. Effector functions of infused T cells can be expected to include tissue damage similar to that encountered in T-cell-mediated autoimmune diseases. In the case of allogeneic lymphocyte infusions, GVHD and bone marrow aplasia can occur [90].

On-target toxicities were expected with CD19 CAR T cells and include B-cell aplasia, tumor lysis syndrome (TLS) and cytokine release syndrome (CRS). Intravenous immunoglobulin can be used to replace deficient antibody function. TLS has been managed successfully according to standard supportive therapy, including hydration, alkalinization, allopurinol, and rasburicase as required [91]. A unique feature of the TLS following CAR T-cell therapy is that this can be delayed for significant periods of time [21]. At the University of Pennsylvania, one patient developed fulminant TLS nearly 2 months following CAR infusion. In all cases, TLS is accompanied by fever and elevated levels of inflammatory cytokines such as IFN-γ and IL-6.

In patients with B-cell malignancies, CRS occurs at the time of peak levels of CAR T cells in blood and bone marrow. The optimal management of CRS is still an open question. Corticosteroids and cytokine blockade with etanerocept and tocilizumab are currently being evaluated for patients with ALL and CLL. A grading system for CRS was recently reported, and a clinical management algorithm was suggested [140]. On-target but off-organ toxicity to normal tissues remains an issue for which novel strategies to mitigate toxicity, such as by regulating CAR expression or T-cell survival, are needed.

A number of theoretic off-target toxicities with CAR T cells are possible. The introduction of CARs by integrating retroviral or lentiviral vectors, transposons, and electroporation all create the risk of transformation. This risk appears to be low based on the long-term follow-up data in patients treated with the CD4ζ CAR, where there have been no cases of genotoxicity in more than 540 patient-years of observation [56]. Another theoretic risk, particularly with CARs containing synthetic signaling domains, is the induction of a T-cell lymphoproliferative syndrome or T-cell leukemia. T-cell leukemia has occurred in the setting of retroviral-mediated gene transfer into HSC for congenital immunodeficiency [54]; however, it has never been reported following gene transfer into mature T cells. Finally, the risk of replication competent retrovirus (RCR) or lentiviruses exists, as this has occurred previously with severe consequences in non-human primates who were infused with HSC cells transduced with a retroviral vector containing RCR [92]. With improved packaging lines and plasmid design, this has not occurred following adoptive transfer of retroviral modified T cells in 297 humans enrolled in 29 different clinical protocols [93].

Clinical trials with transgenic TCR-modified T cells

A principal limitation of adoptive T-cell therapy for most cancer histologies is that the tumors are poorly antigenic or that the highest avidity T cells have been deleted during thymic selection; therefore, there are no T cells with high avidity for tumor-specific antigens and there may be no T cells with the desired specificity left in the patient after chemotherapy. Most T cells have TCRs of micromolar affinity for peptide–MHC ligands, but genetic engineering can generate TCRs of nanomolar affinity. Two approaches to improve the affinity of TCRs are now being tested in clinical trials. In one approach, mice that express both human MHC and TCR genes have been developed and can be vaccinated to elicit TCRs from a repertoire that is not tolerant to human self or tumor-associated determinants [94]. It is also possible by mutation to improve the affinity of TCRs obtained from low-avidity T cells specific for self-proteins [95]. The affinity for MHC class I TCR viral epitopes can be enhanced into the sub-nanomolar range, with improved clinical activity noted when the affinity-enhanced TCRs are expressed in T cells in preclinical studies [96]. However, the introduction of TCRs specific for self antigens that are of too high affinity can result in the deletion of transferred $CD8^+$ T cells in vivo, whereas $CD4^+$ T cells expressing the same TCR survived [97].

Clinical trials using TCR gene transfer to target differentiation antigens (MART-1, gp100, CEA) and the cancer-testes antigen NY-ESO-1 have been reported [98–100]. Some antitumor effects were observed and on-target toxicity to normal melanocytes in the skin, eye, and ear that required local corticosteroid therapy was observed in a significant fraction of patients who received the highest avidity TCRs. Consistent with this, the introduction of a high-affinity carcinoembryonic antigen (CEA)-specific TCR into autologous T cells to treat colorectal cancer resulted in severe colitis, presumably from recognition of normal epithelial cells that express CEA, and the trial was halted when stopping rules were reached after three patients [101]. These data, together with the CD19 CAR data showing persistent B-cell aplasia, indicate that for engineered T cells the therapeutic index is less than with natural T cells, and that only targets with highly restricted expression to tumor cells will have acceptable toxicity. Consistent with this notion, a TCR specific for an HLA A*0201 restricted NY-ESO-1 epitope, which is expressed in many human cancers but not in normal tissues apart from testes, was used to engineer autologous T cells for treatment of metastatic melanoma and synovial sarcoma. Objective responses were observed in five of 11 melanoma patients and four of six sarcoma patients without toxicity to normal tissues [100]. A phase II trial testing the same NY-ESO-1-specific TCR in myeloma patients that is ongoing at the University of Pennsylvania has also documented potent on-target antitumor effects without significant toxicity (ClinicalTrials.gov NCT01352286).

A concern with the transgenic TCR approach has been that it might generate additional, novel receptor specificities by pairing of the transgenes with the endogenous TCR chains, creating so-called "mixed dimers." In mice, a lethal GVHD syndrome can occur due to the formation of mixed dimers [102]. However, it is encouraging that no toxicity was observed in the above TCR trials that have together enrolled more than 50 patients. There are several potential solutions to address this concern. First, alpha beta TCRs can be transferred into gamma delta T cells, taking advantage of the observation that alpha and beta polypeptide chains do not pair with gamma delta TCRs [103]. Second, there are elegant approaches to prevent the endogenous TCR from pairing with the transgenic TCR. In one case, this was prevented by knocking down the expression of endogenous TCR chains using inhibitory RNA [104], and another approach to eliminate the problem of mispairing of TCR chains is to abolish expression of the endogenous receptors using specific zinc-finger nucleases that disrupt the endogenous TCR alpha and beta gene loci [105]. Serious off-tumor toxicity with engineered TCRs has occurred [141–143]. In addition, serious case of CRS occurred following the infusion of MART-1 modified T cells [144].

Incorporating CAR and TCR T cells into stem-cell transplant regimens

Genetically engineered T cells have the potential to augment the efficacy of autologous HSCT procedures. The antitumor activity of autologous CD19 CAR T cells in patients with advanced B-cell malignancies and TCR T cells in myeloma illustrates the potential to incorporate the administration of T cells modified with a CAR or TCR to reduce relapse after autologous stem-cell transplant. The importance of lymphodepleting chemoradiotherapy for improving the persistence and antitumor efficacy of transferred T cells was established in studies in melanoma and lymphoma [36,37], and this suggests that intensive conditioning administered before autologous HCT could provide an ideal environment for administering engineered T cells. Furthermore, in mice, mice effector $CD8^+$ T-cell function is enhanced by concomitant HSC infusion [106].

Leukemia relapse remains a major cause of failure after allogeneic HCT and the long-sought goal of augmenting the graft-versus-leukemia (GVL) effect without aggravating GVHD remains elusive [107]. It is possible that infusion of allogeneic TCR- or CAR-modified T cells could enhance the efficacy of allogeneic HSCT. This is supported by data that indicated that infusion of costimulated but non-gene-modified allogeneic T cells was safe in a phase I trial [108]. In that study, 18 patients with relapse after allogeneic SCT were given infusions of activated donor T cells costimulated with anti-CD3- and anti-CD28-coated beads. Patients were given adoptive transfer on day 12 after reinduction chemotherapy. Activated donor lymphocyte infusion (DLI) was dose escalated from 1×10^6 to 1×10^8 $CD3^+$ cells per kilogram in five levels. Seven patients developed acute GVHD (five grade I–II, two grade III), and four developed chronic GVHD. Eight patients achieved complete remission, including four of seven with acute lymphocytic leukemia, two of four with acute myelogenous leukemia, one with chronic lymphocytic leukemia, and one of two with NHL. Several trials are now under way to evaluate the safety and antileukemic potential of CAR-modified allogeneic T-cell infusions.

Strategies to use engineered T cells as a bridge to transplant

The development of CAR- and TCR-transduced T cells, specific for leukemia-related antigens, introduces the possibility of utilizing these cells in a variety of clinical circumstances important in transplantation. The first are for patients who have relapsed ALL and who are candidates for allogeneic transplantation. Given the limited therapeutic efficacy of reinduction regimens, the development of engineered T cells may provide an appropriate bridge to transplant by inducing a remission that can then be preserved by an allogeneic transplant. In patients who have undergone a transplant, it may be possible to utilize the CAR- or TCR-transduced T cells for treatment of either relapse that occurs after transplant, instead of DLI, utilizing an antigen-specific donor-derived immune response separate and distant from the allogeneic reactivity against leukemia cells mediated by DLI. In addition, as monitoring for minimal residual disease becomes more standardized and important, both in the management of patients pre-transplant receiving standard therapy and post-transplant, donor-derived CAR-transduced T-cell DLI could be utilized to treat molecular evidence of recurrent disease before overt relapse occurs. The possibility of utilizing viral-specific T cells as the transduced cell population can further reduce the likelihood of concomitant graft-versus-host reaction and may be manipulable by various vaccine strategies. These same principles could be extended to other target antigens as they evolve for both myeloid leukemias and also multiple myeloma.

In the autologous setting, there are fewer data available about the efficacy of these cells in the treatment of patients with recurrent B-cell lymphoma, although the antigenic determinants may be the same. Nevertheless, CAR-based T-cell therapy that facilitates a response, if not remission, in patients relapsing after standard therapy for large-cell lymphoma may also serve as a bridge to transplant. Similarly to the allogeneic setting, investigators could utilize CAR-transduced T cells specific for CD19 as post-transplant immunotherapy to help reduce the chances of relapse by introducing a CD19-specific T-cell reconstitution as part of the general immune reconstitution that occurs after transplant. Currently, trials are ongoing to address this approach in the post-transplant setting, both for lymphoma and also for multiple myeloma targeted to NY-ESO1, utilizing a TCR transduction strategy. Thus, chimeric antigen receptor-transduced T cells may have a role in the autologous setting in preparing patients for transplant or by augmenting the efficacy of transplant to prevent relapse after autologous transplant.

Moving to the dark side: adoptive therapy with T regulatory cells

Mature donor effector T cells cause GVHD, but they are also the main mediators of the beneficial graft-versus-tumor (GVT) activity of allogeneic bone marrow transplantation. Naturally occurring, thymic-derived Treg cells that express CD4, CD25, and the forkhead box protein 3 (FoxP3) are potent suppressors of GVHD and other immune responses. In mice, the infusion of freshly isolated or ex vivo expanded donor Treg cells was highly effective in preventing acute or chronic GVHD [109]. In a mouse model, the infusion of donor-derived Treg cells prevented GVHD without abrogating GVT effector function, an effect that was mediated primarily by the perforin lysis pathway [110]. Further, in vivo activated donor or host Treg cells were able to ameliorate ongoing chronic GVHD.

Clinical-scale culture technologies using rapamycin and artificial APCs (Figure 69.1) were developed at the University of Minnesota to expand efficiently naturally occurring regulatory T (nTreg) cells from donor peripheral blood or cord blood [111]. A trial tested nTreg cell infusion in patients receiving double umbilical cord blood (UCB) transplant demonstrated the safety and feasibility of the adoptive transfer of ex vivo expanded nTreg cells [112]. In this protocol, 23 patients received infusions of nTreg cells that were isolated from a third UCB unit, enriched, expanded ex vivo, and reinfused. The UCB nTreg cells engrafted and were detected in circulation for 2–14 days after infusion. The UCB trial demonstrated that expanded nTreg cells were safe up to a dose of 3×10^6/kg, meeting the primary end-point of the study. No infusional toxicity was observed, nor was there an increased risk of infection, relapse, or early mortality. Patients given adoptive transfer of nTreg cells had a reduced the incidence of grade II–IV acute GVHD (43% vs. 61%, $p = 0.05$) compared with 108 historical controls treated identically except for Treg cells [112].

Phase II trials are scheduled to test the safety and efficacy of nTreg cell adoptive transfer to prevent GVHD in patients at high risk for grade II–IV GVHD. The main issues to address are whether Treg cells decrease the GVL effect and lead to higher rates of tumor relapse or increase the risk of opportunistic infections. A theoretic concern is that in some models, Treg cell plasticity has been reported, and the Treg cells can lose Foxp3 expression and differentiate into pathogenic Th17 cells [113].

Dose and scheduling issues

Information on the dose and schedule dependence of adoptively transferred cells is widely scattered in the literature, and from this literature one concludes that there is no standardized dose system. There is, however, evidence from animal models in non-lymphopenic hosts suggesting that multiple doses of adoptively transferred T cells have superior antitumor effects to a single infusion of T cells [114]. Doses of adoptively transferred cells are usually reported as the total number of viable cells administered, or as the total number of viable cells administered per kilogram body weight or per square meter of body surface area. However, total endogenous lymphocyte numbers do not correlate well with body surface area, but rather display a strong inverse correlation with age. Other variables add to the complexity, particularly the fact that, in the case of T cells or other adoptively transferred cells with high replicative potential, the infused dose might not relate well to the steady-state number of cells that engraft and persist. Therefore, dose considerations are more complex than in other areas of transfusion medicine, where, for example, the maximal level of transfused red cells or platelets occurs immediately following infusion. In our studies of adoptively transferred autologous CAR T cells, we often find that the number of cells in the host peaks 2–3 weeks after infusion of the cells [20]. This is because the engraftment potential and the replicative potential of the infused cells depend on complex host variables such as the number of niches available in the host for engraftment, and the antigenic stimulus for clonal expansion or deletion. In most rodent tumor models, T-cell proliferation in the host after transfer is obligatory for therapeutic efficacy (reviewed in [2]) and, with rare exceptions where adoptively transferred effector T cells are used as a non-replicating drug rather than replicating cells [115], this is presumed also to be required in humans.

Cytokines given to the host can also have a major impact on the persistence of adoptively transferred T cells. Others have found that the persistence of adoptively transferred human $CD8^+$ T cells is enhanced by coadministration of IL-2 [116]. However, we have found that when autologous human $CD4^+$ and $CD8^+$ T cells are given in combination, persistence is not increased by concomitant IL-2 therapy [117]. Finally, studies have shown that IL-2 can induce the proliferation and maintenance of effector $CD8^+$ T cells but might actually delete memory T cells and increase the number of Treg cells [118]. By contrast, IL-15 and IL-7 seem to select for the persistence of memory $CD8^+$ T cells and might decrease the number of Treg cells in mice [119] and non-human primates [120].

Striking schedule-dependent increases in efficacy and the frequency of adverse effects from adoptively transferred cells have been reported when T-cell infusions are given to lymphopenic hosts [51,121,122]. Many studies in rodent tumor models show that the coadministration of cytotoxic therapy can enhance the effects of adoptively transferred cells [123]. Cyclophosphamide and/or fludarabine is generally administered to the host several days before the adoptively transferred T cells. The drugs have multiple effects that seem to promote the antitumor effects of the adoptively transferred T cells [7].

Curti et al. [124] studied the optimal time to harvest autologous $CD4^+$ T cells in relation to the timing of cyclophosphamide administration in patients with advanced cancers. T cells were harvested at steady state, or when on the decline or when on the recovery from the cyclophosphamide-induced leukopenia, and they found the greatest in vivo $CD4^+$ T-cell expansion after infusion occurred when cells were harvested as patients entered the cyclophosphamide-induced nadir. These findings are relevant to T-cell infusions post-HSCT. In a pediatric population, we find that infusion on day +2 is superior to day +12 or +90 after high-dose chemotherapy [50].

Conclusions and future directions

Adoptive T-cell therapy is the ultimate challenge to the implementation of personalized medicine, and is a natural extension of HSCT. The field of adoptive therapy with engineered T cells is poised for substantial clinical advances that are now possible because of improved cell culture and gene transfer methods. In some cases, engineered autologous T cells may be able to replace the need for allogeneic HSCT, so it is conceivable that autologous HSCT with T-cell infusions could reach the efficacy of allogeneic HSCT but without the risk of GVHD. However, the major challenge currently facing the field is to conduct randomized clinical trials demonstrating sufficient clinical benefit to justify the logistics and expense of customized cellular therapies.

Based on a more advanced understanding of lymphocyte development, another area of opportunity will be the infusion of engineered precursor cells that are programmed to have the functions of effector lymphocytes. Examples include the adoptive transfer of engineered common lymphoid progenitor cells [125], memory stem T cells [13,28], and "retrogenic T cells" produced by administration of embryonic stem cells or HSCs engineered to express transgenic TCRs or chimeric antigen receptors after in vivo differentiation to T cells [126–128].

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CHAPTER 70

Relapse of Hematologic Malignancy After Allogeneic Hematopoietic Transplantation

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Introduction

Allogeneic hematopoietic transplantation is an effective, potentially curative treatment for hematologic malignancies. Advances in supportive care have markedly improved treatment-related mortality associated with the procedure, but relapse of the underlying malignancy remains a major problem and is the major cause of treatment failure [1–4]. The prognosis relates to the aggressiveness of hematologic malignancy, responsiveness to salvage chemotherapy, and immune manipulations of the graft-versus-malignancy effect.

Allogeneic hematopoietic transplantation involves the administration of high-dose chemotherapy and/or radiation with reconstitution of hematopoiesis by donor-derived cells. The high-dose therapy does not generally eradicate the malignancy, and the immune graft-versus-leukemia (GVL) effect is important to eliminate residual drug-resistant disease [5]. Evidence supporting this premise includes the higher rate of relapse in patients with identical twin donors and in those receiving T-cell-depleted transplants [6]. Natural killer cells are important in the process of GVL against myeloid leukemias [7,8] and may mediate GVL effects in T-cell-depleted transplants. Graft-versus-host disease (GVHD) remains a major obstacle to successful allogeneic hematopoietic transplantation. Calcineurin inhibitors and antithymocyte globulin have reduced the incidence and severity of GVHD, but the intensity of post-transplant immunosuppressive therapy may increase the risk of relapse [9,10]. The minor histocompatibility antigens involved in graft-versus-leukemia and graft-versus-host reactivity likely overlap [11] and patients with GVHD (particularly chronic GVHD) are less likely to relapse [6]. The major challenge in hematopoietic transplantation is to separate effectively the beneficial GVL effect from the toxicity of GVHD. Several approaches are under investigation aimed at augmenting the allogeneic graft-versus-malignancy effect while preventing GVHD.

Mechanism of relapse post-transplant

Relapse generally results from residual malignant cells that survived the preparative regimen and are not eliminated by the GVL effect. In a minority of patients, relapse appears to occur in donor-derived cells [12–14]. The diagnosis of donor cell relapse is generally made in sex-mismatched cases and based on the finding of the donor sex chromosomes in the leukemia cells. When multiple genetic assays for chimerism are applied, both donor and host markers are present in some cases, making interpretation complex [15]. The mechanism of leukemogenesis in donor cells is unknown; it could include recapitulation of the original leukemogenic process in donor cells, transfer of genes or chromosomes from recipient leukemia to donor cells, or the effect of a leukemic microenvironment. One interesting case was reported in which the patient and the donor shared a germline CEBPA mutation. Donor hematopoietic cells evolved into overt acute myeloid leukemia (AML) by developing a second CEBPA mutation in the recipient bone marrow microenvironment. These were identical with the acquired mutations identified in the patient's original leukemia [16].

Relapse may occur by immune escape from GVL effects. This may be due to loss of expression of leukemia-related antigens [17]; one case was reported associated with loss of expression of a human leukocyte antigen (HLA) haplotype by deletion of chromosome 6 [18]. Another mechanism of immune escape is development of tolerance in donor-derived T cells post-transplant. Antigen-specific T cells may be deleted over time, which has been associated with relapse post-transplant [19]; such patients could potentially be treated by donor lymphocyte infusion (DLI) or second transplants, administering non-tolerized donor T cells.

Chronic myeloid leukemia

Relapse post-transplant has been most extensively studied in patients with chronic myeloid leukemia. Prior to the advent of tyrosine kinase inhibitors, allogeneic hematopoietic transplantation was commonly employed as primary treatment of this malignancy. DLIs were initially studied in this disease [20]. Over 70% of patients with minimal residual disease (MRD) or relapsing into a chronic phase achieve a durable complete remission [21]. Patients who had relapsed to accelerated or blastic phase disease had a lower probability of achieving remission (33 and 16%, respectively [22]). The major complications are GVHD and myelosuppression [23]. GVHD is more common with higher T-cell doses, short intervals from transplant to DLI, and use of unrelated donors. With matched sibling donors, initial infusion of a relatively low cell dose (≤10^7 T cells/kg) with subsequent escalating doses if needed produces a lower rate of GVHD than higher cell doses and has the same ultimate efficacy of achieving remission [24–26]. Tyrosine kinase inhibitors can induce complete remission in patients who relapse post-transplant, in patients who are not resistant to these agents [27]. The leukemia generally recurs if the tyrosine kinase inhibitor is discontinued. Promising results have been reported for patients

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with tyrosine kinase-resistant chronic myelogenous leukemia (CML) using a reduced-intensity conditioning regimen and DLI if there was residual disease present post-transplant [28,29].

Acute myeloid leukemia and myelodysplastic syndrome (MDS)

Allogeneic hematopoietic transplantation is a major treatment for AML/MDS. Relapse is the major cause of treatment failure and extensive research has been conducted to evaluate approaches to prevent or treat relapse post-transplant.

Disease recurrence is a devastating event after allogeneic hematopoietic stem-cell transplantation for AML or advanced MDS. The median time to relapse is approximately 4 months and the majority of relapses occur within 2 years after transplant. The prognosis is usually poor [30,31]. Overall 5-year survival of all patients relapsing post-transplant for AML or MDS at our center is 6%. However, many patients can respond to further treatment and a substantial minority can still achieve a durable remission, usually after a second allogeneic transplant or donor lymphocyte infusion [32].

A number of patient-, disease-, and treatment related factors identify patients at highest risk for relapse post-transplant, summarized in Table 70.1. The major disease-related factors predicting for relapse include disease status (active disease vs. remission), cytogenetics, and high-risk molecular abnormalities, particularly the FLT3-ITD mutation [33]. The cytogenetic abnormalities associated with relapse post-transplant differ from those reported with standard chemotherapy. Only the presence of complex cytogenetics with four or more abnormalities was predictive of relapse in a large Center for International Blood and Marrow Transplant Research (CIBMTR) registry analysis [34]. Intriguingly, the occurrence of cytomegalovirus or other herpesvirus infections is associated with a reduced risk of leukemia relapse [35]; the mechanism of this benefit is unknown, but is likely related to immune activation.

Donor factors may affect the risk of leukemia relapse. The use of donors with predicted natural killer (NK) cell alloreactivity [36,37] or expressing certain activating killer immunoglobulin-like receptor (KIR) genes or KIR haplotypes related to NK cell reactivity is associated with a reduced risk of relapse [38–40].

Non-myeloablative and reduced-intensity preparative regimens are commonly used to allow treatment of older and medically infirm patients who might not be able to tolerate myeloablative regimens [41]. Few randomized studies have been performed [42], but this approach may be associated with a higher risk of relapse, but counterbalanced by lower treatment-related mortality [43].

Table 70.1 Risk factors for relapse after allogeneic transplantation for AML/MDS

Patient
Older recipient age
Disease
Disease not in first remission
Refractoriness to salvage chemotherapy
Unfavorable cytogenetics
FLT3-ITD mutations
Minimal residual disease detected pre- or post-transplant
Transplant
Gender donor–recipient combinations other than female to male
Specific KIR haplotypes in the donor
Single versus double umbilical cord transplants
Use of non-myeloablative or reduced-intensity preparative regimen
In vivo or in vitro T-cell depletion
Mixed chimerism post transplant in CD34⁺ cells
Occurrence of cytomegalovirus infection

The presence of pre-transplant MRD is associated with an increased risk of leukemia relapse post-transplant. Walter et al. studied a large cohort of adults with AML in first complete remission undergoing myeloablative conditioning [44]. Five-year estimates of overall survival (OS) were 26 and 79% for MRD-positive and -negative patients, respectively, and 2-year estimates of relapse were 58 and 14%, respectively. Although the presence of MRD was closely associated with other adverse risk factors, this approach could identify patients at high risk for relapse.

Chimerism analysis post-transplant has been reported to predict leukemia relapse, but data are conflicting. Loss of donor chimerism or persistent mixed donor–recipient chimerism may precede acute leukemia relapse, but the kinetics of engraftment in different cell populations vary with different preparative regimens [45]. Mixed myeloid chimerism, particularly an increasing proportion of host myeloid cells, has been associated with leukemia relapse [46]. The kinetics of donor–recipient chimerism within CD34⁺ cells may provide information regarding leukemia recurrence [46–48]. Mixed lymphocyte chimerism is common with the commonly used busulfan–fludarabine preparative regimen. Mixed lymphoid chimerism alone has not predicted for relapse in our experience [49].

Relapse of AML or MDS is classically diagnosed by morphologic criteria. Morphologic diagnosis of relapsed MDS post-transplant can be challenging. Patients may have cytopenias for many reasons and dysplastic features are often present in patients in remission post-transplant; a morphologic diagnosis of MDS may mistakenly be made. If the disease-related clonal cytogenetic abnormality is present in recipient-derived cells, this definitively demonstrates persistent or recurrent disease. Chimerism studies are useful; if the recipient has 100% donor myeloid cells, overt relapse of the recipient MDS cannot be present. Patients with AML in morphologic remission who have residual cytogenetic abnormalities or aberrant phenotype by flow cytometry have residual disease and a poor prognosis [50].

The definition of relapse is evolving due to the development of increasingly sensitive methods for the identification of MRD [51–57]. Real time quantitative polymerase chain reaction-based molecular techniques have the highest sensitivity, but their use is limited to patients with a molecular marker of their disease. This approach can detect MRD, identifying leukemia-related translocations [58], NPM1 [55,59], and FLT3 internal tandem duplication (ITD) [60]. Overexpression of the non-mutated Wilms tumor gene 1 (*WT1*) is found in 80–100% of AML cases, and has been found to predict relapse [61–63].

Patients relapsing post-allogeneic hematopoietic transplantation have a poor prognosis. Duration of remission after hematopoietic stem-cell transplantation is an important predictor of survival after recurrence; the best results occur in patients with remission duration longer than 6 months. Figure 70.1 shows the survival of patients with a time from transplant to relapse less than or greater than 6 months. One-year survival was 8% versus 30%. Adverse prognostic factors include receiving the initial transplant while in relapse, having poor cytogenetic and molecular markers, and receiving transplants from alternative donors [2,3,64].

Recipient age, performance status, comorbidities, and presence of active GVHD are important factors in choosing salvage treatments [65]. Indolent relapses are more amenable to intervention than rapidly proliferating leukemias. Patient preference is an important consideration; transplant recipients have already gone through prolonged active treatment, often at a remote transplant center; some patients are inclined to continue aggressive therapy,

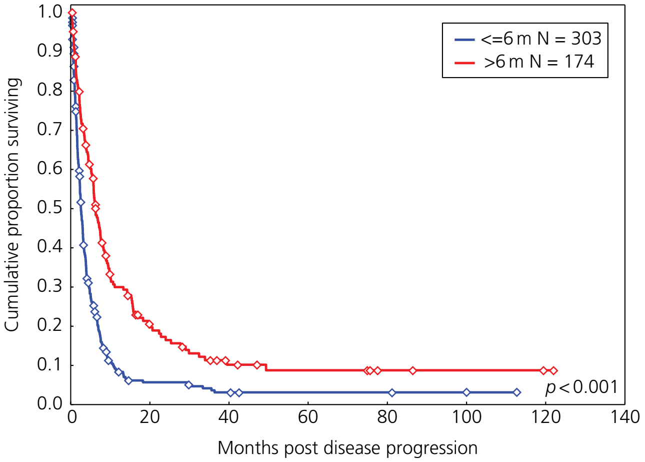

Figure 70.1 Survival of AML/MDS patients relapsing following allogeneic hematopoietic transplants at the M.D. Anderson Cancer Center since 2000. Those relapsing less than 6 months post-transplant (dark curve) have a particularly poor prognosis.

whereas others realistically prefer to return to their homes and receive palliative care.

Selection bias affects the outcomes in all series reporting results of treatment of relapse post-transplant. A minority of patients are enrolled in clinical trials, and an even smaller group achieve remission and long-term survival [30,65–67]. The choice of salvage treatments reflects institutional experience and varies widely. Several approaches have been used, including immunosuppression withdrawal, salvage chemotherapy, DLI, second allogeneic hematopoietic transplants, and combinations of these modalities [68].

Despite anecdotal reports of success, immunosuppression withdrawal is unlikely to induce clinical benefit in patients with AML/MDS with morphologic relapse [66] and should not delay more effective interventions. GVHD often complicates early or rapid withdrawal of immunosuppressive therapy, and may jeopardize the success of subsequent treatment. The worst situation is to have relapse and acute GVHD. We recommend continuing immunosuppressive therapy in patients who have active GVHD and a slow taper of immunosuppressive treatment in others.

Results of conventional chemotherapy remain poor in patients relapsing post-hematopoietic transplantation and the reported complete remission (CR) rates (0–60%) are highly influenced by remission duration and patient selection [69]. Patients may respond to salvage chemotherapy, particularly if they had chemosensitive disease before the initial transplant. In that case, retreatment with anthracycline- and cytarabine-based regimens may be effective.

Patients with FLT3-ITD mutations may respond to treatment with an FLT3 tyrosine kinase inhibitor. Sorafenib produced a prolonged remission in two of four relapsed FLT3-ITD-positive AML patients after allogeneic hematopoietic cell transplantation [70]. We were unfortunately unable to replicate sorafenib treatment results, and had no CR in 16 patients [71]. Other FLT3 tyrosine kinase inhibitors are under study. Patients tend to relapse quickly when the tyrosine kinase inhibitor is stopped, but these agents may induce transient responses that can be a bridge to a second allogeneic transplant.

5-Azacytidine has been used for the treatment of patients with MRD or overt relapse post-transplant. It is a DNA hypomethylating agent that can induce leukemic cell differentiation and may also increase the “immunogenicity” of tumor cells [72,73], augmenting GVL activity [74]. We evaluated the feasibility and possible efficacy of using low-dose 5-azacitidine (doses ranging from 16 to 40 mg/m^2) to treat and/or prevent disease recurrence after allogeneic hematopoietic cell transplantation in AML patients; five of nine patients with recurrent disease responded [75]. Lübbert et al. [76] administered 5-azacitidine followed by DLI to treat 26 patients with AML relapsing after allogeneic hematopoietic transplantation, and observed acceptable toxicity with prolongation of survival among responders. Bolaños-Meade et al. [77] reported 10 patients with myeloid malignancies (mostly AML) who received azacitidine for relapse post-allogeneic hematopoietic transplantation; six patients achieved CR and five remained disease free at a median follow-up of 624 days. Platzbecker et al. reported azacitidine (75 mg/m^2) to treat MRD based on a decreasing CD34-donor chimerism post-transplant [78]. Among 19 evaluable patients, 53% had complete clearance of MRD, defined as an increase in CD34-donor chimerism. These data led to consideration of this agent for post-transplant maintenance treatment, given with the goal of preventing relapse.

Chemotherapy alone generally produces only short-term responses in AML/MDS patients relapsing post-transplant and the best results have been achieved when a DLI or a second allogeneic transplant is performed to consolidate a chemotherapy-induced response. DLIs are frequently given to augment GVL effects as treatment of relapse post-transplant [79]. Although highly effective for the treatment of CML, AML has only intermediate sensitivity to GVL effects. DLIs are generally ineffective for relapsed disease when given alone, although there are some long-term survivors when given following salvage chemotherapy [65]. Results are very dependent on patient selection. DLIs may be complicated by GVHD, infection, and pancytopenia. Most responses do not translate into long-term survival [80]. Schmid et al. reviewed 399 patients with AML relapsing post-transplant. Estimated survival at 2 years was 21% for patients receiving DLI and 9% for those not receiving DLI. Better outcomes were associated with age <37 years, relapse occurring more than 5 months post-transplant, and use of DLI. A lower tumor burden at relapse, female gender, favorable cytogenetics, and being in remission at time of DLI were predictive for survival in a multivariate analysis. Two-year survival was 56% if DLI was performed in remission or with favorable karyotype, and 15% if DLI was given in aplasia or with active disease [65]. Levine et al. reported 57 patients with relapsed advanced myeloid malignancies receiving cytarabine-based chemotherapy and granulocyte colony-stimulating factor (G-CSF)-mobilized DLI; 27 patients experienced CR. GVHD was observed in 56% of the patients. Treatment-related mortality was 23%. The OS at 2 years for the entire cohort was 19% [81]. There is a higher rate of DLI-induced GVHD with unrelated or mismatched donors compared with matched siblings [82]. There are no randomized comparative trials to support the use of DLI versus chemotherapy alone or second allogeneic transplants [65,81,83].

A number of strategies have been studied to improve the efficacy and reduce DLI-induced GVHD. Adoptive transfer of ex vivo-selected T cells recognizing minor histocompatibility antigens (mHAs) may avoid the risk of GVHD while mediating an antileukemia effect [84,85]. Warren et al. reported the results of a phase I clinical trial in which the safety of adoptively transferring donor-derived $CD8^+$ cytotoxic T cells (CTCs) was evaluated. T-cell clones recognizing mHAs expressed by recipient hematopoietic cells but not by recipient dermal fibroblasts were given to patients with recurring AML after myeloablative allogeneic hematopoietic transplantation [86]. Generating therapeutic doses of T cells was complex but feasible. Unfortunately, GVHD with pulmonary toxicity was seen in three of seven patients, and the authors were also unable to show sustained in vivo persistence of transferred $CD8^+$ T cells. Next-generation sequencing may be able to identify unique targets for immunotherapy for each donor–recipient pair [87].

Second hematopoietic transplants can induce durable CR in a fraction of AML/MDS patients with relapse post-transplant. Registry data and single-center retrospective analyses show that the likelihood of success with a second transplant is increased by achievement of a morphologic CR (or minimal disease state) prior to the second transplant, and also by a CR duration longer than 6 months after the first allogeneic transplant; 20–30% of patients can achieve long-term survival in this setting [66,88–92]. We believe that this is the most effective treatment for patients in good general medical condition with chemosensitive relapse and should be considered the standard of care for these selected patients. Figure 70.2 shows progression-free survival of AML/MDS patients relapsing post-transplant at the M.D. Anderson Cancer Center who received second hematopoietic transplants. Patients who had achieved CR or CR with incomplete hematopoietic recovery after salvage chemotherapy had 21% long-term survival. Selected patients with low bulk residual disease post-salvage chemotherapy or untreated early relapse may also benefit; 16% of these patients survived disease free for over 5 years. Patients with overt relapse or chemoresistant disease are not good candidates for second transplants and should be offered supportive care or considered for clinical trials of novel therapeutic approaches.

There is no consensus regarding the optimal preparative regimen for a second allogeneic transplant; myeloablative and reduced-intensity regimens have been used. Myeloablative regimens produce lower relapse rates, but treatment-related mortality is higher than with reduced-intensity conditioning [90]. Most centers use a different regimen to that with the initial transplant and reduced-intensity regimens have generally been used in older patients. At our center, if a busulfan-based regimen was used for the initial preparative regimen, we generally employ a reduced-intensity melphalan and fludarabine regimen for the second transplant in adults. For young, fit patients, a high-dose total body irradiation-based regimen may be considered.

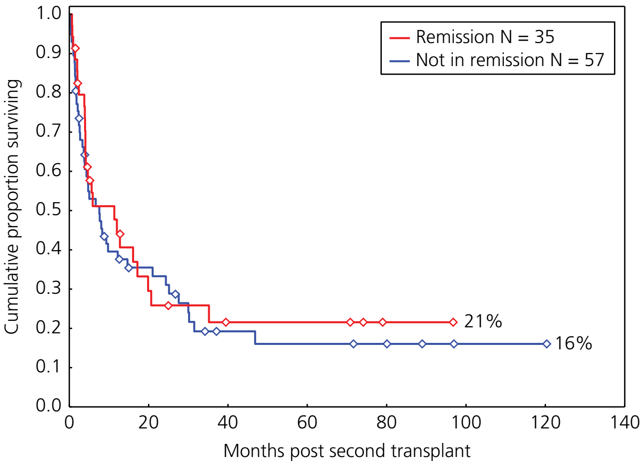

Figure 70.2 Progression-free survival for patients receiving second allogeneic hematopoietic transplants who had achieved a remission with salvage chemotherapy (light curve), or who had low bulk disease but were not in remission (dark curve).

Donor availability is a major issue after unrelated donors or umbilical cord blood (UCB) transplants. A second transplant from the same donor is not an option for UCB recipients. There has been speculation that the use of a different donor or cell source may improve the outcome of a second transplant. A different donor would likely express different minor histocompatibility antigens and might produce a potentially more effective GVL effect. A recent study by Christopeit et al. reported that there was no benefit in using a different donor for a second transplant, and the use of related donors had better outcomes than with unrelated donors [93]. An alternative strategy would be to perform an HLA non-identical cord blood or haploidentical transplant with the goal of potentially inducing a more potent immunologic effect. Large clinical trials of this approach have not been reported.

The major problem is that most patients with relapse post-transplant will not achieve even a transient remission with salvage chemotherapy. One approach is to administer intensive myelosuppressive therapy to cytoreduce the leukemia and achieve a hypoplastic bone marrow., The patient could then receive a reduced-intensity allogeneic transplant at the nadir of their blood counts before the leukemia could regenerate. This approach has been effective in some studies for initial transplants in patients with relapsed AML [94], but has not been specifically studied in patients with relapse post-transplant.

Acute lymphoblastic leukemia

Of all the major leukemias, acute lymphoblastic leukemia (ALL) appears least affected by GVL effects [6,95]. Detection of MRD before or after transplantation identifies patients at higher risk for relapse [96]. The European Bone Marrow Transplantation Group recently reported that median survival of less than 6 months' prognosis is related to advanced disease at initial transplant, time to relapse post-transplant, and percentage blasts in the peripheral blood [97]. DLIs have generally been ineffective; the CIBMTR analyzed 44 patients receiving DLI. Two of 15 patients receiving no other treatment achieved CR and the OS was 13% at 3 years [98]. Novel cell therapy approaches, such as the use of redirected T cells reactive against CD19 by a genetically modified chimeric antigen receptor, are under investigation [99]. Second allogeneic transplants have been performed in selected patients, who usually achieved a subsequent remission. Approximately 20–25% achieved durable remissions [90,100].

Lymphoid malignancies

There has been limited experience with the treatment of relapse post-transplant in patients with lymphomas, Hodgkin disease, chronic lymphocytic leukemia (CLL), and multiple myeloma.

Allogeneic transplants have produced a high rate of durable remission in follicular lymphoma and mantle cell lymphoma. Several groups have reported responses in selected patients relapsing after T-cell replete allogeneic transplants. Relapse has been more frequent in patients receiving alemtuzumab-based regimens [101]. There was a low rate of treatment-related mortality, but a high risk of relapse; DLIs were effective in achieving durable CR in over 50% of patients with follicular lymphoma or mantle cell lymphoma. It was reported that 19 of 24 patients with relapse after this regimen achieved a complete remission and 64% were disease free at 4 years post-transplant [102,103]. There were poor results with DLI for the treatment of aggressive lymphomas [101].

Allogeneic transplantation with non-myeloablative or reduced-intensity conditioning is frequently used for patients with advanced CLL [104]. Relapse or persistent disease is a common problem, particularly after an alemtuzumab-containing preparative regimen [105]. Khouri et al. reported that 20 of 43 patients had durable responses to immune manipulation including immunosuppression withdrawal, rituximab treatment, or DLI [106]. Several patients had durable remissions after rituximab treatment alone, suggesting augmentation of GVL effects. Cell therapy using anti-CD19 chimeric antigen receptor T cells are promising against a broad range of lymphoid malignancies and chronic lymphocytic leukemia [107].

Allogeneic hematopoietic transplantation is frequently performed for Hodgkin disease that has recurred following autologous hematopoietic transplantation. Selected patients with recurrent disease responded to DLI [108,109]; Anderlini et al. reported response in four of nine patients [109]. There is recent interest in combining DLI with brentuximab vedotin [110].

Multiple myeloma

Allogeneic hematopoietic transplantation has been studied in patients with multiple myeloma. There remains a high risk of relapse, but a fraction of patients may achieve a durable CR. A graft-versus-myeloma effect of allogeneic hematopoietic transplantation exists against multiple myeloma [111]. DLIs can produce CRs [112]. Most responders also developed GVHD [113]. Beitinjaneh et al. reported on 23 patients with multiple myeloma who received DLI after allogeneic hematopoietic transplantation. Eight patients received preemptive DLI for residual disease and 15 received DLI for the treatment of recurrent or progressive disease. Five patients (22%) achieved a complete or a very good partial response, and eight patients (34%) had stable disease after the DLI. Patients who received DLI for residual disease had a higher response rate than those treated for disease progression (50% vs. 7%) and a longer median survival (28.3 vs. 7.6 months) [114].

Prevention of relapse

There is major interest in potential interventions that could be performed post-transplant to reduce the risk of recurrent malignancy post-transplant. The approaches under study include maintenance treatment with chemotherapy, hypomethylating agents, immunomodulatory therapies, vaccines, and cellular therapies. These approaches have been most extensively studied in patients with AML.

Interventions could be applied to patients considered at high risk for relapse or in patients with detection of MRD. Hematologic relapse is often preceded by a period with detectable MRD. A preemptive strategy to monitor and treat low bulk disease may improve outcomes compared with waiting for overt relapse to occur, while avoiding exposure of cured patients to treatment-related toxicities.

Prevention is more accurately treatment to eradicate occult residual malignancy after transplant. A proposed intervention must not jeopardize the graft or hematologic/immune reconstitution and have acceptable toxicity. Interventions to prevent relapse have to be implemented early, especially for patients who were not transplanted in CR, given that the median time to relapse post-transplant is only 4 months.

As indicated, azacitidine has direct antileukemia effects and may favorably enhance GVL effects against myeloid malignancies. Azacitidine treatment also causes expansion of T-regulatory cells and inhibits he development of GVHD [115], making it attractive as post-transplant therapy. We conducted a phase I study in 42 relapsed or refractory AML patients who underwent reduced-intensity allogeneic hematopoietic transplantation. The maximum tolerated dose was defined at 32 mg/m^2/day for five consecutive days every 4 weeks for at least four cycles, starting on the sixth week after transplant. The dose-limiting toxicity was thrombocytopenia. We are now performing a prospective randomized trial of high-risk AML/MDS patients who are in remission and medically stable at 1 month post-transplant. Patients are randomized to receive monthly courses of azacitidine for 1 year versus no maintenance therapy (ClinicalTrials.gov identifier: NCT00887068).

As indicated, patients with FLT3-ITD mutations are at high risk for relapse after allogeneic hematopoietic transplantation. A number of novel FLT3 tyrosine kinase inhibitors are being studied for the prevention of relapse in the post-transplant setting [116,117]. No data regarding their tolerability and efficacy are available at present. These trials will need to be attentive to toxicities, including myelosuppression and potential drug interactions [118,119].

Early immunosuppression withdrawal, with or without prophylactic DLI, has been employed with variable results [120]. T cells are mediators of GVL effects. In vivo T-cell depletion with anti-T-cell antibodies has been reported to increase the risk of relapse post-transplant, particularly following reduced-intensity conditioning [10]. One approach to separate GVL from GVHD is to perform an initial T-cell-depleted transplant with in vivo or in vitro T-cell depletion to achieve engraftment without GVHD. A planned prophylactic DLI can be administered approximately 6 weeks later to augment GVL effects. This approach is under study. As discussed above, DLI may be complicated by GVHD, and the efficacy of this approach to prevent relapse has not been investigated in randomized trials [60]. This platform of delayed DLI does allow the evaluation of engineered T-cell infusions to target specifically the leukemia or reduce GVHD. Antigen-specific T cells targeting the malignant cells can be infused. T cells can also be transduced to express herpesvirus thymidine kinase [121] or a modified caspase-9 [122], which can act as a suicide switch; the genetically modified T cells can be eliminated by administration of an otherwise non-toxic activating drug if GVHD or other toxicity occurs [123].

Peptide vaccines may improve the GVL effect with little toxicity. Specific cytolytic T-cell responses may be induced to leukemia-associated antigens that are over- or aberrantly expressed by malignant cells, such as the PR1 peptide in AML [124,125]. Numerous reports have documented disease remissions following the use of peptide vaccines, but controlled trials have not been completed and widespread use of this strategy is not yet available [126,127]. Molldrem et al. are investigating the PR1 peptide both as a myeloid leukemia-associated antigen capable of generating clinically relevant immune responses [19], and also as a potential target of a therapeutic monoclonal antibody [128]. Rezvani et al. [126] conducted a clinical trial investigating HLA-A0201-WT1 peptide vaccine administered in conjunction with PR1 vaccine to leukemia patients and observed an increase in functional WT1-CTLs following vaccination in five of eight patients. Disease regression, as measured by WT1 expression, was correlated with an increase in anti-WT1 and PR-1 cytolytic T cells. Another potential strategy uses donor PR1 peptide vaccination prior to stem-cell harvest, in an attempt to enhance the GVL effect.

Donor-derived NK cells mediate antitumor effects against a broad range of hematologic malignancies and do not appear directly to cause GVHD [129]. Predicted NK cell alloreactivity is associated with a reduced risk of relapse in AML patients following haploidentical transplants [37]. The risk of AML relapse post-transplant was decreased for recipients from related and unrelated donors with group B KIR haplotype [39,130,131]. Adoptive therapy with NK cells can induce remission in patients with advanced AML [132] and has been used to prevent relapse post-chemotherapy [133].

A limitation is the relatively small cell dose possible that can be collected from normal donors by apheresis. We and other investigators are studying ex vivo expansion of NK cells [134] for infusion with allogeneic hematopoietic transplantation.

Conclusions and future directions

Relapse remains the major cause of treatment failure after allogeneic hematopoietic transplantation as treatment for malignancy. Immunomanipulation and donor lymphocyte infusions are effective in many patients with CML and indolent lymphoid malignancies. The occurrence of overt relapse post-transplant has a grim prognosis in acute leukemias, but selected patients may respond to further chemotherapy and a fraction can achieve durable CR with a second hematopoietic transplant or DLI. There is considerable interest in maintenance strategies or preemptive therapy using targeted chemotherapy, immunomodulatory treatments, or antigen-specific immunotherapy. Prospective clinical trials are in progress to evaluate each of these modalities.

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CHAPTER 71

Hematopoietic Cell Transplantation for Thalassemia

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Introduction

The thalassemias refer to a diverse group of hemoglobin disorders characterized by a reduced synthesis of one or more of the globin chains (α, β, γ, δβ, γδβ, δ, and εγδβ) and are the most common monogenic disorders to cause a major public health problem in the world [1]. Globally it is estimated that there are 270 million carriers of hemoglobin disorders, of which 80 million are carriers of β thalassemia. Currently recommended medical therapy of thalassemia consists of lifelong regular blood transfusions to maintain hemoglobin levels between 9 and 10 g/dL together with chelation therapy aimed at reducing iron accumulation as a consequence of chronic transfusions. Desferrioxamine (DFO), the first available chelator, administered as continuous subcutaneous infusion for at least 8–12 h daily has improved life expectancy up to and beyond the second decade of life, and has dramatically improved the quality of life for children with thalassemia. Recently introduced in clinical practice the once-daily oral chelator deferasirox has been shown to remove iron from the liver and the heart with improved compliance, although its efficacy compared to desferrioxamine in the long term has not yet been determined. However, in many countries where the transfusion and the iron chelation treatment is given irregularly, mainly due to the high cost, the majority of patients with homozygous β thalassemia die in childhood, from chronic anemia and its complications and from the organ iron overload.

Hematopoietic cell transplantation (HCT) has been used in attempts at curing thalassemia. The first successful marrow transplant for thalassemia was reported in 1982 by Thomas and his colleagues [2], and the first reports of series of transplants were by Lucarelli et al. [3–5]. Some discussion of the epidemiology, molecular biology, pathophysiology, clinical features, and supportive therapy is necessary in an attempt to define how, when, and for whom to pursue bone marrow transplantation (BMT) for thalassemia.

Epidemiology and etiology

Even though the relationship has not been proven conclusively for β thalassemia, the geographical distribution coincides historically with malarial areas and there is more convincing evidence for β thalassemia and sickle-cell disease. Because of a selective advantage of heterozygotes against the severe form of malaria, the frequencies of β thalassemia are particularly high in the malarial tropical and subtropical regions of the Mediterranean area, the Indian subcontinent, Africa, and Southeast Asia [1]. The prevalence of β-thalassemia carriers is low in people of Northern European origin (1:1000), while in the Northern Mediterranean countries the carrier prevalence varies between 1% and 19%. In the Middle East countries carriers are around 3%, while in central Asia the prevalence is 4–10%. In the Indian subcontinent and Southeast Asia, β thalassemia coexists with hemoglobin E (HbE) in carrier rates that range from 1–40% [6]. Worldwide about 60,000 children with a major thalassemia and 250,000 with a sickle cell disorder are born annually, giving a rate of more than 2.4 affected children per 1000 births [7]. The epidemiology of the disease is changing due to recent population migrations. In Europe, the United States, and Australasia, thalassemia has become an important part of clinical practice.

Molecular and clinical biology

More than 200 mutations affecting the β-globin gene are known to result in a phenotype of β thalassemia. With the exception of the 619 bp deletion, which accounts for 20% of the β thalassemias in Asian Indians [8], the common mutations of β thalassemias are caused by point mutations as single-base substitutions, minor insertions or deletions of a few bases within the gene or its immediate flanking sequences and are classified according to the mechanism by which they affect gene regulation: transcription, RNA processing, or RNA translation [9]. These genetic defects lead to a variable reduction in β-globin production ranging from a minimal deficit (β^+ thalassemia) to complete absence (β^0 thalassemia).

The pathophysiology of β thalassemia relates to a quantifiable deficiency of functional β globin, which leads to an imbalanced globin chain production and an excess of α-globin chains [1,10]. The latter are not able to form viable tetramers and instead precipitate in the red cell precursors forming inclusion bodies that cause mechanical damage and the premature destruction of red cell precursors in the bone marrow leading to ineffective erythropoiesis. The red cells that survive to reach the peripheral circulation are prematurely destroyed in the spleen, which becomes enlarged, eventually leading to hypersplenism. Thus, anemia in β thalassemia results from a combination of ineffective erythropoiesis, peripheral hemolysis, and an overall reduction in hemoglobin synthesis leading to intense proliferation and expansion of the bone marrow with the resulting skeletal deformities. These secondary complications of bone disease,

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splenomegaly, endocrine and cardiac damage can be related to the severity of anemia and the iron loading that results from increased gastrointestinal iron absorption and blood transfusions.

Clinical features

The clinical manifestations of β thalassemia are extremely diverse, ranging from the transfusion-dependent state of thalassemia major to the slightly less severe transfusion-dependent state of thalassemia intermedia or to the asymptomatic state of thalassemia trait. β thalassemia is usually inherited in an autosomal recessive manner, although a few dominantly inherited forms can also occur. The most severe form of disease is characterized by the complete absence of β-globin production and results from the inheritance of two β^0-thalassemia alleles, homozygous or compound heterozygous states. These combinations usually result in β-thalassemia major and the patients present within 6 months of life with severe anemia, and if not treated with regular blood transfusions, die within the first 2 years. The red cells are microcytic with marked anisochromasia. The bone marrow shows marked erythroid hyperplasia, and the serum ferritin level is elevated. The hemoglobin pattern of patients with β-thalassemia major consists of a variable increase in HbF, which can account for 8% to 90% of the total hemoglobin concentration and show a variable quantity of HbA_2 up to 6% [1]. In children and young adults the expansion of hyperplastic bone marrow results in skeletal deformities and bone disease. Because of chronic anemia and iron overload, endocrinopathies (such as hypopituitarism, hypothyroidism, hypoparathyroidism, diabetes mellitus), cardiomiopathy, and testicular and ovarian failure become common as the child with thalassemia grows older [1,11]. Chronic lung disease and pulmonary hypertension are other complications that are being increasingly recognized in older β-thalassemic patients, and are thought to be related to the increased tendency for thrombosis and small pulmonary emboli [12].

Non-transplant approaches

The current conventional treatment of β-thalassemia major consists of lifelong regular blood transfusions combined with daily available chelation therapy. In 1964, Wolman reported that patients maintained at a higher baseline hemoglobin level had less severe complications and were in better health than those maintained at lower hemoglobin levels [13]. Stimulated by this observation, Piomelli and his colleagues studied a transfusion regimen designed to prevent the baseline hemoglobin from falling below 10 g/dL. When this hypertransfusion regimen was instituted in infancy and rigorously implemented, cardiomegaly and bone malformation were greatly reduced, although normalization of growth was not achieved and the development of hypersplenism was not prevented [14]. The target hemoglobin level of 10 g/dL was designed to depress endogenous erythropoiesis and thereby avoid marrow hypertrophy with accompanying marrow space extension. This objective was partially achieved, but complete suppression of endogenous red cell production requires that hemoglobin be maintained at levels greater than 13 g/dL. There are major obstacles in the way of achieving such levels. Raising the baseline hemoglobin level from 10 g/dL to 11 g/dL requires a 20% increase in the red cell transfusion requirement [15], and there are many reasons for minimizing transfusion in patients with thalassemia.

Hypertransfusion regimens have a major impact on iron traffic and distribution in patients with severe hemolytic anemia. The complications of iron overload such as endocrine deficiency, hepatic and pancreatic damage, and heart disease are the principal causes of morbidity and mortality in patients with thalassemia receiving this form of therapy. The transfusion of 165 mL of packed red cells per kilogram per year is associated with an annual iron intake of approximately 180 mg/kg [15]. More recently, clinical experience suggested that a moderate transfusion regimen by maintaining a hemoglobin level of 9–10 g/dL throughout life may reduce iron loading in patients with β-thalassemia major without producing excessive expansion of erythropoiesis [16].

Iron accumulation resulting from both repeated blood transfusions and increased gastrointestinal absorption in β-thalassemia major patients may cause serious organ damage and ultimately death. Effective removal of this excess iron can improve survival of these patients. Until the last decade DFO was the only effective iron-chelating drug available in patients with β-thalassemia major. DFO has a strong and selective affinity for iron and does not appear to have any major side effects. However, DFO displays poor bioavailability when given orally and has a rapid plasma clearance. Therefore, DFO is efficient only when administered parenterally over several hours in order to keep a constant serum level. Since the maintenance of steady plasma levels of this agent is essential for the effective management of iron traffic, it must be continuously administered either intravenously or subcutaneously using a special pump (overnight 8–12-h infusion for 5–7 nights). Intensive long-term chelation with DFO proved beneficial for a large number of patients, as evidenced by the gradual decrease of their serum ferritin and liver iron with improved survival [17].

There are two common reasons for the failure of chelation therapy with DFO. In emerging countries where thalassemia is a substantial public health problem, the high cost of the drug and of the supplies for its administration make it unavailable for most patients. Despite wide availability of DFO in Western countries, some patients are unable to comply sufficiently with the prescribed treatment because the effective chelation is unpleasant and cumbersome, requiring daily subcutaneous or intravenous administration leading to poor compliance with therapy. It was therefore obvious that an orally administered chelator was a pressing need.

Two oral iron chelators are currently available for clinical use. Deferiprone or L1 (DFP) is a bidentate chelator, which forms a chelator/iron complex excreted mainly in the urine. At a standard dose (75 mg/kg/day, in three doses) iron stores may decrease in some patients, remain stable in others, and increase in still others [18]. DFP is approved in Europe and many other countries, often as a second-line treatment for iron overload. In general, DFP is less effective in removing liver iron, although great individual variations occur. Toxic effects include agranulocytosis, neutropenia, and arthropathy, which demand close monitoring. Studies in patients treated with DFP have shown significant improvement in cardiac magnetic resonance imaging, consistent with a reduction in cardiac iron overload and improved cardiac function, in comparison with DFO, supporting a potential cardioprotective role of DFP [19].

Another oral chelator is now available – deferasirox (ICL670), which is a member of a new class of tridentate iron chelators [20]. It is orally bioavailable with terminal elimination half-life between 8 and 16 hours, allowing for once-daily administration. A recent phase III randomized study comparing deferasirox to deferoxamine in pediatric and adult patients with β-thalassemia major receiving regular blood transfusions showed that at 20–30 mg/kg/day, deferasirox can keep most but not all patients in even or negative iron balance in rough equivalence with moderate doses of deferoxamine.

The most common adverse events included rash, gastrointestinal disturbances, and mild non-progressive increases in serum creatinine [21]. While efficacy and limits of DFO therapy in transfusion-dependent β-thalassemia major patients are well known, long-term data on these two new oral chelators in treating iron overload are not yet available and they should be investigated in large-scale prospective randomized studies.

The beneficial effect of high levels of HbF in β thalassemia and sickle cell anemia has been recognized for many years. Pharmacologically, three classes of agents have been shown to be capable of inducing HbF to therapeutic levels: erythropoietins, short-chain fatty acid derivatives, and chemotherapeutic agents. However, the sustained pharmacologic induction of HbF to therapeutic levels in β thalassemia has been disappointing. Despite addressing the effective erythropoiesis, correction of the anemia has been difficult because of suppression of erythropoiesis by the currently available pharmacologic agents.

Gene therapy for β thalassemia requires gene transfer into HSCs using integrating vectors that direct regulated expression of β globin at therapeutic levels. Unlike oncoretroviral vectors, human immunodeficiency virus-based lentiviral (LV) vectors can transduce non-dividing cells, and were shown to stably transmit the human β-globin gene and a large LCR element, resulting in correction of the mouse thalassemia intermedia phenotype, with variable levels of β-globin expression [22]. A recent study reported successful lentiviral β-globin gene transfer associated with RBC transfusion independence in a patient with thalassemia intermedia in France [23]. However, there was the emergence of a partially dominant clonal cell population raising concerns regarding the safety of lentiviral vectors, particularly with regard to genotoxicity.

Programs for prevention of thalassemia are presently based on prospective carrier screening and prenatal diagnosis. Although traditional prenatal diagnosis of β thalassemia has been highly effective in some countries the fact that affected pregnancies must be terminated to avoid the birth of affected children makes it unacceptable to most persons. Recently developed preimplantation genetic diagnosis (PGD) with in vitro fertilization has already become an alternative to traditional prenatal diagnosis, allowing only unaffected pregnancies and avoiding the risk for pregnancy termination [24]. PGD completed with HLA typing allows selection of healthy and compatible embryos in families with affected children with the certainty that a source of stem cells will be available for transplantation [24].

Hematopoietic cell transplantation (HCT)

At present, allogeneic HCT is the only rational therapeutic modality for the eradication of β-thalassemia major. It is a form of gene therapy that uses allogeneic stem cells as vectors for genes essential for normal hematopoiesis. Eventually, the vector may well be autologous stem cells transformed by the insertion of normal genes but there is no indication that this approach will be a clinical option in the foreseeable future. All of the transplant procedures in Pesaro discussed in this chapter were performed with bone marrow and the term "bone marrow transplantation" (BMT) is used for these procedures.

There are no controlled trials of HCT versus medical treatment for thalassemia major and few studies comparing quality of life. Regular blood transfusion and iron chelation have improved both survival and quality of life of patients with thalassemia and have changed a previously fatal disease with early death to a chronic, although progressive, disease compatible with prolonged survival [7,25]. Despite the prolonged life expectancy, a recent study from the UK Thalassaemia Register showed a steady decline in survival starting from the second decade, with fewer than 50% of patients remaining alive beyond 35 years mainly because of poor compliance with chelation therapy [26]. In the developing world where thalassemia is more common, most children die before the age of 20 years because of the unavailability of safe blood products and/or expensive iron-chelating drugs. Two recently developed oral iron chelators could allow better compliance with chelation, and therefore could have a favorable impact on survival of patients with thalassemia. However, even an ideal iron chelator with rigorous adherence only substantially reduces, but does not eliminate, the iron overload of patients on lifelong transfusions. The acute toxicities of transplantation are challenged by the observation that in the developed world patients with thalassemia are well served by medical treatment, and in some countries patients receiving regular blood transfusion and chelation could have a better survival rate [17,27]. Unfortunately, these same patients with increasing age currently experience poor outcome on conventional treatment, even in well-resourced countries with universal access to good medical treatment [11,26].

The first two transplant procedures for the treatment of thalassemia with marrow from matched related donors were performed in December 1981, in Seattle, WA, and in Pesaro, Italy. The Seattle approach was based on the assumption that the risks associated with BMT would be increased by the iron overload and by sensitization to human leukocyte antigens (HLAs) induced by hypertransfusion. Therefore, it was decided that early clinical studies would be conducted in very young patients who had received very few transfusions. On December 3, 1981 a 14-month-old child with β-thalassemia major who had been transfused with a total of 250 mL of packed RBCs received BMT from his HLA-identical sister following conditioning with a single dose of IV demethylbusulfan (5 mg/kg) and cyclophosphamide (200 mg/kg, total dose) in Seattle [2]. The treatment was completely successful.

The Pesaro approach was based on an assessment that restricting transplants to untransfused patients was impracticable. On December 17, 1981 the Pesaro team performed a transplant in a 14-year-old thalassemic patient who had received 150 RBC transfusions, using marrow from his HLA-identical brother [3]. This patient rejected the graft and was the first of an extensive series of transplants for thalassemia that provides most of the data supporting this chapter.

Preparatory regimens

To eradicate disordered marrow and achieve engraftment preparatory regimens have generally included total body irradiation (TBI) in association with high-dose chemotherapy. However, the late effects of TBI are well established and may include growth impairment, cataracts, hypothyroidism, and secondary malignancies [28,29], although the risk of these toxicities has not yet been fully explored for cytotoxic regimens that do not involve TBI. These hazards are particularly objectionable in very young patients with potential for a long life span. Busulfan (BU) is an alternative to TBI [30–33]. BU is an alkylating agent with exquisite specificity for the most primitive precursors of the myeloid–erythroid axis. It has been used in low doses for more than 30 years for the treatment of patients with chronic myeloid leukemia, and its toxicity and effectiveness have been well documented in that setting. Studies in rodents demonstrated that marrow-lethal doses of BU have minor toxicity to the lymphoid system and cause little immunosuppression [34]. Canine studies of transplants between dog leukocyte antigen (DLA) identical littermates demonstrated 50% engraftment after BU alone and 95% engraftment when antithymocyte serum was added to the conditioning regimen [35]. Santos et al. reported the first clinical trials of very high-dose BU in the context of BMT [30]. In these studies, patients with acute myeloid leukemia (AML) received allogeneic marrow transplants after immunosuppression with CY (200 mg/kg over 4 days), and oral BU (16 mg/kg over 4 days) was administered as additional antitumor therapy. Early results with this therapy were encouraging, and in successful attempts to reduce early transplant-related toxicity, Tutschka et al. reduced the CY dose to 120 mg/kg over 2 days [36]. To optimize BU treatment, therapeutic drug monitoring (TDM) of oral BU with dose adjustment is widely practiced but with variable results due to the unreliability of oral BU pharmacokinetic assessment because of erratic intestinal absorption and possible dose loss with emesis. A recently developed IV formulation of BU exhibits less interpatient as well as dose-to-dose variability, leading to more reliable and consistent PK estimations [37]. High BU exposure (area under the concentration versus time curve (AUC) $>1500\,\mu$mol/min) was associated with greater toxicity, whereas low exposure (AUC <900 μmol/min) with graft rejection or relapse [38,39]. Consequently, targeting the therapeutic range of exposure may improve clinical outcomes. We prospectively studied the pharmacokinetics (PK) and clinical outcomes of intravenous busulfan (IV BU) in 71 children with pre-existing liver damage who underwent HCT for thalassemia [40]. IV BU was administered every 6 hours as part of a conditioning regimen with PK-based dose adjustment to target a conservative AUC range (900–1350 μmol/min). We found that the first dose of BU clearance (Cl) was significantly higher than the subsequent daily Cl that remained unchanged in following days. Consequently AUC targeting performance was 59% at the first dose of IV BU. One third of patients required dose escalation based on dose 1 AUC whereas mainly dose reduction was needed in the subsequent days due to a "normalization" of the BU clearance in following days. The fluctuations in drug exposure and doses adjustment followed the pattern of BU Cl. To avoid such large fluctuations in drug exposure and frequent dose readjustments, we suggested targeting the lower range of exposure (900 μmol/min) for the dose adjustment based on dose 1 AUC. This procedure should prevent unexpected high exposures at subsequent doses observed in this patient population. For the subsequent dose adjustment, the target dose is the conventional one (i.e., 1125 μmol/min) because the Cl remained unchanged after dose 1. Busulfan is a highly lipophilic molecule that easily crosses the blood–brain barrier and could cause seizure. Therefore during busulfan treatment antiseizure prophylaxis should be administered.

Further, CY has been a component of most conditioning regimens for transplanting patients with hematologic malignancies. Santos et al. reported on its use in high doses (200 mg/kg over 4 days) as the sole antitumor agent in patients receiving allogeneic transplants for leukemia and demonstrated that it was sufficiently immunosuppressive to permit sustained allogeneic engraftment [41]. Also, CY is most commonly employed for the treatment of lymphoid malignancies and solid tumors, and it has been used as a component of combination chemotherapy for the treatment of acute leukemia. It is not considered a highly effective agent against myeloid malignancies, although single-drug studies are not available in this context. The dose-limiting toxicity of CY is to the heart and not to the marrow. Mice, monkeys, and humans recover hematopoiesis promptly after the highest doses of CY because CY does not eliminate HSCs. Therefore CY alone is not an appropriate conditioning modality for BMT for the treatment of thalassemia because the thalassemic marrow would recover rapidly. On the other hand, BU is an agent that has a good possibility of eradicating a diseased erythron but when used alone is not likely to be sufficiently immunosuppressive to permit sustained allogeneic engraftment. A combination of BU and CY can eradicate the thalassemia and facilitate sustained allogeneic engraftment.

Many of the hematopoietic manifestations of thalassemia resemble those of hematopoietic malignancy. The disease is characterized by extreme marrow hyperplasia with aggressive extension of a rapidly proliferating erythron into intra- and extra-medullary areas not usually occupied by marrow. This results in major bone remodeling together with marked hepatomegaly and splenomegaly. By analogy with the behavior of malignant tissue, it might be hypothesized that this large mass of rapidly proliferating hematopoietic tissue would be more difficult to eradicate than normal hematopoietic tissue, and more likely to recur after transplantation. Although post-transplant thalassemic recurrence is a problem, it occurs in circumstances that differ from those usually observed with leukemic relapse. The most common presentation of leukemic relapse is return of host-type leukemia in the presence of a persisting immune system of donor origin. In contrast, the recurrence of thalassemia usually occurs in the context of a return of host-type immune and hematologic reconstitution. Thus, this event has aspects of both relapse and rejection and it is customary to speak of this phenomenon as rejection.

Stem cell sources

There are three possible sources of stem cells to use for transplantation: bone marrow, peripheral blood, and cord blood. Which stem cell source is best for transplantation for thalassemia? Bone marrow is the most frequently used stem cell source for patients with thalassemia. Currently the use of peripheral blood has become the preferred source of stem cells in most transplant centers especially for hematologic malignancies. In the last decade cord blood-derived hematopoietic stem cells have become more frequently utilized for allogeneic transplantation. There are some pros and cons to each source. Easy donation, harvesting high stem cell dose and faster hematologic recovery are pros to peripheral blood stem cell transplantation (PBSC). However, the risk of both acute and especially chronic GVHD is somewhat higher with PBSC than with bone marrow transplants.

No need for separate donation, easy procurement, low incidence of acute GVHD despite 1 or 2 antigen mismatches are advantages of cord blood transplantation. Drawbacks of cord blood transplantation include longer time to engraft with associated high risk for infection and bleeding complications, and unavailability of the donor cells in case of engraftment failure of cell therapy.

A recent study from the Eurocord cooperative group analyzed the outcome of 33 patients with class 1 or class 2 thalassemia, and 11 with SCD who received cord blood cells from a sibling following BUCYATG or BUFLU regimen [42]. In 17 patients TT was added to the conditioning regimens. Seven (21%) out of 33 patients with thalassemia experienced graft failure. A low percentage of patients experienced either acute (11%) or chronic (6%) GVHD, which is encouraging. The 2-year estimate of event-free survival was 79%. Although transplanted patients were in class 1 and class 2, the incidence of graft rejection was higher than after an HLA-matched sibling bone marrow transplantation. An increased risk of graft failure after cord blood transplantation, compared with that in patients given BMTs, has previously been reported. The 1 log lower cell dose in cord blood grafts, which is a well-known disadvantage, probably has a negative influence on sustained engraftment in recipients with non-malignant diseases.

More recently results of transplantation in 183 patients with thalassemia receiving either PBSC ($n = 87$) or bone marrow ($n = 96$) have been reported [43]. Patients were given GVHD prophylaxis with CSA ($n = 60$) or CSA + MTX ($n = 123$). The incidence of grade 2–4 acute and extensive chronic GVHD was 74% versus 57% and 49% versus 17% in the PBSC and bone marrow arms, respectively. Taken together these data clearly demonstrate that the use of bone marrow should be the first choice for transplantation for patients with thalassemia.

Engraftment

Most patients undergoing transplantation for the treatment of thalassemia have been transfused repeatedly. Experience with BMT for the treatment of aplastic anemia in heavily transfused patients demonstrated that a history of many pre-transplant transfusions increased the probability of graft rejection [44,45]. There is a substantial incidence of graft rejection in thalassemia patients after most preparatory regimens for marrow grafting, and this seems to be related to the stage of disease at the time of transplant. As discussed earlier, in most cases of graft rejection the thalassemic marrow will regrow and subsequent survival will be long (albeit with thalassemia). Occasionally, patients will reject grafts without recurrence of thalassemia. Unless rescued by a second transplant such patients will die from the consequences of marrow aplasia. The treatment of patients after graft rejection will differ depending on whether the rejection is accompanied by regeneration of host-type hematopoiesis or by marrow aplasia. Our experience with this situation is discussed later.

Transplant-related morbidity and mortality

HCT can be a dangerous undertaking. Regimens capable of eradicating a diseased marrow and facilitating persistent engraftment are necessarily toxic, and the consequences of successful allogeneic marrow engraftment include acute and chronic GVHD, syndromes associated with severe immune incompetence. Both prophylaxis against GVHD and methods for its treatment are immunosuppressive. Transplant-associated toxicity may be aggravated by GVHD and by measures aimed at the prevention of this complication. Such toxicity can be categorized either as regimen-related toxicity (RRT) or as GVHD.

In studies of HCT for the treatment of hematologic malignancies, RRT from the preparative regimens has been well described [46,47]. The lungs and liver are the organs most at risk for toxicity induced by TBI and BU while the heart is the main site of CY-induced damage. Increasing patient age, previous exposure to cytotoxic agents and the presence of latent viruses such as hepatitis C and cytomegalovirus adversely influence these toxicities. Patients transplanted for the treatment of thalassemia derive benefit from the fact they are usually young and without prior exposure to cytotoxic agents. However, because of previous intensive transfusion therapy they will have a high probability of carrying harmful viruses and have organ damage induced by extreme iron overload.

Clinical transplant experience

Experience of the first 20 years (1982–2002)

By far the largest body of experience in the treatment of thalassemia with BMT has been accumulated in Pesaro, Italy. From December 1981 to June 2001, 915 patients with homozygous β thalassemia received marrow transplants. The thalassemia-free survival of the entire group of patients (aged 1 through 35 years at the time of the transplantation) is 69%. For 880 patients, the donors were HLA identical (853 siblings, 27 parents), 29 were HLA partially matched relatives, and six were HLA-identical unrelated donors. Most patients were treated with one of two types of regimens: one, used for 547 patients, prescribed BU 14 mg/kg and CY 200 mg/kg and the other used BU with a lower dose of CY for patients believed to be especially susceptible to the toxic complications associated with this high dose of CY.

In early experience, the best results were obtained in younger patients [5,48,49]. Of the first six patients older than 16 years, five died of transplant-related complications (GVHD, infections) within the first 100 days. In view of this experience, early studies concentrated on patients under the age of 17 years.

Children and adolescents. Results in young patients were very encouraging, and in 1990, Lucarelli and his colleagues reported their experience through August 1988 in treating 222 consecutive patients under the age of 16 years [5]. All these patients received HLA-identical marrow, in 10 cases from parents and in the other cases from siblings, after conditioning with regimens containing BU 14 mg/kg and CY 200 mg/kg. One hundred and forty-one donors were heterozygous for β thalassemia and 81 donors were normal homozygotes. Five different regimens were used to prevent acute GVHD.

Analysis of the influence of pre-transplant characteristics on the outcome of transplantation was conducted in 116 patients who were all treated with exactly the same regimen. It was demonstrated that hepatomegaly and portal fibrosis were associated with a significantly reduced probability of survival. In multivariate analysis a history of poor compliance with the chelation regimen could not be distinguished from hepatomegaly as a predictor of survival and rejection-free survival. The influence of pre-transplant characteristics on the outcome of transplantation was reexamined in late 1989 [50] by which time 161 patients <17 years of age had been treated with the same regimen. The quality of chelation was characterized as regular when deferoxamine therapy was initiated no later than 18 months after the first transfusion and administered subcutaneously for 8–10 h continuously for at least 5 days each week. The chelation variable was defined as irregular for any deviation from this criterion. The degree of hepatomegaly (greater than or not greater than 2 cm), the presence or absence of portal fibrosis in the pre-transplant liver biopsy, and the quality of chelation (regular or irregular) given through the years before transplant were identified as variables permitting the categorization of patients into three risk classes. Class 1 patients had none of these adverse risk factors, class 3 patients had all three, and class 2 patients had one or two adverse risk factors. This analysis confirmed the prognostic significance for transplant outcome of risk class and the strong influence of the quality of chelation therapy.

By the end of December 2003, 524 patients <17 years of age had received HLA-identical transplants using regimens containing BU 14 mg/kg and CY 200 mg/kg. There were 145 class 1 patients, 333 class 2 patients, and 46 class 3 patients. The probabilities of survival, thalassemia-free survival, rejection and non-rejection mortality for class 1 patients were 90%, 87%, 3%, and 10%, respectively, and for class 2 patients they were 87%, 85%, 3%, and 13%, respectively (Table 71.1). The probabilities of survival, thalassemia-free survival, rejection and non-rejection mortality for the 46 patients in class 3 were 53%, 51%, 7%, and 42%, respectively, calculated at

Table 71.1 Pesaro experience of BMT for thalassemia from HLA-matched related donors between May 1985 and December 2003

	Class 1	Class 2	Class 3 (age <17 years) (since 1997)	Adult (age >17 years) (since 1997)
Patients, *n*	145	333	33	15
Protocols	6	6	26	26
Survival %	90	87	93	65
Thalassemia-free survival %	87	85	85	65
Death %	10	13	6	28
Rejection %	3	3	8	7

Table 71.2 Current treatment protocols

	Pre-conditioning	Conditioning	GVHD prophylaxis
Class 1 and Class 2 age >4 years	None	IV Bu Cy200	CSA/MP/short MTX
Class 1 and Class 2 age ≤ 4 years	None	IV Bu TT10 Cy200	CSA/MP/short MTX
Class 3 age ≤17 years	d-35 to -11 Az, Hu; d-16 to -11 Flu 150	IV Bu TT10 Cy160	CSA/MP/short MTX
Adults (age >17 years) Class 2–3	d-35 to -11 Az, Hu; d-16 to -11 Flu 150	IV Bu TT10 Cy 90	CSA/MP/short MTX

Az, azathioprine; Hu, hydroxyurea; Flu, fludarabine; Bu, busulfan; Cy, cyclophosphamide; TT, thiotepa; CSA, cyclosporine; MP, methylprednisolone; MTX, methotrexate.

15 years. In an attempt to improve results in class 3 patients, new treatment regimens consisting of BU 14 mg/kg and reduced doses of CY (160 or 120 mg/kg) were used between September 1989 and April 1997. These regimens improved the probability of survival in class 3 younger (age <17 years) patients from 53% to 79%, but were associated with an increase of rejection probability from 7% to 30%, probably due to inadequate immunosuppression and failure to eradicate the massive erythroid hyperplasia characteristic of these patients [51].

In April 1997, a new preparative regimen (Protocol 26) was adopted for class 3 patients <17 years of age in an attempt to decrease the 30% rejection rate without a corresponding increase in reappearance of the thalassemic clone. This protocol involved an intensified preparation with 3 mg/kg of azathioprine and 30 mg/kg hydroxyurea daily from day −45 from the transplant, fludarabine 20 mg/m2 from day −17 through day −13, followed by the administration of BU 14 mg/kg total dose and CY 160 mg/kg total dose. Prophylaxis against GVHD consisted of CY 7.5 mg/kg IV on day 1, methotrexate 10 mg/m^2 IV on days 3 and 6, and cyclosporine (CSP) 5 mg/kg IV daily from day −2 through day 5 reduced to 3 mg/kg/d IV until oral administration of 12.5 mg/kg/day could be tolerated. Continuous 24-h infusions of 40 mg/kg of deferoxamine via central venous catheter were initiated on day −45 and a regimen of hypertransfusion with RBCs was used to keep the level of hemoglobin between 14 and 15 g/dL. During this time interval patients received growth factors, granulocyte colony-stimulating factor (G-CSF) and erythropoietin twice weekly to maintain stem cell proliferation in the face of hypertransfusion, thereby facilitating the effect of the hydroxyurea. Protocol 26 was devised on the assumption that preparation with BU 14 mg/kg and CY 160 mg/kg was inadequate to eradicate thalassemic hematopoiesis in class 3 patients <17 years of age. Preliminary results of transplantation in 33 such patients treated with Protocol 26 showed the probabilities of survival, thalassemia-free survival, rejection and non-rejection mortality were 93%, 85%, 8% and 6%, respectively (Table 71.1 [52]). Interestingly this regimen improved the Kaplan–Meier probability of thalassemia-free survival for patients with class 3 thalassemia aged younger than 17 years at the time of the transplantation from 58% to 85% together with a reduction of the probability of rejection from 30% to 8% as compared with previous preparative regimens. The immunosuppressive qualities of fludarabine may have contributed to the effectiveness of this regimen.

Adult patients. Adult thalassemia patients have more advanced disease with both disease- and treatment-related organ complications mainly due to prolonged exposure to iron overload. From November 1988 through September 1996, 107 patients older than 17 years received transplants from matched donors. The median age for this population was 20 years (range 17–35 years). Eighteen patients were in class 2 and these received BU 14 mg/kg and CY 200 mg/kg. The other patients were in class 3 and received BU 14–16 mg/kg and CY 120–160 mg/kg. The probability of survival, thalassemia-free survival, rejection and non-rejection mortality for the entire group of these patients were 66%, 62%, 4%, and 37%, respectively [53,54]. For the 18 class 2 adults, the probabilities of survival and of thalassemia-free survival were both 76%.

From April 1997 all adult patients were prepared for transplantation according to Protocol 26 with the only difference that the dose of cyclophosphamide was reduced to 90 mg/kg total dose. The probability of survival, thalassemia-free survival, rejection and non-rejection mortality in 15 high-risk group patients were 65%, 65%, 7%, and 28%, respectively [55].

Current experience

In 2003, the authors of this chapter and two other transplant physicians moved from Pesaro to Rome where we started a new Transplant Program in the International Center for Transplantation in Thalassemia and Sickle Cell Anemia at the Mediterranean Institute of Hematology. Whereas the Pesaro experience was predominantly carried out on an Italian patient population, the Rome experience was characterized by two features: the patients were ethnically very heterogeneous, and the vast majority of these patients was not regularly transfused/chelated, or highly sensitized due to receiving RBC transfusions without the use of leukodepletion filters. Therefore, these patients were likely to have a higher risk of graft rejection as a result of sensitization to minor histocompatibility antigens. Taking these characteristics into consideration, we have modified the treatment protocols for transplantation of such patients (Table 71.2). As of September 2012, 24 class 1, 34 class 2, and 47 class 3 patients, younger than 17 years, received a first HSCT from an HLA-matched sibling donor at our institution. Initially 26 class 3 patients treated with original Protocol 26 had an elevated (15%) rejection probability. Therefore we modified this protocol in 2007 by increasing the dose of fludarabine in preconditioning and adding thiotepa to the conditioning regimen to further decrease rejection in this group of patients. Since then we have treated 20 class 3 patients with the modified Protocol 26. The preliminary results showed that none of the patients had rejection and thalassemia-free survival was 84% [56]. Figures 71.1, 71.2 and 71.3 show the probability of survival, thalassemia-free survival, rejection, and TRM for class 1, class 2, and class 3 patients, with a median follow-up of 65 months (range, 1–97 months) for surviving patients.

Outcome of haploidentical transplantation for thalassemia will be discussed later.

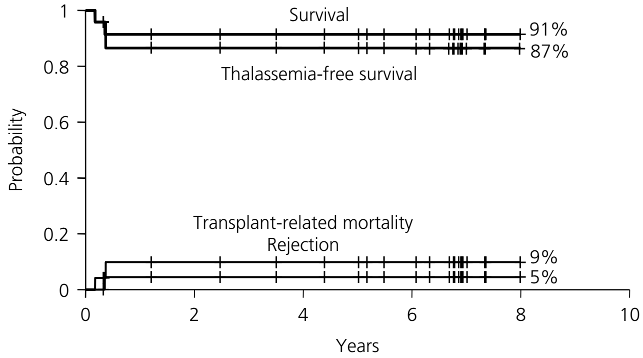

Figure 71.1 Estimates of survival, thalassemia-free survival, non-rejection mortality and rejection for 24 class 1 patients with median age of 3 years (range, 2–6 years) and treated with oral (14 mg/kg) or IV busulfan (weight-based dosing), cyclophosphamide 200 mg/kg and ± thiotepa 10 mg/kg.

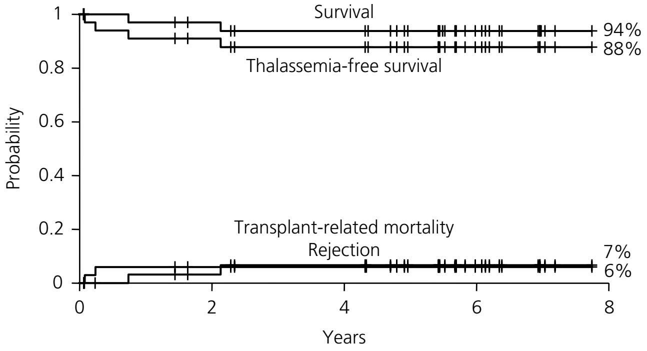

Figure 71.2 Estimates of survival, thalassemia-free survival, non-rejection mortality and rejection for 34 class 2 patients with median age of 7 years (range, 2–16 years) who were treated with oral (14 mg/kg) or IV busulfan (weight-based dosing), cyclophosphamide 200 mg/kg and ± thiotepa 10 mg/kg.

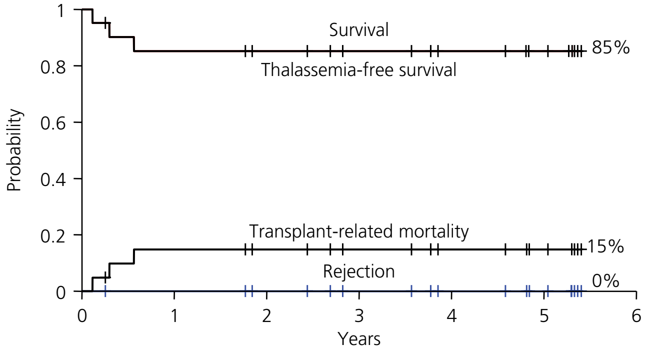

Figure 71.3 Estimates of survival, thalassemia-free survival, non-rejection mortality and rejection for 20 class 3 patients with median age of 9 years (range, 6–16 years) who were treated with the modified Protocol 26 (preconditioning with Hu30Az3Flu150 and conditioning with IV BuTT10Cy160).

Graft failure or rejection. Patients with engraftment failure and without functioning marrow have a bleak prospect because an early second transplant with a second course of conditioning is usually not a reasonable option. However, occasionally patients have late graft failure without thalassemia recurrence and in this situation second transplant attempts with intensive conditioning may provide the only treatment option to offer a chance of prolonged survival.

Patients who reject their grafts and have a return of host hematopoiesis do not have an urgent need for second transplants, and such interventions can be delayed until the toxic effects of the conditioning regimen for the first transplants have resolved. At least a year should be allowed to elapse between the first and second transplant. Our historical experience with second transplants showed a low thalassemia-free survival rate and a high rate of graft rejection [57].

In 2003, we devised a new treatment protocol for second transplants in patients with thalassemia aimed at reducing the high graft rejection rate and increasing disease-free survival. Sixteen patients with thalassemia recurrence following rejection of the first graft, with a median age of 9 years (range, 4–20 years), were given second transplants using bone marrow or PBSC [58]. All but two patients received stem cells from the same donor. The sustained engraftment rate was high (94%), with only one patient having graft failure. The rates of survival, thalassemia-free survival, graft failure, and TRM were 79%, 79%, 6%, and 16%, respectively showing that this treatment protocol is highly effective in terms of sustained engraftment and event-free survival.

Graft-versus-host disease. Graft-versus-host disease (GVHD) is one of the principal causes of morbidity and mortality after allogeneic HCT. In a recent prospective study we have evaluated GVHD in children underwent BMT for thalassemia [59]. All patients were given cyclosporine (CSA), methylprednisolone and a modified short course of MTX (CYorMTX on day +1, and MTX on days +3 and +6) as GVHD prophylaxis. The incidence of grade II–IV and III–IV acute GVHD was 35% and 9%, respectively. In multivariate analysis, high CD3+ (HR 4.6; 95% CI 1.4–14.7; $p = 0.010$) and CD34+ (HR 4.3; 95% CI 1.4–12.7; $p = 0.011$) cell doses were associated with grade II–IV aGVHD. Using a quartile cutoff point analysis we found a minimum threshold for $CD3^+$ (38×10^6/kg) and $CD34^+$ ($4 + 10^6$/kg) cells above which the incidence of grade II–IV aGVHD is significantly increased. In an attempt to decrease the incidence of acute GVHD patients receiving $CD3^+$ and/or $CD34^+$ cell doses above these thresholds are now given day +11 MTX. Cumulative incidence of extensive cGVHD was 10%.

Mixed chimerism (MC) after BMT in thalassemia. Ablation of all host HSCs is usually considered necessary to establish conditions for stable and complete marrow engraftment of donor stem cells [complete chimerism (CC)]. However, we have observed that MC is not unusual in transplanted thalassemic patients. Further, MC may be transient (TMC) when it evolves into CC or graft rejection, or persistent MC (PMC) when the coexistence of donor and recipient cells is longer than 2 years, with hemoglobin levels sufficient for a good quality of life without RBC transfusion [60]. When PMC was detected using sensitive techniques [61], it could be demonstrated in all the nucleated hematopoietic cell subpopulations of the marrow and peripheral blood. In informative situations, all circulating mature RBCs were of the same blood group as the donor, even when 70% of burst-forming units – erythroid (BFU-E) were of host origin.

In a group of 335 mostly consecutive patients, all with 2 or more years of post-transplant follow-up, the incidence of MC was 32.2% at

2 months after HCT. This proportion decreased to 10.1% at 2 years after transplantation and remained stable thereafter. Within this group there were 185 class 1 or 2 patients, all treated with the same pretransplant conditioning regimen (BU 14 mg/kg and CY 200 mg/kg). For these patients, the incidence of MC at 2 months after the transplant was 21%, while it was 54.9% in the group of the remaining 150 patients who were treated with the same dose of BU and a reduced dose CY (120 mg/kg or 160 mg/kg). The incidence of PMC 2 years after transplant was 10.1% in each of the two groups of patients, although they were not homogeneous for the preparative regimen. Graft rejection did not occur in any of the 227 patients with CC early after HCT whereas it occurred in 35 of the 108 patients (32.4%) with MC detected during the first 2 months after HCT.

Rejection was related to the number of residual host cells (RHCs) present in patients with TMC early after HCT [61]. A classification scheme for MC was adopted [61] in which the presence of <10% RHCs was designated as level 1, RHCs between 10% and 25% was categorized as level 2, and RHCs >25% was categorized as level 3. Of the 108 patients who at 2 months after the transplant were MC, 61 patients had level 1, 27 patients had level 2, and 20 patients had level 3 chimerism. In the group of level 1 patients, 57% eventually developed CC, 13% rejected the transplant, and 30% became PMCs. In the group of level 2 patients, 44% became CC, 41% rejected the transplant, and 15% remained PMCs. Eighteen of the 20 patients with level 3 MC rejected the transplant, while only two developed PMC.

Thirty-four ex-thalassemic patients after transplant have maintained PMC for 2–13 years and are transfusion-independent with hemoglobin levels ranging from 8.3 g/dL to 14.7 g/dL [61]. Fifteen of these patients showed level 3 PMC with large numbers of recipient precursor cells. In some patients, the proportion of donor engrafted cells decreased to levels usually predictive of complete rejection. One of these 15 patients who received BMT in 1998 showed progressive increasing of RHCs on annual follow-ups, although her hemoglobin level maintained between 10 and 11 g/dL until late 2005, when the patient became anemic with the percentage of donor cells of 5%. The patient became again transfusion-dependent and a successful second transplant from the same donor was performed in May 2006 [62]. This latest rejection with autologous reconstitution demands lifelong monitoring of the chimerism status in patients with PMC. It is not known why MC is transient in some patients, and remains persistent in others. Clones of regulatory T cells may develop in some patients with PMC and establish a state of reciprocal tolerance. Further studies are needed of the mechanisms underlying this state of tolerance to permit the design of protocols that will produce it predictably. The discovery of this phenomenon has potential importance for the future use of gene therapy and for the adoption of less toxic conditioning regimens as preparation for transplantation. These findings suggest that patients with a high risk of rejection can be identified soon after transplantation permitting the application of preemptive treatment strategies aimed at preventing rejection.

HCT from alternative donors

HCT from alternative related donors. A major limitation to the successful use of transplantation is the limited number of HLA-matched related donors. Between 30% and 35% of patients with hemoglobinopathies have such a donor. The curative potential of transplantation for hemoglobinopathies has encouraged the use of hematopoietic cell donations from donors other than HLA-matched sibling donors (alternative donors).

Our previous experience with transplantation in thalassemia using alternative related donors (either phenotypically identical or 1- to 3-antigen mismatched donors) was not successful. Outcomes were poor with high rates of rejection and GVHD, and low thalassemia-free survival rates [63]. The results of this study clearly showed that the conditioning regimens used were not adequately myeloablative and immunosuppressive to ensure a high sustained engraftment rate, prevent severe GVHD, and promote a high thalassemia-free survival probability. Therefore, we adopted a new intensified treatment protocol (Pc 26.1) for alternative donor transplantation in thalassemia. The Pc 26.1 was initially designed for second transplants [59], but the very encouraging results led us to also apply it in patients receiving transplants from donors who are not HLA-matched siblings. Sixteen thalassemia patients with median age of 9.6 years (range, 1.4–24 years) who received BMT from phenotypically HLA-identical ($n = 11$) or 1-antigen mismatched relatives ($n = 5$) were treated according to this new protocol [64]. All patients had sustained engraftment. One patient died due to chronic GVHD-related causes. The probability of overall survival, thalassemia-free survival, and transplant-related mortality were 94% (95% CI, 63–99%), 94% (95% CI, 63–99%) and 6% (95% CI, 1–26%), respectively. These data showed that the novel treatment protocol Pc 26.1 effectively and safely prevented graft rejection and ensured a high thalassemia-free survival rate in patients who received BMT from related donors who were not HLA-matched siblings. Importantly, the use of phenotypically matched or 1-antigen mismatched related donors resulted in outcomes similar to the use of matched sibling donor grafts. Our data support the use of related HLA-phenotypically matched or 1-antigen mismatched donors as an acceptable alternative to matched unrelated donors in patients with thalassemia.

Haploidentical stem cell transplantation. To further extend the donor availability for those patients who lacked a matched suitable donor, in 2002 we started a haploidentical T-cell-depleted HCT program for thalassemia. To be eligible for this study patient should lack an HLA-matched either related or unrelated donor or have red-cell alloimmunization rendering transfusion impossible or life-threatening. Twenty-two patients with transfusion-dependent thalassemia and a median age of 7 years (range, 3–14 years) were given stem cell grafts from haploidentical mothers ($n = 20$) or brothers ($n = 2$) between 2002 and 2008. All of the patients received an intensified preparative regimen consisting of 3 mg/kg azathioprine and 60 mg/kg hydroxyurea daily from day −59 from the transplant, and fludarabine (FLU) 30 mg/m^2 from day −17 through day −13, aimed at reducing hyperplastic bone marrow and gradually increasing immunosuppression to avoid unacceptable peritransplant drug toxicity. During this time frame, the patients also received hypertransfusion to suppress endogenous erythropoiesis, and granulocyte colony-stimulating factor and erythropoietin to maintain stem cell proliferation, thus facilitating the effect of hydroxyurea. Conditioning regimen consisted of BU14/thiotepa (TT)10/CY200 and antithymocyte globulin (ATG-Fresenius). Patients received cyclosporine for GVHD prophylaxis for the first 2 months after transplantation. As stem cell source, eight patients received T-cell-depleted PBSC (CD34$^+$ immunoselected) and CD3$^+$/CD19$^+$ immunodepleted bone marrow stem cells. The last 14 patients received CD34$^+$ selected peripheral and bone marrow stem cells, using the CliniMACS® procedure. Median infused cell doses were: CD34$^+$ 15.2 × 10^6/kg, CD3$^+$ 1.8 × 10^5/kg, and CD19$^+$ 0.27 × 10^6/kg in the first eight patients, and

$CD34^+$ 14.2×10^6/kg, $CD3^+$ 2×10^5/kg, and $CD19^+$ 0.19×10^6/kg in the last 14 patients. Overall, six patients rejected their grafts, two patients died of Epstein–Barr virus-related cerebral lymphoma or cytomegalovirus pneumonia, and 14 patients are alive and cured of thalassemia. The rates of survival, thalassemia-free survival, rejection and non-rejection mortality were 90%, 61%, 29%, and 14%, respectively [65]. Although younger patient age and megadose of $CD34^+$ cell-containing grafts, immune reconstitution, especially $CD4^+$ cell recovery, was delayed beyond 1 year in most patients [66]. These data showed the feasibility of haploidentical stem cell transplantation for patients with thalassemia and, therefore, this procedure should be considered for those younger patients who lack a suitable HLA-matched related or unrelated donor.

HCT from unrelated donors. Results of HCT from unrelated donors for the treatment of malignant disease have improved steadily, mainly due to the introduction of high-resolution molecular techniques for histocompatibility testing and improvements in the management of post-transplant complications. These results have stimulated the use of HCT from unrelated donors for the treatment of hemoglobinopathies. The Italian Cooperative Group for Bone Marrow Transplantation has recently reported results obtained in 32 thalassemic patients (aged 2–28 years) who received bone marrow from unrelated donors [67]. Four patients were in class 1, 11 were in class 2, and 17 were in class 3 of risk. The first 4 patients received Bu and CY, and the remaining 28 patients BU 14 mg/kg, TT 10 mg/kg and CY. The dose of CY was 200 mg/kg for class 1 and class 2 patients and it was 120 mg/kg or 160 mg/kg for class 3 patients aged >16 years old. Prophylaxis against GVHD included CSP and a short course of MTX. The probability of thalassemia-free survival and non-rejection mortality was 66% and 25%, respectively for the entire group of patients. Although the difference was not significant, five of the six patients who died belonged to class 3. The incidences of grade II–IV acute and chronic GVHD were 41% and 25%, respectively. Patients who shared at least one extended haplotype had less acute or chronic GVHD and better survival. Updating the observations of these centers to 44 patients, the probabilities of survival, thalassemia-free survival, rejection and non-rejection mortality are 79%, 68%, 12%, and 23%, respectively. These data are encouraging and show that the results of HCT from well-selected unrelated donors might be comparable to those of HLA-identical sibling transplants, especially in patients with less advanced disease.

Recently this group has reported data on 27 adult thalassemia patients (aged 17–37 years) transplanted from unrelated donors selected by high-resolution HLA molecular typing [68]. Fifteen patients received BU14 mg/kg, thiotepa 10 mg/kg, and CY 160 mg/kg (nine patients) or CY 120 mg/kg (six patients). In view of the high mortality in these patients the subsequent 11 patients were given BU 14 mg/kg and CY 120 mg/kg. GVHD prophylaxis consisted of CSP and a short course of MTX. Three patients were also given antithymocyte globulin. The probability of survival, thalassemia-free survival, transplant-related mortality and rejection were 70%, 70%, 30%, and 4%, respectively. The incidence of grade II–IV acute and chronic GVHD was 37% and 27%, respectively. Although limited these data show that unrelated marrow transplantation not only in younger patients but also in adult patients from well-selected donors may offer a success rate similar to those obtained from HLA-identical sibling transplantation. The main limitation of the experience with unrelated BMT for thalassemia is that, using such stringent criteria, only about one third of thalassemia patients who started the search found a suitable donor in a median time of 3–4 months. One possibility to increase the donor pool could be adopting less stringent criteria of HLA matching for donor selection allowing one or two allelic disparities, but the results of such transplants remain to be determined.

HCT from unrelated cord blood. Hematopoietic stem cell transplantation from an unrelated cord blood (UCB) donor is, now, standard practice for the treatment of hematologic malignancies. The number of UCB transplants has increased dramatically, because several studies have demonstrated that results from HLA-mismatched UCB transplants were comparable to those from HLA-matched unrelated bone marrow transplants in children [69]. Advantages such as faster availability, tolerance of one to two HLA mismatches, and low incidence of acute GVHD made UCB transplants attractive for patients with some non-malignant diseases [70]. In a recent Eurocord/CIBMTR/NY Blood Center study researchers evaluated the outcome of 51 patients with thalassemia ($n = 35$, median age 4 years) and sickle cell disease ($n = 16$, median age 6 years) treated with unrelated cord blood [71]. Seven patients received 6/6 matched (HLA-A, -B antigen, and -DRB1 allele levels), 18 patients 5/6 matched, 25 patients 4/6 matched, and one patient 3/6 matched cord blood grafts following myeloablative ($n = 39$) or reduced-intensity ($n = 12$) conditioning regimens. Most patients were also given ATG or alemtuzumab before transplantation. Overall and disease-free survival for patients with thalassemia and sickle cell disease were 62% and 94%, and 21% and 50%, respectively. However, a more recent study from China reported very encouraging results. Thirty-five thalassemia patients with median age of 5.5 years (range, 1.2–14) were given 6/6 matched ($n = 8$), 5/6 matched ($n = 16$), 4/6 matched ($n = 27$), and 3/6 matched cord blood grafts following myeloablative conditioning with BUCYATG [72]. The incidence of rejection was 17%, and it was 82% for grade II–IV acute GVHD and 35% for chronic GVHD. The probability of overall survival and thalassemia-free survival was 88.3% and 78.9%, respectively. Genetic homogeneity in the Chinese population might explain the differences in outcomes between the two studies.

Non-myeloablative or reduced-intensity hematopoietic stem cell transplantation. Unlike hematologic malignancies where mixed chimerism may predict relapse, in non-malignant disorders, the development of stable mixed chimerism has a potential for ameliorative effect that was well documented for β-thalassemia major and sickle cell anemia [61,73]. It appears that when stable mixed chimerism is established, even a minority of donor cells is sufficient to overcome an underlying genetic defect. These observations have provided the rationale for using non-myeloablative or reduced preparatory regimens in patients with thalassemia or sickle cell anemia, which may reduce the toxicity of transplantation especially in patients with advanced disease. Non-myeloablative conditioning followed by allogeneic transplantation has been tested in limited clinical series that rely upon immunosuppressive rather than ablative preparatory regimens to facilitate donor cells engraftment. Recently, seven patients (aged 3–20 years) with sickle cell anemia or thalassemia and given stem cell transplantation following a preparative regimen consisting of fludarabine, low-dose (i.e., 200 cGy) total body irradiation, and antithymocyte globuline (in two patients) have been reported [74]. Although transplant-related toxicity was minimal, and many patients had transient engraftment, all eventually rejected their donor graft. In a recent study, results of reduced-intensity HCT using peripheral blood stem cells from siblings or unrelated matched donors after preparatory regimen with fludarabine (180 mg/m^2 total

Table 71.3 Transplants for thalassemia. Reports from centers other than Pesaro

Center [Ref.]	Patients	Survival	Disease-free survival
Pescara, Italy [77]	115	0.89	0.86
Cagliari, Italy [78]	37	0.88	0.88
USA [79]	68	0.94/0.81*	0.81/0.57*
UK [80]	54	0.95	0.82
Tehran, Iran [81]	60	0.83	0.73
Vellore, India [82]	50	0.76	0.68
Malaysia [83]	28	0.86	0.75
Hong Kong [84]	44	0.86	0.82
Thailand [85]	28	0.92	0.82
Greece [86]	75	0.96	0.92
France [87]	108	0.87	0.69

*By risk category, survival = best risk/worst risk.

dose), IV busulfan (6.4 mg/kg total dose) and an equine antithymocyte globuline (160 mg/kg total dose) in patients with SCA (three patients) or β thalassemia (one patient), were not encouraging: only one out of four patients had sustained engraftment [75].

These results indicate that stable donor engraftment after non-myeloablative or reduced-intensity HSC transplantation is difficult to achieve among immunocompetent patients with hemoglobinopathies. Therefore, patients with thalassemia or sickle cell disease need more intensive myeloablative conditioning regimens because graft failure in these patients is not a negligible problem even when a conventional myeloablative preparative regimen is administered [4,5,67]. These conditioning regimens should be studied in selected higher risk thalassemia patients with advanced disease along with organ damage who might have a higher transplant-related toxicity after conventional myloablative transplantation.

Experience at other transplant centers

At present more than 3000 BMTs for thalassemia have been performed worldwide with the probability of overall and thalassemia-free survival greater than 90% and 80%, respectively in low/intermediate risk patients [76]. Table 71.3 reports the outcome of transplantation from different countries [77–87]. In Italy the Pescara group has been particularly active, reporting 115 patients transplanted through 2006 [77].

Management of the ex-thalassemic patient after HCT

After transplantation, ex-thalassemic patients still carry the clinical complications acquired during years of transfusion and chelation therapy. Among the issues requiring long-term management in such patients are iron overload, chronic hepatitis, liver fibrosis, and endocrine dysfunction.

There is no reason to expect that BMT will eliminate the excess iron acquired during years of thalassemia, since spontaneous iron elimination occurs very slowly. In our experience, serum ferritin and transferrin saturation spontaneously return to normal levels only in class 1 patients [88]. Persisting tissue iron overload can cause significant morbidity and mortality similar to that seen in hereditary hemochromatosis [89]. Thus iron removal is indicated in all transplanted thalassemic patients who have evidence of persisting iron overload, and initiating a sequence of phlebotomies or restarting chelation with deferoxamine at 18 months post-HCT achieves this best.

Excess iron can be completely removed from the body and a body iron content within the normal range can be achieved. The necessary duration of treatment is directly correlated with the magnitude of the iron overload, and ranges from a few months to several years [90]. In most ex-thalassemics, reduction or normalization of the iron pool results in marked improvement in serum levels of liver enzymes and in the histological activity index [91]. The serum aminotransferases and the histological activity index normalized after iron depletion in about half of the patients who were seropositive for hepatitis C virus (HCV), suggesting that iron is a cofactor of HCV for liver disease [91]. Ex-thalassemics with early cardiac involvement, characterized by systolic and/or diastolic dysfunction, show complete regression of these subclinical cardiac abnormalities after iron depletion [92].

Infection with HCV is common in thalassemic patients, particularly in those transfused before second-generation enzyme-linked immunosorbent assay (ELISA) tests became available for detecting HCV in donated blood. In thalassemia, liver damage due to HCV infection is exacerbated by iron overload, and liver disease is a recognized cause of mortality and morbidity. In fact, analysis of the natural history of liver fibrosis following BMT for thalassemia showed that iron overload and hepatitis C infection are independent risk factors for liver fibrosis progression, and their concomitant presence results in a striking increase in risk [93].All the donors in this series of patients were HCV seronegative. After HCT, approximately 10–15% of HCV-infected patients become HCV seronegative [94]. This may reflect complete elimination of the virus, but we cannot be sure of this. However, seropositivity has not returned in these patients. The chronic risk of conditions associated with HCV infection, such as chronic hepatitis, cirrhosis, and hepatocellular carcinoma, persists in the remaining patients. The precise risk of developing one of these complications in ex-thalassemics is uncertain. Transplanted thalassemics have a long, probably normal life expectancy and mild chronic liver disease has to be considered in this perspective. Chronic HCV infection and transplant-related complications are probably the only factors that may limit survival in ex-thalassemics. Thus, avoidance of progression of liver damage to cirrhosis must be a primary goal. Treatment for chronic hepatitis C has improved over the last two decades. Present standard therapy is peginterferon alfa/ribaverin for adults and children without thalassemia [95]. Concern about ribavirin-induced hemolysis and worsening of the anemia and iron overload limit the use of this therapy in patients with thalassemia. Ex-thalassemics with complete donor chimerism and chronic hepatitis C will experience a similar spectrum of side effects to those of patients without thalassemia. Therefore ex-thalassemics should be offered treatment with the peginterferon alfa/ribaverin combination after they have completed their iron depletion program.

It is well known that growth failure and endocrine dysfunction are common in thalassemia major patients treated by conventional treatment. While the endocrine dysfunction is usually due to iron overload as a result of chronic transfusions, in growth failure lifelong desferrioxamine treatment also plays a negative role in these patients. Although the role of busulfan-containing regimens on growth velocity after transplant remains controversial, the negative effect of this regimen on gonadal damage is well documented. Age at the time of transplant in β-thalassemia patients is an important predictor of growth. In fact, children receiving transplant before 8 years of age showed a normal growth rate, while older children, class 3 patients, and patients who developed chronic GVHD had impaired growth [96]. Gonadal damage is a common side effect of busulfan- cyclophosphamide conditioning. Indeed, approximately one third of boys and two thirds of girls failed to spontaneously enter puberty following transplant [97]. Nevertheless, some patients can restore their fertility after transplant which is supported by our observations of nine successful pregnancies and six spontaneous paternity in our patients (unpublished data). These data demonstrate that patients exposed to BUCY regimen are not inevitably infertile.

One of the major late complications of BMT is chronic GVHD, which is the principal cause of morbidity and non-relapse mortality. Most of our patients developed a limited form of chronic GVHD and the probability of a moderate or severe form was only 8% and 2%, respectively [59,98].

With the increasing number of transplant survivors there is a risk for secondary malignancies. Patients who received BMT for β thalassemia and sickle cell disease in Pesaro had a low incidence (1.3%) of malignancies (unpublished data). The type of malignancies consisted of three early and one late non-Hodgkin lymphomas, four solid tumors (squamous cell carcinoma, Kaposi sarcoma, melanoma, and colon cancer, respectively), and two acute myeloid leukemias. Six of these patients are alive and well.

The role of HCT in the treatment of thalassemia

Hematopoietic cell transplantation is the only treatment able to definitively cure thalassemia. The results of transplantation from HLA-identical family members are clear. Class 1 patients have a very high probability of cure with a very low early and late morbidity and mortality. There is no reason to deny these patients the advantages of a life free from daily tedious, expensive, and uncomfortable therapy. We still do not know the probability that a patient receiving conventional therapy will deteriorate into a worse risk category, but the fact is that transplant centers are often confronted with patients in risk classes 2 and 3 who represent failures of conventional treatment. Delaying transplantation until the patient is in a risk category beyond class 1 substantially reduces the probability of transplant success and jeopardizes the reversibility of liver and cardiac damage. We therefore believe that all patients with β thalassemia who have HLA-identical related donors should be transplanted as soon as possible. Patients without such donors who have well-selected unrelated donors should also be considered for HCT.

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CHAPTER 72

Hematopoietic Cell Transplantation for Sickle Cell Disease

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Introduction

Sickle cell disease (SCD) is caused by a single point mutation in codon 6 of the β-globin chain. This mutation directs an amino acid substitution of valine for glutamic acid, which promotes the formation of long hemoglobin polymers under hypoxic conditions [1–3]. This propensity for polymer formation deforms the red blood cell (RBC) and causes significant alterations in red cell integrity, rheologic properties, and life span [4]. It is probably directly or indirectly responsible for the vasculopathy that forms in virtually all body organs. This heritable disorder of hemoglobin affects one in every 375 African Americans in the United States. Worldwide, nearly 300,000 infants are born annually with SCD, the majority in Africa [5]. In the United States, 2000 new births occur annually [6].

Ever since the initial report in 1984 of successful hematopoietic cell transplantation (HCT) in a child who had acute myelogenous leukemia and SCD who was cured of both, hematologists and transplantation physicians have struggled with how to make this curative therapy available to a wider group of affected individuals [7]. During the intervening years, a better understanding of the natural history of SCD has emerged, and views about who might benefit most from this intensive procedure have evolved to include more, not fewer, individuals [8]. However, this appreciation of the severity of SCD has not translated into greater utilization of HCT, particularly in children. This has occurred, in part, due to improved survival to adulthood among children identified by newborn screening programs and with the broader administration of hydroxyurea in children and adults to mitigate the frequency of vaso-occlusive clinical events. Of interest, the utility of HCT was questioned in the recent *Evidence-Based Management of Sickle Cell Disease* treatise published in 2014 by the National Heart, Lung, and Blood Institute in which the expert panel concluded that "additional research is still needed that addresses the potential risks of this therapy (e.g., failure of engraftment and chronic graft-versus-host disease) before HCT can become a widely used therapy" [9]. However, the median survival among adults with SCD has not changed significantly over the past three decades [10]. Thus, the curative potential of HCT for SCD remains an important therapeutic option for the indications of stroke, recurrent vaso-occlusive events, and other clinically significant complications that impact life span and quality of life in the long term. Ultimately, as most patients who might otherwise be suitable candidates for HCT lack a sibling donor, the most important progress must occur in the arena of alternative donor HCT for SCD, which remains in a nascent phase of development. Thus, this chapter concludes with reference to ongoing clinical trials that might expand HCT to include more of those who might benefit.

Epidemiology and etiology

Although first reported in the medical literature in 1910 as a disorder characterized by its peculiar alteration in RBC morphology, SCD was recognized many generations earlier in Western Africa, where it was called by various local names that reflected its most common feature, which is recurrent pain. Thus, well-established tribal names for the disorder including "The Ga *Chwechweechwe*, the Fante *Nwiiwii*, the Ewe *Nuiduidui*, the Akan *Ahotutuo* all reflect the onomatopoeia of the repetitive gnawing pains characteristic of sickle cell crisis" [11]. The path of discovering the molecular determinant of SCD focused on the most likely suspect, hemoglobin, the most abundant protein in the misshapen RBC. In 1949, Pauling showed that the sickle RBC contained hemoglobin that had an altered electrophoretic mobility, and reasoned that SCD must be a disorder of hemoglobin [1]. Shortly thereafter, Neel and his colleagues showed that RBCs from patients with SCD could be induced to sickle, and also showed that this phenomenon was inherited as an autosomal recessive disorder [12]. Finally, in 1957, Ingram showed that the cause of SCD was an amino acid substitution of valine for glutamic acid at the β-6 position of the globin chain, which subsequently was determined to follow a single base pair change in the coding DNA [3].

Based upon estimates, it appears that the sickle mutation independently originated from regional populations in Africa and Saudi Arabia approximately 70,000–150,000 years ago [13]. It is generally believed that the persistence of the sickle gene in the African and Mediterranean regions follows from its conferring a reduced risk of infection and mortality from infection by *Plasmodium falciparum*, the malaria parasite [14]. However, infants and young children with sickle cell anemia (homozygous HbSS) in particular are at high risk of dying from malaria, which accounts in part for the significant early mortality of sickle cell anemia in certain regions of Africa [14]. Nonetheless, protection from malaria appears to be conferred in a dose-dependent manner relating to the HbS content in the RBC, with those having sickle cell anemia conferring the greatest natural resistance to malaria infection.

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Table 72.1 Birth prevalence of β-globin gene variants in a California newborn population, by ethnicity, 1990–1996

Ethnicity	Gene variant					
	Hb SS	*Hb AS*	*Hb S*-β^0-**thalassemia**	*Hb SC*	*Hb SD*	*Hb SE*
Asian	0/207,551	1/1,336	0/207,551	0/207,551	0/207,551	0/207,551
Asian Indian	0/15,843	1/725	1/15,843	0/15,843	0/15,843	0/15,843
Black	1/700	1/14	1/4,056	1/1,297	1/63,885	1/63,885
Hispanic	1/45,622	1/183	1/729,953	1/364,976	1/1,459,900	1/1,459,900
Middle Eastern	0/21,677	1/360	0/21,677	0/21,677	0/21,677	0/21,677
Native American	1/16,529	1/176	0/16,529	0/16,529	0/16,529	0/16,529
White	1/158,127	1/625	1/553,447	0/1,106,895	0/1,106,895	0/1,106,895

Source: Lorey et al. 1996 [16]. Reproduced with permission of John Wiley & Sons, Ltd.

Hb, hemoglobin; Hb S, sickle hemoglobin. Gene variants: *Hb SS*, sickle cell anemia (includes *Hb S*-β^0-thalassemia); *Hb AS*, sickle cell trait; *Hb SC*, sickle hemoglobin C disease; *Hb SD*, sickle hemoglobin D disease; *Hb SE*, sickle hemoglobin E disease.

It is estimated that between 80,000 and 100,000 persons in the United States are affected by SCD [15]. The frequency of the sickling hemoglobin disorders, as illustrated by the experience of the California Newborn Screening Program, is presented in Table 72.1, and indicates that the majority of affected individuals in the United States are of African descent [16]. However, individuals of Mediterranean, Caribbean South and Central American, Arab, and East Indian descent also are at risk for SCD. Together, these disorders also contribute to a larger national and worldwide public health concern, by virtue of the impact of SCD on individuals and healthcare systems. In the United States alone, the medical cost of caring for approximately 80,000 affected individuals exceeds $1 billion [17].

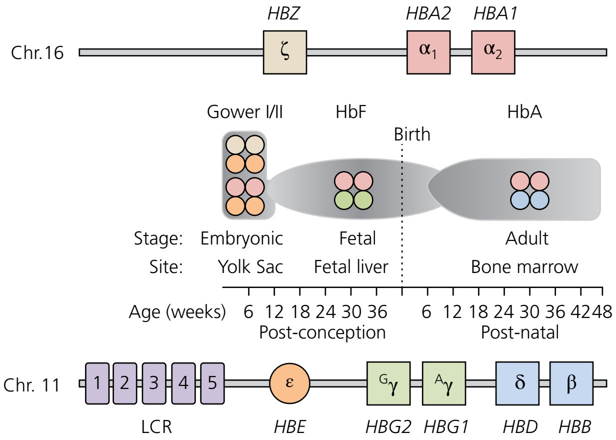

Figure 72.1 Schematic representation of the human α-globin and β-globin loci with temporal expression during ontogeny. The gene order of the α-globin locus on chromosome 16 and the β-globin locus on chromosome 11 is shown with each temporal stage, as indicated on the timeline at bottom. The types of hemoglobin tetramers produced for each indicated site are shown. LCR, locus control region; HbF, fetal hemoglobin; HbA, adult hemoglobin. Source: Wilber et al. 2011 [141]. Reproduced with permission of the American Society of Hematology.

Molecular and cellular biology

The genes that encode the α- and β-globin polypeptides that together account for the hemoglobin tetramer reside on human chromosomes 16 and 11, respectively [18]. Expression of the α- and β-globin proteins is coordinately regulated to avoid polypeptide chain imbalance, the proximate cause of ineffective erythropoiesis in profound inborn examples of chain imbalance such as thalassemia major. The globin loci contain arrays of genes that are expressed sequentially during the course of erythroid ontogeny, and this regulation includes a developmental switch at the transcriptional level of control, which coincides with an alteration in the site of erythropoiesis during embryonic development and in RBC morphology, common characteristics of erythropoiesis in vertebrate animals (Figure 72.1). With regard to SCD, the most clinically important developmental switch occurs during the transition from fetal γ globin to adult β globin at birth, at which time the antisickling fetal hemoglobin is replaced by the polymer-prone HbS, and the clinical features of SCD begin to emerge.

Elucidating the molecular mechanism of the γ to β globin switch has been the focus of intense investigation and a better understanding of the control of fetal hemoglobin expression was recently elucidated. There is general agreement that γ-globin transcription is silenced in erythroid progenitor cells developing in the postnatal marrow, and the presence of a transcriptional repressor was long suspected, but until recently, elusive. Genome-wide association studies performed in individuals with and without hemoglobin disorders discovered two loci outside the globin locus located on chromosome 2 (BCL11A) and chromosome 6 (HBS1L-MYB) that accounted for a significant fraction of individual variation in HbF level [19–21]. BCL11A is a transcription repressor, and when its level of expression was decreased in human primary adult erythroid progenitor cells, HbF expression levels were increased [22]. When BCL11A expression was eliminated by homologous recombination in mice, physiologic silencing of murine embryonic and transgenic humanγ-globin genes was impaired [23]. In addition, BCL11A binds to sites in the human β-globin locus specifically deleted in hereditary persistence of fetal hemoglobin (HPFH) syndromes [24,25]. Thus, it is very likely that BCL11A coordinates the hemoglobin switch, perhaps by participating in a multiprotein complex. It is very clear that the abrogation of this switch with persistent γ-globin transcription has an ameliorative effect on the clinical expression of SCD as occurs when HPFH is co-inherited with SCD. As a consequence of these recent discoveries, targeting the γ to β molecular switch by pharmacologic discovery to elicit persistent HbF expression is under development [26]. In addition, replacement gene therapy to repress BCL11A expression or to express γ globin or other antisickling globins in gene delivery systems that ensure long-term expression for a potent and durable antisickling effect are nearing clinical phase I testing [27].

In the absence of an antisickling hemoglobin, there is a strong propensity for sickle hemoglobin polymerization, and consequently vaso-occlusion, which is the clinical hallmark of this disorder. The phenomenon of vaso-occlusion originates in sickle hemoglobin, which, in the deoxygenated state, tends to form polymers that deform the RBC and diminish its biochemical and structural integrity. While the causal relationship between sickle hemoglobin and vaso-occlusion is not disputed, the contributing factors and sequence of events that ultimately impair the flow of blood in both small and large blood vessels are complex but are being elucidated. The deoxygenated sickle polymer induces cellular potassium and water efflux, caused both by acidification and cellular swelling, which activates the potassium–chloride co-transport efflux pathway [28], and by cellular membrane distortion, which, with the accompanying transient calcium influx, activates the calcium-dependent Gardos channel pathway of potassium and water efflux [29]. The resulting cellular dehydration increases the cell density and sickle hemoglobin concentration, thereby promoting sickle hemoglobin polymerization. The clinical influence of these cellular conditions, as defined by the percentage of circulating dense RBC, is expressed by a strong association with complications such as skin ulcers and priapism [30].

Sickle hemoglobin polymerization also induces perturbations in erythrocyte cellular membranes, which promote adhesive interactions with vascular endothelial cells (reviewed in [31]). These interactions impact factors that control the vascular tone of blood vessels, and generally elicit vasoconstriction. Other cellular blood components are affected by these events, importantly granulocytes and activated platelets that interact with adhesive sickle erythrocytes and endothelial cells. Reticulocytes released prematurely from marrow also express membrane proteins that promote erythrocyte–endothelial cell interactions [32]. These cellular interactions ultimately generate the release of cytokines that promote an inflammatory response and amplify the cascade of vaso-occlusion and tissue injury. None of these factors alone can explain vaso-occlusion, although they may contribute singly or in a combinatorial manner. As a result, the cause and severity of vaso-occlusion may vary from event to event, and from individual to individual.

The recurrent ischemic/hypoxic insults that occur with intravascular sickling cause chronic organ injury. One of the signaling molecules associated with hypoxia is adenosine, and this signaling molecule is expressed at high levels during vaso-occlusive episodes [33]. Under normal physiologic conditions, an ischemic/hypoxic event generates ATP and thereby adenosine, which promotes the production of 2,3 bisphosphate glycerate (2,3-BPG) by signaling through an erythroid-specific stimulatory G-protein receptor, ADORA2B. Upon its accumulation in the RBC under physiologic conditions, 2,3-BPG causes the oxygen-hemoglobin dissociation curve to shift rightward and thus it facilitates oxygen delivery in a hypoxic environment. However, the liberation of adenosine under these conditions in sickle cell anemia elicits a pathophysiologic response, as it promotes deoxygenation of sickle hemoglobin, promotes sickle hemoglobin polymerization and thus vaso-occlusion. Recent studies by Xia and colleagues showed that 2,3-BPG levels are elevated in SCD, and in a murine sickle model, this effect can be abrogated by exposure to theophylline and other ADORA2B receptor antagonists or by the administration of PEG-adenosine deaminase, a pegylated enzyme that lowers adenosine levels [34]. It was demonstrated that by lowering the adenosine concentration in a mouse model of SCD, it was possible also to reduce 2,3-BPG, increase oxygen-binding capacity, and attenuate intravascular sickling. Thus a novel pathway for developing new drugs that reduce sickle polymerization and vaso-occlusion is under study.

Another consequence of sickle hemoglobin polymerization is hemolysis, and considerable evidence supports the notion that the most important consequence of hemolysis is vasoconstriction and vascular remodeling. This is due to impaired nitric oxide (NO) bioavailability, which appears to be the central feature of endothelial dysfunction [35]. There is resistance to replacement of NO by exogenous NO donors such as sodium nitroprusside and nitroglycerin, and this resistance is correlated with plasma hemoglobin levels [36]. Intravascular hemolysis releases hemoglobin and erythrocyte arginase into blood plasma, where the former scavenges NO, and the latter depletes plasma of L-arginine, the obligate substrate for NO synthase; together, these processes severely reduce NO bioavailability (Figure 72.2) [37]. Ordinarily, the gas exchange reaction of NO with oxyhemoglobin is inhibited by the diffusion constraints created by an

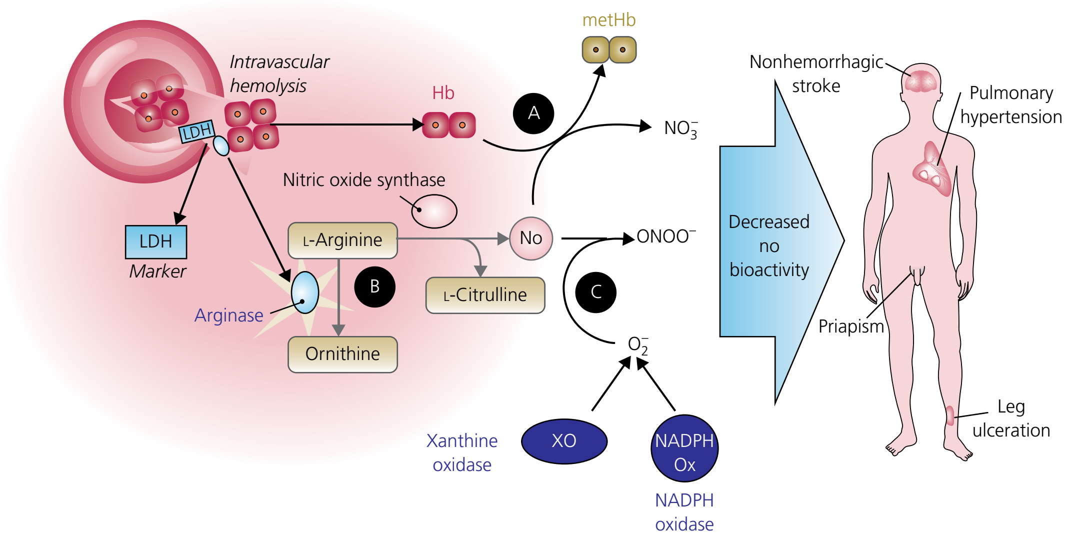

Figure 72.2 Intravascular hemolysis reduces nitric oxide bioactivity. Nitric oxide is produced by isoforms of nitric oxide (NO) synthase, using the substrate L-arginine. Intravascular hemolysis simultaneously releases hemoglobin, arginase, and lactate dehydrogenase (LDH) from red cells into blood plasma. Cell-free plasma hemoglobin stochiometrically inactivates NO, generating methemoglobin and inert nitrate (A). Plasma arginase consumes plasma L-arginine to ornithine, depleting its availability for NO production (B). LDH also released from the red cell into blood serum serves as a surrogate marker for the magnitude of hemoglobin and arginase release. NO is also consumed by reactions with reactive oxygen species produced by the high levels of xanthine oxidase activity and NADPH oxidase activity seen in sickle cell disease, producing oxygen radicals like peroxynitrite (ONOO-) (C). The resulting decreased NO bioactivity in sickle cell disease is associated with pulmonary hypertension and other clinical complications such as priapism. Source: Kato et al. 2007 [142]. Reproduced with permission of Elsevier.

intact RBC membrane. However, intravascular oxyhemoglobin abrogates this constraint and causes a 1000-fold increased rate of NO destruction [38]. An important consequence of this intravascular cascade is pulmonary hypertension, a clinical condition in adults with SCD that is associated with a 37% 6-year mortality in adults (see "Clinical description" later) [39]. The relationship of NO scavenging to vascular constriction and pulmonary hypertension has been questioned in vigorous commentary on this topic [40,41]. An opposing view is that pulmonary hypertension in SCD is rare and is a consequence of pulmonary embolization or in situ thrombosis that accompanies endothelial dysfunction and leukocyte activation in SCD. This argument was strengthened by clinical observations that hydroxyurea administration, which diminishes hemolysis, does not appear to protect from pulmonary hypertension [42], and in a trial of inhalational NO to replete blood levels that did not show an impact on the duration or severity of painful vaso-occlusive episodes [43].

Endothelin-1 is a potent pulmonary vasoconstrictor that acts as a counterpart to NO. Thus, in contrast to NO, its level is increased in pulmonary hypertension. It is also induced by hypoxia-induced factor-1α (HIF-1α), a factor that is expressed by endothelial cells under hypoxic conditions. A novel mechanism of HIF-1α induction and thereby endothelin-1 overexpression involves placental growth factor (PlGF), a recently discovered participant unique to sickle cell disease in particular and hemolytic anemias more generally [44]. PlGF is expressed in the marrow by erythroid cells and its expression helps drive the stress erythropoiesis of severe anemia. In a murine model of PlGF overexpression, endothelin-1 was induced and pulmonary hypertension was observed. In blood samples from 123 patients with SCD, PlGF levels were eightfold higher compared to healthy controls, and were associated with an elevated tricuspid regurgitation velocity (TRV). Thus it appears that NO depletion by hemolysis and other properties related to the exuberant erythropoietic response to severe anemia promote endothelin-1 overexpression and pulmonary hypertension in SCD.

Platelet activation is another important contributor to vaso-occlusion in SCD [45]. NO synthesized by the endothelium and platelets promotes vasodilation, but inhibits platelet activation, platelet aggregation, and endothelial adhesion in a normal vascular environment. However, this process is abrogated in SCD, in part because NO is depleted. Platelet activation is linked to hemolytic rate, suggesting that free hemoglobin may also trigger platelet activation by blocking the inhibitory effect of NO on platelet function [46]. Of interest, there appears to be an association between the severity of pulmonary hypertension and platelet activation, which then might help explain the underlying pathophysiology of this condition, which includes increased vascular tone, vascular proliferation, and *in situ* thrombosis, all of which may be precipitated by hemolysis-associated NO depletion [47]. A similar pathophysiology occurs in the cerebral arteries after stroke in SCD, which also includes intimal proliferation and arterial thrombosis. Thus, a common thread in the development of sickle vasculopathy has been suggested by these observations in disparate target organs, which is characterized by vasoconstriction, intimal proliferation with smooth muscle hyperplasia, and thrombosis *in situ*, even though the clinical manifestations of vascular injury in these organs remain quite distinct.

Clinical description

A comprehensive discussion of all the clinical complications of SCD is beyond the scope of this chapter; however, this brief description supports the notion that SCD is a serious, life-threatening hematologic disorder that significantly reduces the quality of life for most individuals and is associated with, on average, a 30-year decrement in life span [48]. These clinical observations provided the rationale to conduct clinical trials of HCT as a curative intervention for SCD, with the judgment that the risks inherent to HCT are balanced by the considerable difficulties of living with SCD.

Pain

The most common feature of SCD is pain, which varies by frequency and intensity both within and between individuals and sickle genotypes [49]. Febrile illnesses, dehydration, stress, and exposure to extreme temperatures can trigger a painful episode, but more typically an inciting cause is not apparent. Risk factors associated with frequent severe painful episodes, defined as ≥3 episodes per year that were observed in 20% of 381 children with SCD monitored prospectively, included older age, α-thalassemia trait, lowered LDH, and elevated TRV in a recent investigation [50]. Investigations of the frequency and severity of pain episodes by use of a daily diary have shifted perceptions about the spectrum of pain in SCD. The Pain in Sickle Cell Epidemiology Study (PiSCES) studied the longitudinal frequency of pain in a large cohort of adults who entered regular reports of pain in a daily diary for up to 6 months [51]. Patients experienced pain on 54.5% of days but reported an unplanned visit to a healthcare facility for pain treatment on 3.5% of days. Almost one third of patients had pain nearly every day. These observations, reported in children and adults, strongly suggest that painful episodes in patients with SCD occur frequently and are, for the most part, managed at home. These observations also strongly suggest that the clinical impact of pain is much broader than suggested by the Cooperative Study of Sickle Cell Disease (CSSCD), in which painful episodes, defined more narrowly as lasting longer than 2 hours and requiring clinical attention in a medical setting, occurred on average once annually in adults with HbSS, although a small group (5.2%) of individuals had more than 10 painful episodes per year. Thus, pain frequency, as measured by health utilization, vastly underestimates pain frequency and severity. A more accurate view is that painful episodes are frequent and can be characterized by a continuum of frequency and severity, with management at home in contrast to treatment in a healthcare facility at the extremes. Although the administration of hydroxyurea caused a statistically significant reduction in pain intensity and analgesic use, this effect was small (the proportion of days with analgesic use was 0.44 versus 0.40 in placebo and hydroxyurea groups, respectively; $p = 0.0006$) [52]. Thus, frequent painful episodes, particularly when unresponsive to supportive therapy, have been employed as an indication for HCT due to a significantly diminished quality of life.

Pulmonary complications

Acute chest syndrome and pulmonary hypertension

Pulmonary complications, including acute chest syndrome (ACS), pulmonary hypertension, and pulmonary fibrosis, conservatively have been linked as a cause of death in 20–30% of patients with SCD. The most frequent and clinically dramatic expression of pulmonary injury in SCD is ACS, a clinical triad of fever, chest pain, or other respiratory symptoms, and the appearance of a new radiographic infiltrate.

The etiology of ACS is multifactorial, but as often as not, an etiologic agent can be identified if rigorously pursued [53]. In childhood, most episodes are associated with infection, while in adults, ACS generally follows acute painful episodes. Secretory phospholipase A_2 is an enzyme that cleaves fatty acids, and this enzyme may be a biomarker for impending ACS [54]. The release of free fatty acids and the proinflammatory arachidonic acid in the lungs likely contributes to the pathogenesis of ACS, especially in cases of pulmonary fat embolism. Transfusions can prevent ACS when administered during a painful episode accompanied by an elevated serum secretory phospholipase A_2 level, although the feasibility of administering a therapeutic transfusion in a narrow time period based upon an elevated phospholipase A_2 level has been brought into question [55]. For purposes of prognosis, children who experience episodes of ACS early are more likely to experience recurrent episodes, which, if not associated with a higher risk of early mortality, certainly portend morbidity that makes interventions such as hydroxyurea and HCT worthy of consideration. In the long term, adult patients in the CSCCD who had recurrent episodes of ACS experienced restrictive pulmonary disease and abnormal diffusing capacity, which are the most frequent abnormalities noted on pulmonary function testing in affected adults [56]. Still, the global effect of SCD is expressed best in the finding that 90% of individuals had abnormal pulmonary function test results.

The prognostic importance of pulmonary hypertension (PH) as a serious complication of SCD has been established, although the frequency of this complication recently has come under scrutiny. However, as a screening tool to identify high-risk patients, several patient series have shown that adults screened by Doppler transthoracic echocardiography who have a tricuspid regurgitant jet velocity (TRV) that exceeds 2.5 m/s carry a high risk of sudden death. In three large screening studies, approximately 30% of patients had a TRV >2.5 m/s and 10% had a TRV >3 m/s. In all the epidemiologic studies performed to date, the risk ratio of early death in adults with a TRV >2.5 m/s ranged from 9.24- to 15.9-fold [57–59]. More recently, a larger cohort of 483 patients was screened in a study called the walk-PHASST ("walk" test for Pulmonary Hypertension and Sickle Cell Disease with Sildenafil Therapy) screening study [60,61]. In this cohort, the TRV ranged from 2.7 to 3 m/sec in 28.5% of the subjects and in 9.1%, the TRV was >3 m/sec. The 2-year mortality also was significantly increased in the patients with elevated TRV values ($p < 0.000006$). Together, these observations are very likely to help identify a subgroup of adult patients for whom HCT may provide a protective and curative effect in the long term, and for whom risk–benefit considerations are balanced.

Recently, the direct causality of pulmonary hypertension to risk of sudden death in SCD has been questioned as not all individuals with an elevated TRV also have pulmonary hypertension, which is classically defined by right heart catheterization as having a pulmonary arterial pressure of 25 mmHg. A much lower prevalence of PH was suggested by a large screening study in France, which evaluated patients with a TRV >2.5 m/sec by right heart catheterization [62]. The study observed that a minority of patients with elevated TRV (25%) also had a pulmonary arterial pressure >25 mmHg as measured directly by catheterization. Thus, more refined combinations of clinical risk factors for PH may be required to accurately identify those at highest risk of early mortality. PH is linked to elevated serum lactate dehydrogenase (released during hemolysis), and elevated N-terminal pro-B-type natriuretic peptide (NT-proBNP), a hormone that is released in response to cardiomyocyte stretch. As noted earlier, hemolysis appears to be mechanistically linked to PH, a notion that is supported by evidence that elevated serum lactate dehydrogenase also is associated with a high risk of early mortality in adults with SCD. Similarly, a study of banked samples from the CSSCD that analyzed the frequency of NT-proBNP levels >160 pg/mL observed elevated levels in 26% of adults, and high levels were also associated with markers of hemolysis that included elevated LDH, low hemoglobin, and elevated serum bilirubin [63]. An NT-proBNP level >160 pg/mL was an independent risk factor of early mortality (RR 6.24; 95% CI 2.9–13.3; $p < 0.0001$). Applying more stringent clinical criteria that included TRV ≥2.8 m/s, a 6-minute walk distance <500 m, and unexplained dyspnea or oxygen desaturation, 65% of 86 patients who fulfilled these criteria had PH by right heart catheterization [39]. Thus, it is very likely that further refinement of these clinical and laboratory screening tools will enable better accuracy of identifying individuals at high risk for PH and who might benefit from HCT. This is critical because interventions that include sildenafil [60] and bosentan [64] have not improved outcomes in SCD patients with PH.

Stroke

The consequence of vaso-occlusion in cerebral arteries is stroke, a leading cause of morbidity and disability in SCD. The risk of stroke in children with SCD in increased by more than 300-fold, making SCD the most common cause of childhood stroke [65]. The majority of strokes occur in patients with homozygous HbS, and many fewer occur in other SCD genotypes. Clinically overt stroke was observed in 11% of SCD patients by 20 years of age, while 27% had subclinical evidence of "silent" cerebral infarction on brain magnetic resonance imaging (MRI) by age 6 and 37% by age 14, which is defined as a radiographic abnormality that is not associated with neurologic symptoms or a clinical event [66,67]. These injuries are associated with poorer global intellectual functioning. Recently, a cross-sectional study in 652 children with SCD evaluated the frequency of acute silent ischemic events and observed an incidence of 47.3 events per 100 patient-years [68]. While these events are potentially transient, the events are chronic and intermittent, suggesting a continuous risk of brain ischemia and functional impairment.

Co-inherited α-thalassemia appears to confer protection from stroke by possibly improving RBC deformability and decreasing hemolysis [69]. There is also increasing recognition about immune regulation and inflammation with regard to human leukocyte antigen (HLA) genes in diseases such as atherosclerosis and aortic aneurysm [70]. There is a predisposition to Moyamoya disease among Japanese children who inherit the class II HLA allele DQB1*0502 [71]. There was also a strong association with stroke in SCD children who inherited this allele [72]. Likewise, unique HLA alleles DPB1*0401 and DPB1*1701 were associated with increased susceptibility to and protection from small cerebral vessel stroke, respectively.

A number of other candidate genes have been studied by epidemiologic methodologies to assess their association with stroke. Recently, a report of elevated levels of platelet-derived growth factor and brain-derived neurotropic factor were observed in children with elevated cerebral blood flow and stroke, suggesting an association [73]. Vascular cell adhesion molecule-1 (VCAM-*1)*, a gene responsible for expression of adhesion molecules regulating the attachment and migration of leukocytes, is upregulated in response to sickle erythrocytes and appears to be involved in the pathophysiology of SCD-related microvascular occlusion [32]. The VCAM-1 (−1594) *C* variant was associated exclusively with small-vessel stroke risk in a cohort from the CSSCD [74]. Other single-nucleotide polymorphisms in linkage disequilibrium may be present at this locus and suggest a stroke "haplotype" [75]. Further evaluation and confirmation in larger prospective studies is required to document their utilization as a predictor of stroke risk in SCD. However, these may offer utility also in screening those who might benefit from HCT before the onset of central nervous system injury.

Therapeutic alternatives to HCT

RBC transfusions and stroke prevention

It is possible to determine the risk for clinical stroke in children with SCD by measuring the flow velocity in the intracranial arteries by a non-invasive method termed transcranial Doppler ultrasonography (TCD) [76]. The risk of stroke is increased when the time-averaged mean of the maximum (TAMM) flow velocity in the distal internal carotid artery or middle cerebral artery (MCA) exceeds a threshold observed in stenosis. The association of increasing TCD TAMM velocity and increasing risk for stroke formed the basis of the primary stroke prevention trial, the Stroke Prevention Trial in Sickle Cell Anemia (STOP) [77]. The STOP trial tested the hypothesis that periodic blood transfusions would lower the risk of stroke compared with the risk while receiving standard care. The STOP trial was halted prematurely when 11 strokes were observed in the standard supportive care arm and only one in the transfusion arm, indicating a 92% relative risk reduction over 2 years of follow-up on stroke risk with chronic transfusion therapy.

To extend these findings, the STOP investigators conducted a second randomized, controlled trial, the Optimizing Primary Stroke Prevention in Sickle Cell Anemia (STOP 2) trial, to determine whether it was possible to limit the duration of transfusion prophylaxis [78]. Children whose Doppler studies normalized after 30 or more months of transfusion in the STOP trial were eligible for enrollment in STOP 2, and were randomized to halt or to continue regular RBC transfusions. Among the 41 children in the transfusion-halted group, high-risk Doppler findings developed in 14 and stroke in two others by a mean duration of 4.5 (standard deviation ± 2.6; range 2.1–10.1) months after the last transfusion. Neither of these events occurred in the 38 children who continued to receive transfusions, so this trial was terminated early. The authors concluded that discontinuation of transfusion for the prevention of stroke in high-risk children with SCD results in a high rate of reversion to abnormal blood flow velocities on TCD and stroke. These conclusions were supported by extended follow-up observations that included additional strokes in six children during the post-trial follow-up period [79]. In addition, the effect of stopping transfusions on developing new or progressive silent cerebral infarctions by MRI was also determined. At the end of the study, 3 of 37 (9.1%) children developed a new brain MRI lesion in the group treated by transfusions, while 11 of 40 (27.5%) in the group that halted transfusions had new lesions [80]. In addition, there was no change in the total number of MRI brain lesions in the transfusion group, while the number of lesions increased from 27 to 45 in the group that halted transfusions. Thus, regular transfusion therapy also protected from subclinical ischemic brain injury.

It is unclear how long transfusion therapy should be continued as a means of preventing stroke in children with SCD, a question that was not formally resolved by the STOP 2 trial. A trial similar in design to the STOP study has completed accrual to determine whether transfusion is warranted on the basis of MRI lesions in clinically asymptomatic children with SCD, was recently published, and showed that regular RBC transfusions significantly reduced the incidence of cerebral infarct recurrence [82]. Among children who develop a clinical stroke, the risk of a second stroke is at least 20%, even with regular transfusion therapy. In a recent prospective study of 40 patients from seven academic centers with stroke who had regular brain MRI imaging performed over 2 years, the rate of a second stroke or a transient ischemic attack was 40% and new or enlarging cerebral infarcts were observed in 45% of patients [83]. Together, these trials have defined a subgroup of children with SCD who appear to benefit from prevention of neurologic injury by receiving regular RBC transfusions. Thus, like those who have stroke, those at risk for stroke and other brain injury are very likely also to be suitable candidates for HCT, as an alternative to long-term RBC transfusions.

Hydroxyurea

Hydroxyurea is a potent inhibitor of ribonucleotide reductase, which also stimulates fetal hemoglobin production, which in turn inhibits the formation of the sickle hemoglobin polymer. The laboratory effect of hydroxyurea in SCD is to raise the HbF fraction, serum hemoglobin, and the red cell mean corpuscular volume [10,84]. Hydroxyurea also decreases the white blood cell, reticulocyte, and platelet counts while increasing NO production [85], decreasing RBC intracellular dehydration, and decreasing red cell adhesiveness to endothelium. Together, these laboratory effects translate into a clinical benefit of decreased rates of pain and ACS events in severely affected adults and children [10,84]. A long-term follow-up of the Multicenter Study of Hydroxyurea (MSH) showed that, at 17.5 years, while 43% of the original cohort had died, the mortality rates among patients who took hydroxyurea continuously for 10 to 15 years compared to those treated less than 5 years were 6.77 and 1.78 per 100-person years, respectively [10]. A clinical benefit also was observed in a pediatric randomized placebo-controlled trial of hydroxyurea (BABY-HUG) in which the pain rate was nearly halved and the rate of dactylitis was reduced fivefold in the hydroxyurea group, with marginally fewer hospitalizations and RBC transfusions as well [86]. Of interest, there was no significant benefit in protection from progressive splenic and renal injury observed in the hydroxyurea group, suggesting that ongoing subclinical vaso-occlusion was not prevented by regular HU use in young children. Nonetheless, the ready availability of hydroxyurea therapy and its favorable safety profile make it an appropriate intervention in most children and adults.

Unfortunately, the beneficial effect of hydroxyurea is neither universal nor curative, as adult patients with SCD die of disease complications while receiving hydroxyurea therapy [10]. In addition, 81 of 225 children in one series had hydroxyurea therapy withdrawn, primarily due to a lack of clinical response or difficulty with adherence, although there were also failures due to stroke ($n = 2$) and the development of a TCD velocity greater than 200 cm/s ($n = 3$) [87]. Investigation of the quality of life showed that hydroxyurea improved some measures of quality of life, including general health perception and pain recall [88]. However, there was no effect of hydroxyurea on other quality of life measures such as physical functioning or physical role. Even though many experienced an average 50% reduction in acute pain episodes, subjects still had frequent acute pain episodes and high levels of chronic pain. Thus, while hydroxyurea is the sole Food and Drug Administration-approved drug for SCD and its use when indicated should be applied more broadly, it does not appear to be a substitute for the curative potential offered by HCT.

To expand the indications for hydroxyurea, prospective studies have focused on the efficacy of hydroxyurea therapy on recurrent stroke risk and on TCD velocities in children with SCD. The effect of hydroxyurea on hemoglobin levels also makes it feasible to conduct phlebotomy to reduce iron overload in patients who have received chronic transfusions to prevent stroke. In one series of 35 patients, secondary stroke prevention and the reduction of iron stores was facilitated by hydroxyurea, although seven patients experienced a second infarctive stroke after transfusions were replaced by hydroxyurea [89]. In another study, after a minimum period of 6 months for stroke prevention by regular transfusions, hydroxyurea reduced the rate of recurrent stroke in children and young adults to 4.6 per 100 patient-years, a rate which is significantly lower compared to untransfused patients [90]. However, the randomized Stroke Without Transfusions Changing to Hydroxyurea (SWiTCH) trial showed that children with stroke treated by hydroxyurea alone had a higher rate of second stroke compared to those managed with regular RBC transfusions (7/67 vs. 0/66, respectively) [91]. While hydroxyurea may reduce the recurrent stroke risk compared to no transfusion therapy, as demonstrated in a large Jamaican cohort [92], it is an inferior method for preventing second stroke compared to regular transfusion therapy and maintaining the HbS fraction <30%.

Table 72.2 Indications of hematopoietic cell transplantation for sickle cell disease

- Patients with sickle cell disease (HbSS or HbSβ^0-thalassemia) less than 16 years of age
- One or more of the following complications:
  - Stroke or central nervous system event lasting longer than 24 hours
  - Impaired neuropsychologic function with abnormal cerebral magnetic resonance imaging and angiography
  - Recurrent acute chest syndrome
  - Stage I or II sickle lung disease
  - Recurrent vaso-occlusive painful episodes or recurrent priapism
  - Sickle nephropathy (glomerular filtration rate 30–50% of predicted normal)
- Other indications to consider:
  - Abnormal transcranial Doppler scan
  - Pulmonary hypertension
  - Silent cerebral infarction

HCT for SCD

Current indications for HCT

In standard practice, HCT for SCD currently is reserved almost exclusively for patients with clinical features that portend a poor outcome or significant sickle-related morbidity, in part due to the toxicity of this intensive therapy. These clinical indications, which were adapted from the multicenter investigation of bone marrow transplantation for SCD, are listed in Table 72.2 [93]. In addition, these criteria have been applied almost exclusively to children, where the risk–benefit ratio is most advantageous in terms of years of life gained among those who survive with sustained engraftment of donor cells. This is evolving, however, as reduced toxicity regimens become established and a larger proportion of children survive to young adulthood where supportive care measures are limited and the risk of sudden death is high. In a small patient series from France that included 15 adults who received myeloablative conditioning regimens, an event-free survival of 93% was observed [94]. For all patients, clinicians must carefully weigh therapeutic alternatives to HCT, with particular attention to safety, efficacy, availability, and the cost of intervention [95,96].

Current results of HCT for SCD

The worldwide experience of transplantation for SCD is summarized in Table 72.3 [93,97–110] and there are approximately 400 published cases. In the collective experiences of these studies, the transition of HCT from an experimental intervention reserved for severely affected patients, to one in which younger children with early signs of sickle-related morbidity are targeted, has been observed. Several series in Europe and North America have reported very similar results after HLA-identical sibling transplantation in which bone marrow is the source of hematopoietic cells [104–106,111]. There have been no randomized trials performed comparing HCT to supportive care such as hydroxyurea. The principal aim of these multicenter phase I–II clinical studies was to define more completely the risks and benefits of this therapy, and to characterize the natural history of those surviving free of SCD. The results of transplantation were best when performed in children with SCD who had HLA-identical sibling donors. Even though many children who received allografts had significant sickle-related complications such as stroke and recurrent episodes of ACS, the disease-free survival varied from 80–85% in several series. In the most recent series, the disease-free survival has improved to approximately 90% (Table 72.3) [97–99]. However, 5–10% of patients died of complications related to transplantation, with graft-versus-host disease (GVHD) and its treatment as the leading cause of death. Most clinical investigations of HCT for SCD have targeted enrollment of children *in lieu* of adults, but the experience of HCT in adults with SCD is growing [94,100,103].

In the multicenter investigation of HCT for SCD, 59 children who ranged in age between 3.3 and 15.9 (median 9.9) years received HLA-identical sibling marrow allografts between September 1991 and April 2000 [112]. Patients received a myeloablative combination of busulfan (BU), cyclophosphamide, and equine antithymocyte globulin (ATG), and most received a combination of methotrexate and cyclosporine after HCT to prevent GVHD. Fifty of 59 children survive disease-free after HCT. The Kaplan–Meier probabilities of survival and disease-free survival are 93% and 85%, respectively (Figure 72.3). A publication from the Center for International Blood and Marrow Transplantation Research reported very similar results. Outcomes after HLA-matched sibling HCT in 67 patients with SCD transplanted between 1989 and 2002 were described [105]. Rates of acute and chronic GVHD were 10% and 22%, respectively. Sixty-four of 67 patients are alive; 5-year probabilities of disease-free and overall survival are 85% and 97%, respectively.

There were several patient series of HCT for SCD in Europe conducted in parallel. Results among 101 patients who received conventional myeloablative HLA-identical sibling HCT in Europe were remarkably similar to North American reports, with an overall survival probability of 88% and disease-free survival of 80% [104]. A French report included 87 consecutive patients treated between November 1988 and December 2004 at 14 centers [110]. The median age was 9.5 years, and the leading indication for HCT was stroke or transient ischemic attack (*n* = 36) and the development of abnormal cerebral arterial flow velocity as detected by TCD (*n* = 8). Five of the 18 patients who did not receive ATG had graft rejection. The rate of graft rejection declined from 22.6% to 2.9% after the addition of ATG to the conditioning regimen (Figure 72.4(a)). The Kaplan–Meier estimate of event-free survival at 5 years was 86.1%. Multivariate analysis showed that the date of transplantation was the only significant risk factor. The 5-year event-free survival rate was 95.3% in patients treated after January 2000, compared with 76.7% in patients treated earlier (Figure 72.4(b)). A recent series of 11 patients treated at a single center in Rome between 2004 and 2009 reported a disease-free survival rate of 90% after HLA-ID sibling bone marrow transplantation [97].

A retrospective review of 24 Belgian patients with SCD analyzed the impact of pretransplant ATG and hydroxyurea on outcome [113].

Table 72.3 Hematopoietic cell transplantation (HCT) for sickle cell disease: published reports

	Minimal-toxicity conditioning regimen [107,108]	**Reduced-intensity conditioning regimen [109,117,119,143,144]**	**Conventional myeloablative conditioning regimen**				
			US collaborative [112]	**French [111]**	**Belgian [104]**	**CIBMTR [105]**	**Recent series [97–99]**
Number of patients	11*	20[†]	59	87	50	67	46
Patient age (median, in years)	10 (range 3–28)	124 (range 1.8–56)	9.9 (range 3.3–15.9)	9.5 (range 2–22)	7.5 (range 0.9–23)	10 (range 2–27)	10.6 (range 2–17.4)
Conditioning regimen (dose) [135]	Flu (90–150 mg/m^2)/TBI (200 cGy) {5}; Flu (125–150 mg/m^2)/ATG/TBI (200 cGy) [6]	Flu (175 mg/m^2)/BU (8 mg/kg)/ATG/TLI (500 cGy) {7}; Flu (120 mg/m^2)/Mel (140 mg/m^2)/ATG {2}; Flu (120 mg/m^2)/CY (120 mg/kg) {1}; Flu (120 mg/m^2)/Mel (140/70 mg/m^2)/ alemtuzumab {2}; Flu (120 mg/m^2)/BU (3.2 mg/kg) {2} ; alemtuzumab /FLU (150 mg/m^2)/Mel (140 mg/m^2) {2}; Flu (180 mg/m^2)/BU (6.4 mg/kg)/ATG {4}[‡]	BU/CY/ATG {55}; BU/CY/alemtuzumab {4}	BU/CY {12}; BU/CY/ATG {65}	BU/CY {3}; BU/CY/TLI {6}; BU/CY/ATG {14}	BU + CY ± other {63}; other {4}	BU/CY/ATG {44}, BU/CY160 {1}, BU/CY/TT/ATG {1}
Source of stem cells {number of patients}	Marrow{(9}; PBHC {2}	Marrow {10}[§]; CBSC {1}; PBHC {10}	Marrow	Marrow {74}; CBSC {10}; CBSC + marrow {2}; PBHC {1}	Marrow {48}; CBSC {2}	Marrow {54}; PBHC {9}; CBSC {4}	Marrow {42}; PBHC {2}; CBSC {2}
Induction of mixed chimerism	Yes (transient in 10)	Yes	Yes	Yes	Yes	Yes	Yes
Number with graft rejection/disease recurrence	10 (91%)	5 (25%)	5 (8.5%)	7 (8%)	5 (10%)	9 (13%)	1 (2%)
Number with graft-versus-host disease	Acute 1 (grade I), chronic none	Acute 5 (grade II–IV), chronic 4 (3 fatal)	Acute 25%, chronic 12%	Acute 20%, chronic 12.6%	Acute 40%, chronic 20%	Acute 10%, chronic 22%	Acute 33%, chronic 17%
Number of deaths	1 (after a second HCT) (9%)	4 (20%)	4 (7%)	6 (7%)	2 (4%)	3 (4.5%)	3 (6.5%)
Number with event-free survival	1 (9%)	11 (55%)	50 (85%)	74 (85%)	43 (86%)	55 (82%)	42 (91%)

ATG, antithymocyte globulin; BU, busulfan; CBSC, umbilical cord blood stem cell; CIBMTR, Center for International Blood and Marrow Transplantat Research; CY, cyclophosphamide; Flu, fludarabine; Mel, melphalan; PBHC, peripheral blood stem cell; TBI, total body irradiation; TLI, total lymphoid irradiation.

*Includes two patients with thalassemia major.

[†] Includes three patients with thalassemia major.

[‡] Human leukocyte antigen-matched unrelated donor PBHCs were used for HCT.

[§] One patient received a combination of marrow and umbilical cord blood from the same donor.

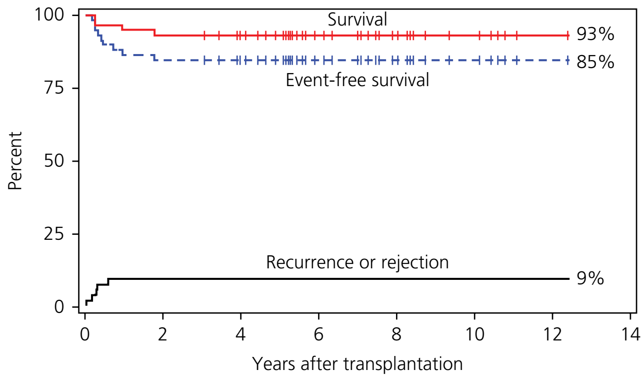

Figure 72.3 Survival probabilities and the cumulative incidence of graft rejection after BMT for sickle cell disease. Kaplan–Meier probabilities for survival and EFS after BMT for SCD are shown. An event was defined as death, graft rejection, or return of SCD. A cumulative incidence curve for graft rejection/disease recurrence is also depicted. Source: Walters et al. 2010 [112]. Reproduced with permission of Elsevier.

The administration of hydroxyurea before HCT was associated with a significantly lower rate of graft rejection, compared with patients who did not receive hydroxyurea, but the administration of ATG before transplantation did not affect the rate of engraftment. Overall 20 of 24 patients had stable engraftment of donor cells after HCT, but none of those who received hydroxyurea before transplantation with ATG in the conditioning regimen had graft rejection or a late recurrence of SCD. This suggests that the augmentation of pretransplantation immunosuppression might be necessary to overcome the immunologic barrier of tolerance to donor histocompatibility antigens. The propensity for graft rejection even after HLA-identical sibling HCT follows the hypothesis that multiple transfusion exposures before HCT induce sensitization to minor histocompatibility antigens expressed on the surface of transfused cells. It appears possible that leukocytes present in blood products are not alone in this ability to cause sensitization, as RBCs may also present histocompatibility antigens via the HLA class I pathway to elicit expansion and activation of host CD8$^+$ T cells [114].

Stable donor–host chimerism after high-dose HCT for SCD

The observation of donor–host hematopoietic chimerism after conventional high-dose HCT has lent substantial support to the notion that persistence of even a fraction of normal erythropoiesis may elicit a curative clinical effect [108,115]. The basis of this effect appears to be twofold: improved survival of healthy donor erythrocytes in the blood compared with sickle erythrocytes, and ineffective erythropoiesis in the SCD marrow that lends a survival advantage to donor erythroid progenitors [116,117]. Approximately 25% of children with SCD developed stable mixed chimerism after HLA-identical sibling HCT [111,112].

In the multicenter investigation of bone marrow transplant for SCD, 13 of 50 patients with clinically successful allografts developed stable mixed chimerism, which is defined as the durable coexistence of donor and host hematopoietic cells well after the discontinuation

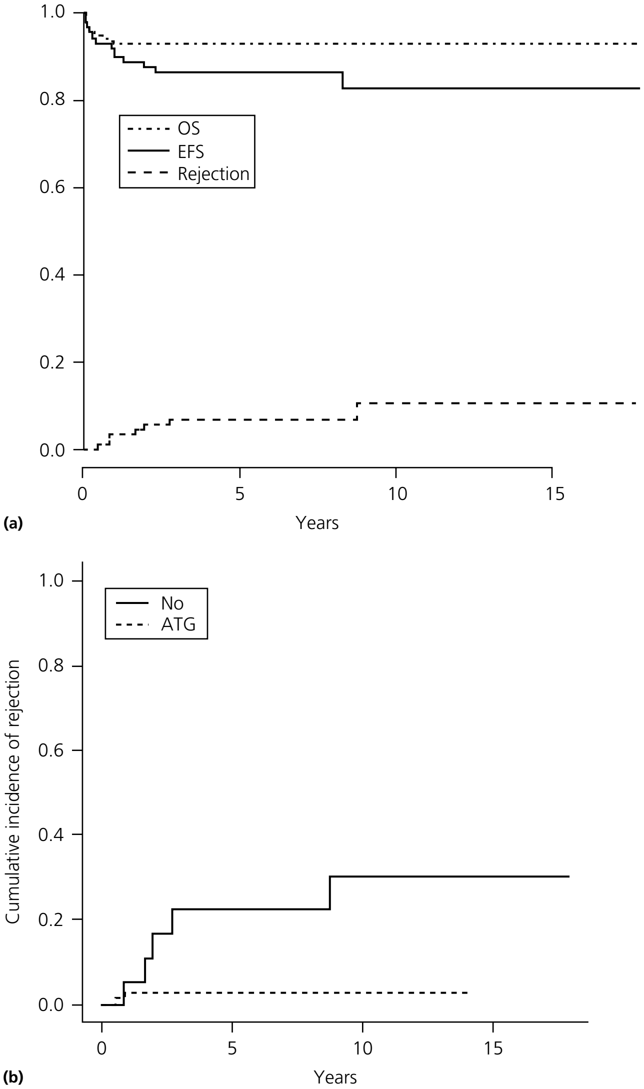

Figure 72.4 (a) Estimated outcomes: OS, EFS, and cumulative incidence of rejection in 87 patients. (b) Comparison of the cumulative incidence of rejection in patients conditioned with and without antithymocyte globulin (ATG). Source: Bernaudin et al. 2007 [110]. Reproduced with permission of the American Society of Hematology.

Table 72.4 Neurologic outcomes among surviving patients ($n = 55$)

Pre-BMT status with duration follow-up (median, range)	Ischemic stroke after BMT	Cerebral hemorrhage (ICH) after BMT	Seizure after BMT	MRI appearance after BMT (median duration, range in years after BMT)
"Clinical" stroke ($n = 29$) (7.0, 3.4 12.4 y)	$n = 1$	$n = 1$ (SAH)	$n = 7$	27/28 studied had stable or improved MRI; 1 not studied (3.2 y, 0.6–7.3)
"Silent" stroke* ($n = 10$) (6.7, 3.4–10.6 y)	$n = 0$	$n = 0$	$n = 4$	All 8 studied had stable (4) or improved (4) MRI; 2 not studied (1.7 y, 0.6–4.9)
Normal ($n = 16$)** (6.6, 3.1–11.1 y)	$n = 0$	$n = 0$	$n = 5$	All 10 studied had normal MRI; 6 not studied (3.1 y, 0.5–5.2)

*"Silent" stroke refers to individuals with MRI evidence of cerebrovascular disease without clinical manifestations.
**Including four with no baseline exam.
BMT, bone marrow transplantation; ICH, intracranial hemorrhage; MRI, magnetic resonance imaging; SAH, subarachnoid hemorrhage; y, years.

of postgrafting suppression. The level of donor chimerism, measured in peripheral blood 6 or more months after transplantation, varied between 90% and 99% in eight patients who had normal hemoglobin levels, with a lower proportion of donor cells (range 11–74%) in five. Among these five, hemoglobin levels varied between 11.2 and 14.2 (median 11.3, mean 12.0) g/dL. None of the patients experienced painful events or other clinical complications related to SCD after HCT. One patient, who had a stroke before transplantation, had no further strokes after HCT and had stable appearance by cerebral MRI, despite having as few as 10% donor cells detectable in the peripheral blood.

In the French series, low levels of residual host hematopoiesis were observed significantly more frequently in patients who did not receive ATG before HCT ($p = 0.04$ at 12 months and $p = 0.002$ at 24 months post-transplant) [111]. A higher fraction of residual host cells was observed in patients who received ATG ($p = 0.03$ at 12 months and $p = 0.001$ at 24 months post-transplant), and this level of donor–host chimerism remained stable in the long term. These observations are consistent with the idea that chimerism, even with a minority of donor cells, can have a significant ameliorative effect so that full engraftment of donor cells is not a requirement for successful HCT, and that the donor chimerism fraction remains stable 12 months and longer after HCT.

Reduced-intensity HCT for SCD

Due in part to the observations discussed earlier, it was reasoned that conditioning therapy might be adjusted to promote stable engraftment of a threshold fraction of donor cells that would be sufficient to prevent vaso-occlusion, and that this regimen could be carried out more safely than conventional high-dose conditioning, particularly in adult patients. Toward this end, several groups have attempted to apply reduced-intensity HCT to SCD, utilizing conditioning regimens of varying intensity [100,107,108,117]. A minimally toxic regimen was first developed in a large animal model and translated successfully into human trials for older adult patients with hematologic malignancies [108]. When applied to SCD, this approach was safe, generated little or no acute GVHD, and in most cases was associated with an initial period of donor engraftment. Unfortunately, in nearly all cases, withdrawal of postgrafting immunosuppression was followed by graft rejection with disease recurrence (Table 72.4). This approach was modified by a group of investigators at the National Institutes of Health that resulted in engraftment of donor cells in the majority of adults with severe disease after long-term administration of sirolimus for postgrafting immunosuppression [100]. Mixed donor–host hematopoietic chimerism was established in 9 of 10 patients who had significant medical comorbidities after HLA-identical sibling mobilized peripheral blood stem cell transplantation. The patients were prepared for HCT with a non-myeloablative combination of alemtuzumab, fludarabine, and total body irradiation, 300 cGy. The mixed chimerism was sufficient to decrease markers of hemolysis (reticulocyte count, total bilirubin and LDH levels) and the hemoglobin levels gradually improved after HCT without RBC transfusions (Figure 72.5). More recently updated results indicate that 23 patients now survive after HCT and that 5 of 17 patients had >50% donor T-cell chimerism and sirolimus was successfully discontinued [118]. In another patient series of reduced-intensity HCT, there was a twofold higher expression of donor β-globin RNA

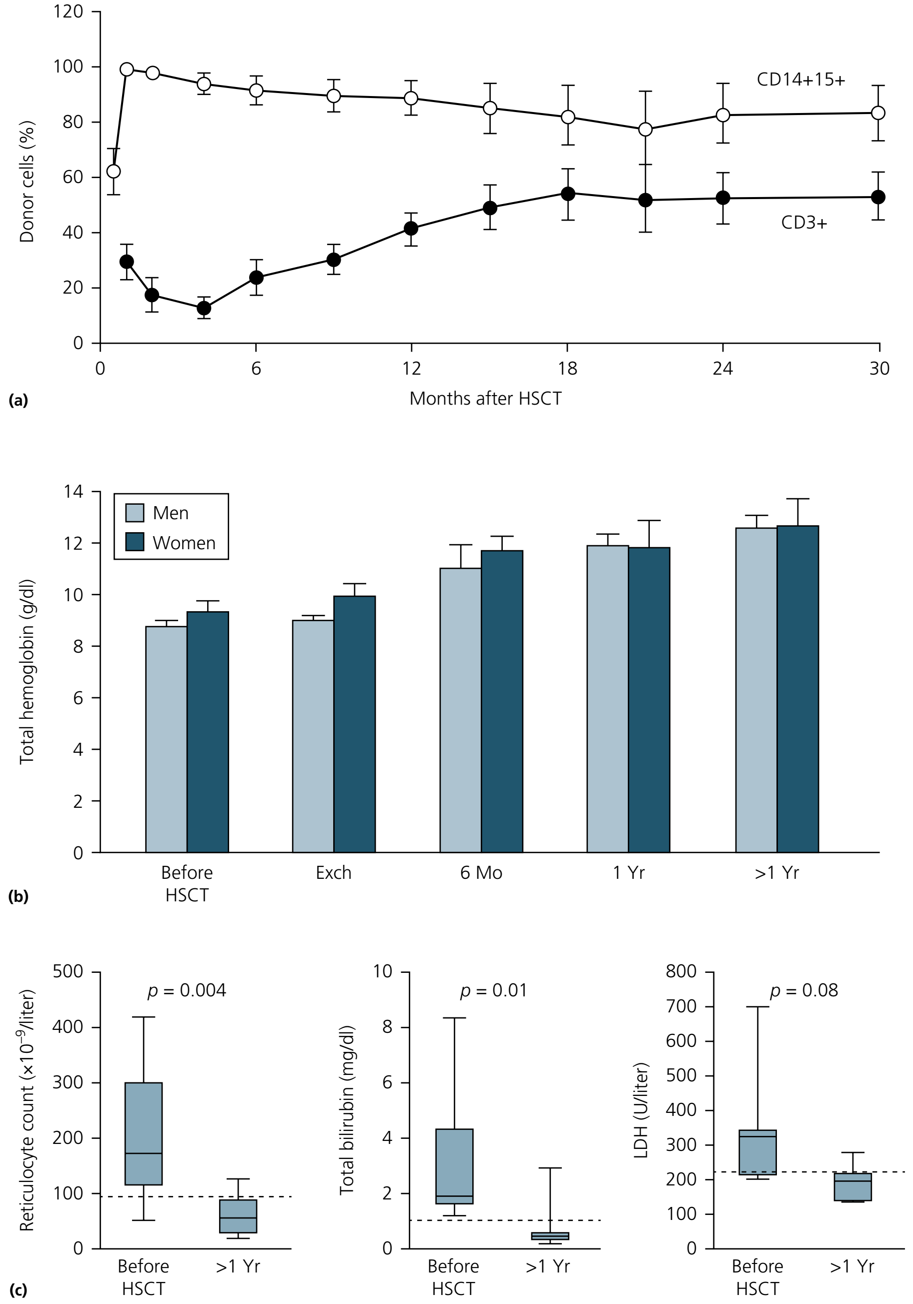

Figure 72.5 Donor cell chimerism and laboratory measurements after hematopoietic stem cell transplantation. (a) shows the mean donor chimerism among $CD3^+$ T cells and $CD14^+15^+$ myeloid cells after HCT. (b) and (c) show pre- and post-HCT laboratory measurements of serum hemoglobin, reticulocyte count, LDH, and total bilirubin. Source: Hsieh et al. 2009 [100]. Reproduced with permission of the Massachusetts Medical Society.

compared with total genomic DNA in the blood after non-myeloablative HCT. Direct bone marrow analysis revealed ineffective erythropoiesis of native HbSS erythroblasts, with a progressive increase in representation of donor RBCs during erythrocyte maturation [117]. These findings were associated with clinical benefit after transplantation, and with improvements in hemolysis, endothelial function, and NO bioavailability; however, they did not persist after graft rejection.

An increasing number of patients have received regimens that are less intense than a high-dose regimen, but retain a moderate degree of the myelosuppressive effect in order to suppress the host-versus-graft reaction and promote engraftment. These regimens require hospitalization, but their risk of regimen-related toxicity is related to the profound immunosuppression of the regimens and an accompanying risk of opportunistic infection (Table 72.4). Donor chimerism was successfully established in children who received a reduced-intensity regimen consisting of BU, fludarabine, ATG, and total lymphoid irradiation (500 cGy) [109]. Seven patients who were between 6 and 18 years of age received HLA-identical sibling bone marrow transplantation with cyclosporine and mycophenolate mofetil for postgrafting immunosuppression. Treatment-related toxicity was minimal, and all the patients had evidence of stable donor engraftment. Another study that included 16 children with non-malignant disorders included one patient with SCD [119].

The patients received melphalan, fludarabine, and alemtuzumab before transplantation, and included seven patients who received unrelated or HLA-mismatched related donor allografts. Twelve of the 16 (75%) patients survive disease-free after transplantation, and none of the surviving patients had graft rejection. This particular approach is being studied in a multicenter clinical trial of unrelated donor HCT for children with symptomatic SCD in the United States (Sickle Cell UnRelated donor Transplant study, SCURT), and the results after unrelated donor umbilical cord blood transplantation recently were reported (see later).

Effect of donor hematopoiesis on vaso-occlusion

After successful HCT for SCD, patients with stable engraftment of donor cells have not experienced clinical complications or required transfusions, even when there was stable mixed donor–host hematopoietic chimerism [111,112]. In addition, investigators have reported improvements in splenic function and osteonecrosis [120,121]. Patients treated by HCT also had stabilization of the underlying cerebral vascular disease. Forty-six of 55 patients who were enrolled in the multicenter study had a cerebral MRI performed a median of 25.4 months after transplantation [112]. These studies were compared with pretransplantation baseline examinations in all but four patients. Surviving patients with stroke who also had stable engraftment of donor cells ($n = 25$) experienced no subsequent stroke events after BMT. However, patients who had graft rejection after BMT were not protected from stroke. One patient with graft rejection experienced a second stroke when the HbS fraction reached 60% after BMT and another patient with graft rejection had a subarachnoid hemorrhage after BMT. In total, 28 of 29 (97%) patients with stroke survived after transplantation, and 26 of 29 (90%) survived stroke-free after BMT. Ten patients had evidence of silent cerebral infarction before transplantation. Of these 10, eight patients had post-BMT studies performed a median of 1.7 (range: 0.5–4.9) years after BMT, which were stable in three patients and four patients had lesions that had evolved to a smaller size by brain MRI. There were no clinical strokes after HCT in this group. Sixteen patients had no documented CNS disease before BMT. All 10 studied in this group had normal MRI appearance a median of 3.1 (range: 0.5–5.2 years) after BMT. Together, these observations show that most patients had stabilization of cerebral vasculopathy after HCT (Table 72.4). These data also suggest that, in those with silent cerebral infarction who are at risk to develop stroke, there is protection from disease progression and a first stroke.

A similar experience was reported from the French group [111]. Among the 36 patients with a history of stroke before HCT, two had a recurrent stroke. One patient experienced a transient ischemic attack 10 days after HCT, while the other, who had severe cerebrovascular injury with Moyamoya disease, had a fatal intracranial hemorrhage 32 days after HCT. With a median follow-up of 6 years, the risk of recurrent stroke was 5.6%, which mirrors the rate of second stroke observed in SCD patients treated by regular RBC transfusion therapy after a first stroke. However, vascular occlusions tended to persist after HCT, and the long-term outcome varied somewhat: in five, the stenoses resolved, in 16 there was no change, and in two cases, the stenoses showed progression. However, none of the patients with durable donor engraftment had a clinical stroke or silent ischemic lesion, including the two patients who had progressive cerebrovascular narrowing. Cortical atrophy worsened in two cases. In addition, cerebral artery velocity was significantly decreased ($p < 0.001$) by 1 year after HCT in the 49 patients who had a pre-HCT TCD examination performed [the TCD velocity declining from 138 (range 88–188) before HCT to 100 (range 66–134) cm/s as measured in the right MCA, and from 138 (range 92–184) to 103 (range 63–143) cm/s as measured in the left MCA]. The TCD velocity also normalized by 3 months after HCT in two patients who had abnormal measurements before HCT, even though both had been treated by regular RBC transfusions before HCT.

In other series, patients with stroke before HCT have also experienced transient ischemic attacks and intracranial hemorrhage in the era before measures to prevent these adverse events were instituted [122,123]. After the institution of preventive measures, neurologic complications still occurred after transplantation, but these tended to be self-limited events such as seizures with no long-term sequelae. One group of investigators observed radiographic progression in some patients with SCD who underwent HCT for stroke [124]. Five of nine patients had either new or slightly increased size of cerebral lacunae or leukoencephaly that stabilized 2–7.5 years after HCT. Thus, it is possible that these observations reflect the evolution of cerebrovascular disease that existed before bone marrow transplantation. Of interest, these cerebral MRI changes were not associated with progressive neurocognitive deficits.

Pulmonary function also was evaluated systematically after HCT in the multicenter collaborative study. Eighty-two percent of the surviving patients had pulmonary function measured a median of 3.2 years after HCT. The mean predicted FEV1 changed from 88% ± 10% to 86% ± 11% ($p = 0.59$) and the mean predicted FVC changed from 78% ± 16% to 81% ± 12% ($p = 0.45$) after transplantation. Before HCT, 11 had restrictive changes, 2 had obstructive changes, and 10 patients had normal lung function at baseline. Compared to pre-HCT measurements, the RV ($p = 0.02$) and RV/TLC ($p = 0.03$) were significantly decreased after BMT. The observed difference in RV and in RV/TLC suggested that there was decreased air trapping and therefore improved lung function after BMT. Overall, patients who had normal baseline pulmonary function were likely to remain normal (8 of 10) after BMT. Patients who had restrictive disease at baseline were likely to have improved or persistent, but not progressive restrictive disease (10 of 11). Taken together, these data show that most patients had stable pulmonary function after BMT. These results contrast with the natural history of SCD, for which there is growing evidence to indicate that the pulmonary toxicity of SCD is progressive, and may be expressed as either restrictive or obstructive pulmonary disease [125,126]. In a series from the Cooperative Study of Sickle Cell disease, only 10% of adults had normal PFT results, with restrictive pulmonary disease (74%) accounting for the most frequent abnormality among 310 adult SCD individuals tested [127].

Growth and development after HCT for SCD

There remains concern about possible growth impairment after HCT for SCD. The potential for growth impairment stems, in part, from the gonadal toxicity associated with high doses of BU, particularly in females. A report compared four groups of children with SCD in an analysis of growth after HCT. Children who were enrolled in the CSSCD study comprised one supportive therapy comparison group. Children who were enrolled in the HUG-KIDS trial made up the remaining two comparison groups [128]. These two groups included children who had pretreatment growth measurements (HUG-KIDS Pre) and the same children who again had measurements for at least 1 year after achieving the maximum tolerated dose of hydroxyurea (HUG-KIDS HU).

Using two-level hierarchical linear models, there were no statistically significant differences in the estimated height (weight) velocity curves between the HCT and the three comparison groups among females; however, there were significant differences between males in the HCT and CSSCD groups The predicted height velocity during follow-up in the HCT group was greater than the velocity in the CSSCD group between 3.3 years and 11.6 years of age. After this period of 8.4 years, there was no apparent growth benefit associated with HCT. As a result, the predicted height velocity in HCT males who were greater than 12 years of age at transplantation was less than the velocity in CSSCD males. Similarly, until the baseline age of 12.1 years, the linear weight velocity in the HCT group was greater than in the CSSCD group. In summary, growth after transplantation for SCD was not impaired, except in older males. These data show that conventional high-dose HCT generally had no adverse effect on height or weight gain in young children.

Gonadotropin and sex hormone levels of surviving patients have also been monitored, and these confirm the toxic effect of BU on gonadal function. In the multicenter study, the median ages at evaluation were 21.6 (range: 16.1–28.5 years) and 21.7 (range: 14.1–27.8 years) among the males and females, respectively. Among these, 13 males (39%) and 14 females (64%) had post-transplantation endocrine studies reported. Eight of 14 females had increased gonadotropin levels and/or below normal estradiol levels, a finding consistent with primary ovarian failure in the majority of postpubertal females. Only 4 of 14 females had normal estradiol levels. However, one female had a successful pregnancy 13 years after BMT and another female with graft rejection gave birth to a healthy baby following preimplantation genetic diagnosis 14 years after BMT, although she had experienced two previous spontaneous abortions several years earlier. Among six evaluable prepubertal girls in the Belgian cohort, five had primary amenorrhea with elevated serum LH and FSH [104]. Two postpubertal females developed secondary amenorrhea.

In the French series, seven postpubertal females (ranging from 13 to 22 years of age) developed amenorrhea after HCT with decreased serum estradiol and elevated LH and FSH levels, and received hormone replacement therapy [111]. In addition, most females who were prepubertal before HCT received hormone replacement therapy to promote the development of secondary sexual characteristics after they reached a bone age of 13 years. However, two younger girls (6.4 and 8.3 years of age) experienced spontaneous pubertal development, raising the possibility that HCT performed in very young patients might not be associated with delayed puberty. Nonetheless, it is anticipated that many if not most of the females will require hormonal replacement therapy after HCT with high-dose BU.

Males may be somewhat more resistant to the adverse effect on gonadal function, although the long-term outcome remains somewhat uncertain. Of seven males from the multicenter study of HCT for SCD the LH and FSH levels were normal and less than normal in four after transplantation. However, only three of 13 had normal testosterone levels, consistent with hypogonadotrophic hypogonadism in most of the pubertal males [112]. Testicular function was also adversely affected in four of six evaluable boys from the Belgian cohort who demonstrated decreased testosterone and elevated FSH levels [104]. However, all the male recipients from the French series had testosterone, FSH, and LH levels in keeping with their bone age and pubertal status [111]. Six Belgian males, five of whom were prepubertal before HCT, had semen analysis performed 14 – 21 years HCT and preparation with BU/CY or BU/CY with total lymphoid irradiation (TLI). The three TLI recipients had azoospermia while two of the three remaining patients were azoospermic and one patient was normal [129]. While none of these series has definitive information about the impact of treatment on fertility, there is an expectation, particularly among postpubertal females that fertility is likely to be adversely affected after myeloablative BU. The possibility of ovarian tissue autografting after myeloablative HCT for SCD was recently reported, however, and may offer an alternative in the future [130].

Delays in growth and development after HCT are amplified by iron overload, illustrated best in children with thalassemia major who have significant transfusional iron loading before HCT [131]. The transfusion iron burden in SCD can approach that observed in thalassemia major, thus a strategy to eliminate iron after successful HCT is warranted [132]. Phlebotomy after HCT is a safe and effective method for reducing iron stores, and should be pursued in patients with SCD who have iron overload [133]. Treatment by iron chelation therapy is an alternative in those who cannot support regular phlebotomy [134].

Alternative sources of donor hematopoietic cells

The use of umbilical cord blood (UCB) *in lieu* of bone marrow in transplantation for SCD is of interest, due primarily to a lowered risk of GVHD after UCB transplantation. However, this benefit is balanced by a higher rate of graft rejection and a longer duration until hematologic recovery that accompanies UCB transplantation. Thus, to overcome these risks, investigators have modulated pre- and postgrafting immunosuppression and intensified supportive care to ensure favorable outcomes.

In a report of 44 patients that included 11 with SCD who received HLA-identical or one-antigen HLA-mismatched sibling UCB allografts, the overall 2-year SCD-free survival rate was 91%, with one patient experiencing recurrent disease [135]. However, among patients who received thiotepa or fludarabine in addition to the BU/CY backbone and who did not receive methotrexate with postgrafting immunosuppression, the outcome after sibling donor UCB transplantation was superior, particularly among recipients with thalassemia major. In addition, only four of 44 patients experienced acute, and two chronic, GVHD after UCB transplantation, and there were no deaths related to GVHD. This experience was expanded in a larger, more recent comparison of outcomes after UCB and marrow transplantation from HLA-ID sibling donors involving 485 patients with SCD or thalassemia [136]. The overall 6-year disease-free survival in 160 patients with SCD who were treated between 1994 and 2005 by either source of hematopoietic cells was 92 ± 2%. The disease-free survival after UCB transplantation was 90 ± 5% in 30 patients and it was 92 ± 2% after bone marrow transplantation in 130 patients. In this investigation, the outcome after UCB transplantation also was influenced by the use of methotrexate for GVHD prophylaxis, which was a significant variable in a multivariate analysis of factors influencing the probability of disease-free survival (HR 3.81; $p = 0.004$). None of the patients developed chronic extensive GVHD and none of the patients died of GVHD after UCB transplantation. Thus, while it appears that UCB and marrow from HLA-ID donors might be used interchangeably to support HCT for SCD, the use of UCB extends a benefit in terms of a lower risk of acute and chronic GVHD.

The current experience of unrelated donor UCB transplantation for SCD is quite limited, but it illustrates the challenges yet to be overcome. In an analysis by the CIBMTR that included patients with thalassemia and SCD, 16 children had SCD [102]. Nine of 16

SCD patients received myeloablative conditioning. Seven of 16 patients achieved neutrophil recovery a median of 22 days after transplantation with full donor chimerism. Eight of 16 patients were alive and disease-free with an overall survival probability of 62 ± 9%. In multivariate analysis, disease-free survival was higher if the UCB unit contained $>5 \times 10^7$ TNC/kg recipient weight (HR 0.4; $p = 0.01$). The cumulative incidence of chronic GVHD in all patients was 16 ± 4%. Finally, enrollment in the UCB arm of the SCURT trial in the United States was stopped early due to a high-rate of graft rejection following a reduced-intensity regimen of alemtuzumab, fludarabine, and melphalan [101]. Eight children received UCB from unrelated donors matched at 5 or 6 of 6 HLA antigens. Five patients experienced autologous marrow recovery after UCB transplantation and one died of chronic GVHD. There were three of eight patients who survived disease-free after receiving this reduced-intensity regimen.

Together, these reports suggest that while a successful outcome is possible after unrelated donor UCB transplantation for SCD if one adheres to strict criteria for HLA-matching, conditioning regimen intensity and a minimum cell dose, these features alone are not sufficient to ensure a successful outcome in the majority of patients. Thus, it is very likely that improved outcomes will rely upon novel conditioning regimens, alternate donor/recipient HLA matching algorithms, screening for anti-HLA antibodies and perhaps an even higher cell-dose target to overcome the problems of graft rejection and GVHD that currently impede progress.

That the broader application of HCT for SCD is limited is best illustrated by the observation that only 14% of SCD recipients are likely to have a HLA-identical sibling donor [137]. Not surprisingly, lacking a suitable donor is the major barrier to HCT for SCD. An expanded availability of HLA-compatible unrelated donor sources is necessary to pursue HCT as a therapeutic option in most patients with SCD. Donors who are matched at six (HLA-A, -B, and DRB1 loci) to 10 HLA antigens (including HLA-C and DQB1 loci) can be identified via the National Marrow Donor Program in approximately 80% of Caucasian recipients; however, the likelihood of identifying a similarly HLA-matched unrelated donor is less likely in other ethnic groups due to underrepresentation of these groups in the volunteer donor pool and greater HLA diversity. The reduced likelihood of identifying a suitable donor is a difficult problem in HCT for SCD and can limit the application of HCT among those who might benefit most [138–140]. However, the feasibility of identifying suitable donors was suggested by surveys in which 60% of SCD patients had at least one potential suitable unrelated donor, and 26% had three or more potential donors, although in practice, it is still very likely that a suitable unrelated donor will not be identified for most individuals with SCD. For these individuals, utilizing hematopoietic cells from an HLA-mismatched related donor for HCT would be particularly useful. This possibility was suggested by a report of HLA haploidentical bone marrow transplantation in severe SCD that was pioneered by the transplant team at Johns Hopkins University [103]. This approach employed a nonmyeloablative combination of cyclophosphamide, antithymocyte globulin, and fludarabine with 200 cGy TBI before HCT. After the marrow infusion, postgrafting high-dose cyclophosphamide was administered to accomplish in vivo T-cell depletion and to facilitate durable engraftment of donor cells without severe GVHD. In a pilot investigation, 14 recipients of haploidentical HCT were treated and all had prompt recovery after HCT, although six developed graft rejection a median of 151 days after HCT and one patient had low-level donor chimerism with severe anemia after HCT. Nonetheless, 50% of the patients had stable donor engraftment and cessation of sickle-related complications, and none had chronic GVHD. If the complication of graft rejection can be reduced, this would broaden the availability of transplantation to a much larger fraction of patients who otherwise satisfy eligibility requirements but lack a suitable unrelated donor.

Thus, it appears feasible to pursue alternate donors suitably matched at HLA alleles in the treatment of individuals with severe SCD. It is quite possible and even likely that the successful translation of unrelated donor HCT for SCD will hinge on our ability to establish bidirectional donor–host immunologic tolerance, and that this will involve the administration of more intensive immunosuppression than we are accustomed to using in this clinical setting. This represents a shift from the notion that marrow ablation and elimination of sickle cell progenitor cell populations are the principal objectives in carrying out a successful HCT. There are many reasons why this might be, not least of which is that the immunologic *milieu* of SCD is one of inflammation, where innate stimulation of graft-versus-host and host-versus-graft reactions is very likely to occur. In the course of inhibiting these reactions by pharmacologic means, controlling the emergence of opportunistic viral and other infections will take on greater importance than has been appreciated previously after transplantation for hemoglobin disorders.

Conclusion

The track record of HCT from HLA-identical sibling donors for clinically significant hemoglobinopathies is among the best of disorders currently treated by HCT. Most patients with SCD survive after HCT with no evidence of the characteristic clinical sequelae of vaso-occlusion. Moreover, as new indications for HCT emerge and the possibility of alternate donor HCT is expanded, there is likely to be a possibility of HCT for individuals where none existed previously. Nonetheless, very few individuals currently are offered this therapy. Decisions about treatment options in the future are very likely to be influenced by perceptions about the nature of SCD as a chronic illness, and whether the short-term risk of mortality inherent to HCT is balanced by the possibility of survival without symptoms or treatment. While HCT is the only curative therapy for SCD, other treatments such as hydroxyurea and regular RBC transfusion may pose fewer short-term risks and should be considered in selected children who lack suitable donors or in those who exhibit favorable-risk characteristics. Controlled clinical trials comparing chronic transfusions, hydroxyurea, and HCT should be conducted to determine the relative values of these treatments and ultimately provide the optimal care for the SCD patient.

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CHAPTER 73

Hematopoietic Cell Transplantation for Immunodeficiency Diseases

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Introduction

Significant advances in the diagnosis and treatment of severe combined immunodeficiency syndrome (SCID) and other congenital lymphoid immunodeficiencies have occurred since the fourth edition of this book was published in 2008 [1]. Advances in the molecular genetics of these diseases have been particularly noteworthy [2–4]. The genetic causes of over 120 primary immunodeficiency diseases (PID) are now elucidated (reviewed in [5–7]). Identification of these genes and their mutations has radically enhanced precision of diagnosis and led to the discovery of variant mutations as well as modifying genes contributing to phenotypic and functional differences between affected individuals. Newborn screening techniques for detection of SCID and other diseases associated with profound T lymphopenia have now been introduced in several US states, permitting diagnosis in the newborn period and definitive treatment with transplants or gene therapy before the patient develops life-threatening acute or chronic infections. Techniques for transplantation of patients with SCID, WAS, and other lethal genetic immune deficiencies also continue to improve; resulting in survival rates of 80–100% following an HLA-matched related HCT and 50–90% following alternative donor transplants. In this chapter, the results of HCT for SCID, WAS, and other combined lymphoid immunodeficiency syndromes (CID) will be updated, the genetic mutations giving rise to SCID and CID summarized, and the problems still facing physicians caring for these children analyzed.

Immunophenotypic and genotypic variants of severe combined immunodeficiency (SCID)

SCID is a diagnosis that encompasses a heterogeneous group of lethal, genetic disorders each characterized by the absence of antigen-specific T and B lymphocyte responses (reviewed in [1,7,8]). Two overall strata of SCID, based on phenotype are recognized [8]: a typical or "classical" form of SCID marked by T-cell lymphopenia (<300/μL) and responses to phytohemagglutinin (PHA) that are <10% of lower limit of normal, and "leaky" forms distinguished by less severe T-cell lymphopenia (<1000/μL) without maternal T-cell engraftment and a PHA response <30% of lower limit of normal. Based on the number of patients treated for SCID in the United States, the incidence of SCID had previously been estimated to range from 1/50,000 to 1/100,000 live births [5]. However, initial results of large newborn screening programs (see later) indicate that the incidence is approximately 1/58,000 [9]. In contrast to the overall population in the United States, Athabascan-speaking Native Americans, have a high frequency of Artemis gene mutations. As a consequence, a higher incidence of SCID (52/100,000) has been reported in this population [10].

Prior to the identification of the multiple genetic defects that can give rise to SCID, children with classical or typical SCID were classified according to the phenotype and function of their circulating lymphocytes, the mode of inheritance, and/or the presence or absence of adenosine deaminase (ADA), an enzyme deficiency known to be associated with SCID [8,11]. Three major forms of SCID were identified, consisting of the most common form of SCID [B^+ SCID with absent T cells (T^-B^+)], the classical form of SCID (T^-B^-), and ADA-deficient SCID, which may present with either phenotype. Subsequently, mutations in over 15 genes have been shown to give rise to SCID (reviewed in [5–8,12–14]). The currently known genetic defects resulting in SCID affect four major aspects of lymphoid development: (1) metabolic disorders causing premature death of lymphoid precursors due to toxic metabolites (e.g., ADA deficiency) or impaired differentiation (e.g., adenylate kinase 2 deficiency in reticular dysgenesis); (2) defective development resulting from abnormal cytokine signaling (e.g., null mutations of the γc, IL-7Rα, Janus kinase, IL-2Rα, IL-2R/IL-15β genes); (3) defective VD-J gene rearrangement in T-cell and B-cell precursors due to recombinase activating gene defects (RAG1/2) or DNA repair gene defects (e.g., Artemis, DNA ligase IV); and (4) abnormal signaling through the T-cell receptor (CD3/TCR subunit defects, and defects of CD45) (reviewed in [5–8,14]). The genetic disorders causing SCID and their usual lymphoid phenotype are summarized in Table 73.1.

Deficiency of adenosine deaminase, an enzyme involved in purine catabolism, was identified as the first known cause of SCID by Giblett et al. in 1972 [11].In these patients, T cells and, to varying degrees, B cells and NK cells are depleted in the course of their development due to their inability to deaminate deoxyadenosine and adenosine to their metabolites deoxyinosine and inosine. Resultant accumulations of deoxyadenosine and adenosine are toxic to these lymphoid cells and curtail their survival. In reticular dysgenesis, mutations in the mitochondrial enzyme adenylate kinase 2 affect the folding and distribution of proteins essential to T and B cells as well as neutrophil and platelet differentiation [15,16].

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Table 73.1 Genetic mutations and phenotypic characteristics of defined variants of severe combined immunodeficiencies

Disease	Circulating T cells	Circulating B cells	Serum Ig	Circulating NK cells	Distinctive features	Inheritance	Genetic defects/presumed pathogenesis
1. T^-B^+ SCID							
(a) γc deficiency	Markedly decreased	Normal or increased	Decreased	Markedly decreased		XL	Null mutations of the GFAC encoding the γ chain of receptors for IL-2, -4, -7, -9, -15, -21
(b) JAK3 deficiency	Markedly decreased	Normal or increased	Decreased	Markedly decreased		AR	Null mutations of the JAK3 signaling kinase gene
(c) IL-7Rα deficiency	Markedly decreased	Normal or increased	Decreased	Normal		AR	Null mutations of the IL-7Rα chain gene
(d) CD45 deficiency	Markedly decreased	Normal	Decreased	Decreased	Normal γ/δ T cells	AR	Null mutations of the CD45 gene
(e) CD3δ/CD3ε /CD3ζ deficiency	Markedly decreased	Normal	Decreased	Normal		AR	Null mutations of genes encoding the CD3δ, CD3ε, or CD3ζ chains of T-cell antigen receptor
2. T^-B^-SCID							
(a) RAG 1/2 deficiency	Markedly decreased	Markedly decreased	Decreased	Normal	Defective VDJ recombination	AR	Null mutations of the recombinase activating gene (RAG) 1 or 2
(b) DCLRE1C (Artemis) deficiency	Markedly decreased	Markedly decreased	Decreased	Normal	Defective VDJ recombination, radiation sensitivity	AR	Null mutations of the gene encoding Artemis DNA recombinase-repair protein
(c) Adenosine deaminase deficiency (ADA)	Absent from birth (null mutations) or progressive decrease	Absent from birth or progressive decrease	Progressive decrease	Normal	Costochondral junction flaring	AR	Null mutations of the ADA gene, elevated lymphotoxic metabolites (dATP, S-adenosyl homocysteine)
(d) Reticular dysgenesis	Markedly decreased	Decreased or normal	Decreased	Decreased	Granulocytopenia, thrombocytopenia (deafness)	AR	Null mutations of the adenylate kinase 2 gene, defective maturation of T, B and myeloid cells (stem cell defect)
(e) DNA ligase IV	Decreased	Decreased	Decreased	Normal	Microcephaly, facial dystrophy, radiation sensitivity	AR	DNA ligase IV defect, impaired non-homologous end joining (NHEJ)
(f) ZAP-70 deficiency	Decreased CD8, normal CD4 cells	Normal	Normal	Decreased		AR	Defects in ZAP-70 signaling kinase
3. Omenn syndrome	Present; restricted heterogeneity	Normal or decreased	Decreased, except increased IgE	Normal	Erythroderma, eosinophilia, adenopathy, hepatosplenomegaly	AR	Hypomorphic mutations allowing residual activity, usually in RAG1 or 2 genes but also in Artemis , IL-7Rα and RMRP genes

Of the $T^-B^+NK^-$ forms of SCID, the X-linked form of ($T^-B^+NK^-$) SCID, accounts for approximately 50% of all cases. This disorder is caused by mutations within the γ chain of the common cytokine receptor, γc [17,18]. The most common autosomal recessive form of $T^-B^+NK^-$ SCID is caused by mutations within the Janus kinase 3 (JAK3) gene [19,20]. Another rare variant of (T^-B^+) SCID with absent circulating NK cells is caused by mutations within the IL-2R/IL-15β subunit [21]. On the other hand, infants with T^-B^+ SCID who have circulating NK cells may have defects within the α chain of the IL-7 receptor [22] or null mutations within the δ, ε, or ζ invariant chains of the CD3 subunit of the CD3/TCR complex [23,24]. Children with $T^{lo}B^+NK^+$ SCID have also been described who have mutations within genes encoding CD45 [25], hypomorphic mutations within the invariant chains of the CD3 receptor (reviewed in [23]) or IL-2Rα [26], and mutations in the CHD7 gene resulting in $T^-B^+NK^+$ SCID with dysmorphic features associated with the DiGeorge and CHARGE syndromes [27].

A T^-B^- SCID phenotype is observed in patients with defects in the recombinases, RAG1 or RAG2 [28–30], mutations of Artemis affecting DNA repair enzymes critical to lymphoid development [31,32] (one of which is particularly prevalent in the Navajo Nation [10]), and the gene encoding ADA (reviewed in [11]). Two other genes affecting DNA repair, DNA ligase IV [13] and Cernunnos-XRCC4-like factor [12] (see cited immunodeficiencies later), usually present as combined immunodeficiencies with detectable T-cell function but progress to a T^-B^- SCID phenotype. ZAP-70 [33–35] and p56lck [36] defects usually result in patients with T cells, but absent CD8 or CD4 cells, respectively. However, genetic analyses of patients diagnosed with SCID have shown that patients with ZAP70 mutations may also present with a full T^-B^- phenotype [35,37]. Abnormal CD7 [38] expression has also been associated with SCID; however, the specific gene mutations involved have not yet been identified.

Several large centers have now analyzed the distribution of SCID genotypes in patients referred for treatment [39–42]. While there is some center-to-center variation, likely reflecting differences in gene frequency among different ethnogeographic groups, X-linked $\gamma c^{-/-}$ SCIDs are the most prevalent (20–46%), followed by the ADA deficient (5–16%), IL-7 $R\alpha^{-/-}$ (5–10%), $JAK3^{-/-}$ (7–13%), RAG1/2 defects including Omenn syndrome (3–23%), and Artemis gene defects (1–12%). Other variants of SCID identified are rare, accounting for no more than 1–5% of any series. In addition, for at least 7% of patients with SCID currently referred, the genetic defects remain to be identified.

Clinical features of SCID

In the absence of a positive family history, children with SCID generally reach medical attention at 3 to 6 months of age coincident with waning protection afforded by passively transferred maternal antibodies. Most children with SCID present with failure to thrive, refractory thrush, persistent diarrhea, recurrent otitis media, and/or interstitial pneumonia secondary to *Pneumocystis jiroveci*, cytomegalovirus (CMV), or common respiratory viruses, such as respiratory syncytial virus (RSV), parainfluenza, adenovirus, or influenza [43–46].

Some genetic causes of SCID exhibit distinctive clinical features, which are also included in Table 73.1. Children with genital and oral ulcers usually have Artemis mutations [10,31,32]. Infants with

Omenn syndrome (scaling erythroderma, lymphadenopathy, organomegaly, eosinophilia, high IgE levels [47]) most frequently have hypomorphic defects within recombinase activating genes (RAG1/RAG2) [28–30] and less commonly within the Artemis [48] or IL7-Rα [49] gene. Microcephaly, developmental delay, and photosensitivity are common in SCID syndromes associated with genes involved in repair of DNA double-strand breaks by non-homologous end joining, such as DNA ligase IV [13,50,51]. Adenosine deaminase deficiency [11,52] can be associated with skeletal, renal, hepatic, and neurologic defects, which may persist despite successful correction of SCID by HCT [53]. Rare children with SCID present with short-limbed dwarfism and ectodermal dysplasia [54]. Patients with reticular dysgenesis, a T^-B^- variant of SCID also present with neutropenia thrombocytopenia and sensorineural deafness [15,55,56]. Cartilage–hair hypoplasia [57,58], which can present as a $T^-B^+NK^-$ SCID, and is caused by mutations in the RNA component of the mitochondrial RNA processing endoribonuclease [59] which maps to chromosome 9P21-P12 [59], also presents with short stature, metaphyseal chondrodysplasia, sparse hair and, in some cases Hirschsprung disease.

In addition, an as yet poorly defined proportion of patients with documented mutations in genes associated with SCID present with levels of T cells and T-cell function that exceed those usually associated with the diagnosis of SCID. Identification of the genes mutated in these “leaky” variants of SCID [8] has also demonstrated the potential of different mutations within the same gene to induce different clinical and immunologic features of disease. Such “leaky” forms of SCID are also extensively documented in murine models bearing mutations in the same genes [60–62]. A striking example of this variation can be observed among patients with null or hypomorphic mutations of RAG1. Null RAG1 [28] mutations can be associated with classical ($T^-B^-NK^+$) SCID, while hypomorphic mutations that preserve some recombinase activity can cause Omenn syndrome as well as a syndrome characterized by oligoclonal T cells, autoimmune cytopenias, and increased risk of CMV infections [28–30,63]. In addition, certain mutations of RAG1 or 2 have been described that result in only mildly diminished recombinase activity. These patients present later in life with normal numbers of T, B, and NK cells but variable degrees of immunoglobulin deficiency and outgrowths of autoaggressive T cells causing an extensive and destructive granulomatous disease [64]. Patients with “leaky” forms of SCID, have also been recognized in several other genetically defined SCID variants, particularly among patients bearing mutations in genes affecting DNA repair including Artemis [48], the DNA protein kinase catalytic subunit (DNA-PKCS), and DNA ligase 4 [12,65]. Such “leaky” features are occasionally observed even in patients bearing mutations in the γc receptor associated with X-linked $T^-B^+NK^-$ SCID and in ADA^- SCID.

Such differences in presentation have also been recorded within single sibships bearing mutations in RAG1/RAG2 [29,30] or Artemis [48]. In addition, significant differences in severity of T- and B-cell cytopenia as well as functional parameters such as responses to mitogens have been reported in siblings with ADA deficiency [33]. These variations within single sibships suggest that other genetic or microenvironmental factors (e.g., thymic stromal function [66] or effects of infection) can modulate expression of mutated and defective gene products, thereby modifying their clinical sequelae.

Unexpectedly high numbers of T cells in a patient with a SCID genotype may also be observed particularly in NK-deficient forms of SCID as a result of the engraftment of transplacentally derived maternal T cells [67–69]. In our own series, over 90% of patients with X-linked, $\gamma c^{-/-}$ SCID have detectable populations of maternal T cells in the blood. While engraftment of maternal $CD4^+$ T cells may cause GVHD, maternal $CD8^+$ T cells rarely cause this complication [68].

Early detection of SCID: the potential impact of prenatal diagnosis and newborn screening on transplant outcome

Historically, recognition of the defects in T- and B-cell function characteristic of SCID permitted early identification of patients with SCID only in siblings of relatives of previously diagnosed patients. Subsequent elucidation of many of the genetic mutations that give rise to SCID has increased the proportion of infants who can be diagnosed in utero [70–73]. For such cases, experienced centers now regularly plan for and provide curative transplants in the newborn period [45,71–74]. The results of such early transplants underscore the advantages of grafting patients with SCID prior to acquisition of infection. In the original study of Myers et al. [75], long-term survival was observed in 21 of 22 infants (95%) transplanted with T-cell-depleted HLA haplotype-disparate parental marrow within the first 28 days of life, compared to 51 of 67 children (76%) who received such transplants at later time points. The significantly improved results for matched and HLA disparate related and unrelated transplants in patients with SCID transplanted ≤3 months of age has subsequently been confirmed by several centers [45,76]. These results also provided a major stimulus for the development of adequately sensitive and specific diagnostic tests that permit screening of large populations of newborns for severe combined immune deficiencies.

Functional T cells, during development in the thymus, undergo V-D-J gene rearrangements to generate an α/β T-cell receptor (TCR). In doing so, these T cells also generate T-cell receptor excision circles (TRECs), which Douek et al. [77] showed could be quantitated and used as a measure of recent thymic emigrants in the blood. Subsequently, Chan and Puck [78] developed an assay permitting measurement of the DNA of one specific TREC, the γRec-ΨJa signal joint TREC, which is produced by at least 70% of developing T cells in the course of generating an α/β TCR. Puck et al. [79] subsequently showed this assay to be useful for screening large populations of newborns, demonstrating that in patients with severe T lymphopenia, including all patients with SCID, TREC levels are low or absent. They also demonstrated that TREC levels could be accurately measured by quantitation of PCR amplified TREC sequences in dried blood samples excised from Guthrie cards used to screen newborns for metabolic diseases. In 2008, Wisconsin initiated a statewide screening of all newborns using this approach [80]. In a report analyzing results of the first 3 years of this program [81], they screened 207,696 infants, of whom 33 patients (incidence 1 : 41,848) were confirmed to have severe T lymphopenia, including five patients with absence of TRECs who were confirmed to have SCID and four patients with DiGeorge syndrome. Other conditions resulting in T lymphopenia included patients with multiple chromosomal abnormalities as well as newborns with complex vascular and lymphatic malformations. The reproducibility, sensitivity, and specificity of the TREC assay for detecting severe lymphopenia were excellent, with false positives largely confined to tests of premature infants of <37 weeks gestation. Recently, in an analysis of over 3 million infants screened in 10 states and in the Navajo Nation, Kwan et al. [9] detected 52 patients with SCID or a leaky variant thereof (incidence 1 : 58,000). Results from this 10-state screening program have identified some geographical differences in the prevalence of specific SCID variants, and also suggest higher incidences among specific ethnogeographic groups, such as the Hispanic population in California [9].

Early estimates of the impact of newborn screening on treatment outcomes are also very encouraging. Although the number of children with SCID, or a leaky variant thereof, who have been identified by newborn screening is limited, the recent report from Kwan et al. [9] indicates that of the 52 patients detected in the 10-state study, 49 were rapidly referred for a transplant, enzyme replacement or gene therapy of whom 87% are surviving at various stages of immune reconstitution.

HCT for SCID

Attempts to correct the lethal immune deficiencies have led to several benchmarks in human HCT, including the first successful allogeneic transplants to employ HLA-compatible marrow from a sibling to correct the immune deficiencies associated with SCID [82] and Wiskott–Aldrich syndrome [83], the successful use of marrow transplants from HLA-compatible unrelated donors [84], and the demonstration that HLA haplotype disparate parental marrow grafts adequately depleted of T cells could reconstitute children with SCID without inducing graft-versus-host disease (GVHD) [85,86]. Initial corrections of a human disease by gene therapy were also achieved in patients with SCID [87]. In this section, we will first discuss results of HLA-matched related transplants, and, thereafter, review results of matched unrelated marrow grafts, HLA haplotype disparate T-cell-depleted grafts and cord blood grafts.

Unmodified HLA-matched related HCT

The first successful human allogeneic HCT was reported in 1968 by Gatti et al. in a patient with X-linked SCID [82], who currently remains alive and well over four decades later. Transplantation of T-cell-replete HCT from an HLA genotypically identical sibling remains the treatment of choice for infants with SCID.

Results of HLA-matched transplants for SCID have significantly improved from the 48% long-term survival reported in 1979 [1,88] to 80–100% in current series [69,89–91]. An update of the long-term results of transplants for SCID in Europe from 1968 to 1999, demonstrated an 81% 3-year survival in 104 recipients of an HLA-matched sibling BMT and over 90% for patients transplanted after 1995 [76,90]. Earlier disease diagnosis, improved supportive care, and particularly, better detection, treatment and prevention of opportunistic infections, have contributed to the improved outcome of SCID transplants from all donor sources. Indeed, in the recent Primary Immune Deficiency Transplant Consortium (PIDTC) analysis of 240 children transplanted for classical forms of SCID between 2000 and 2009, the 5-year survival rates for recipients of HLA-matched related grafts for patients transplanted <3.5 months of age or without active infection were 100% and 100% respectively, compared to 93% for patients with active infection at time of transplant [69] (Figure 73.1a).

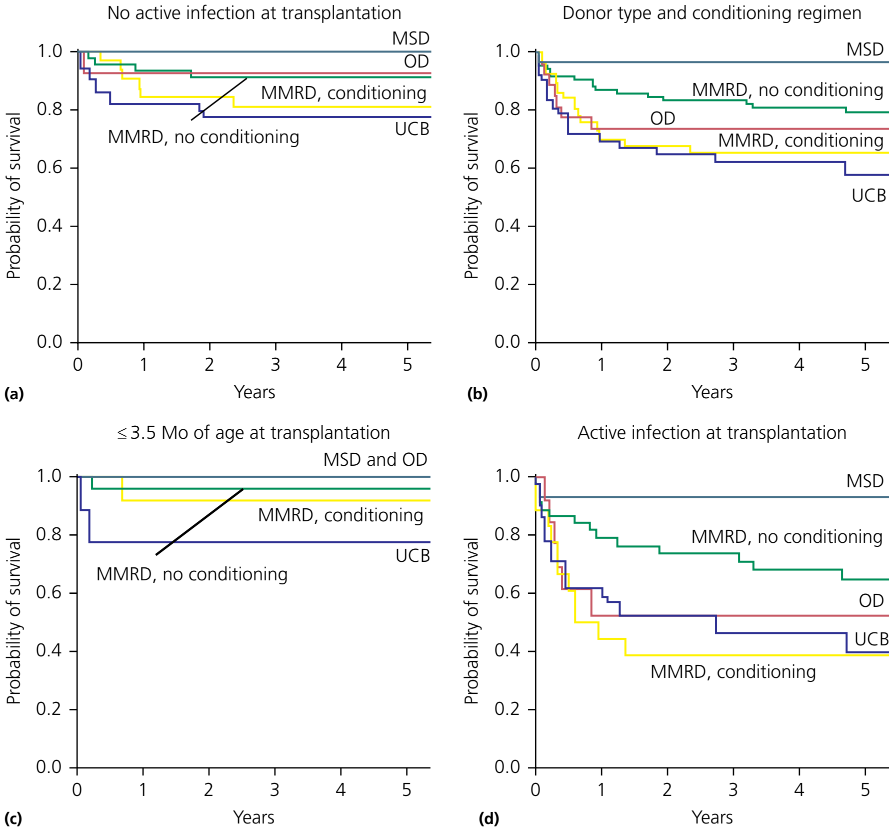

Figure 73.1 Kaplan–Meier estimates of 5-year survival for 240 patients with classical forms of SCID evaluated by the Primary Immune Deficiency Treatment Consortium (PIDTC) who received transplants between 2000–2009. (a) Effect of age at transplant on survival for recipients of matched sibling donor (MSD), other related and unrelated donors (OD), T-cell-depleted mismatched related donors (MMRD) or unrelated cord blood donors (UCB) transplants. (b) Effect of donor type and conditioning on survival in the same groups. (c) and (d) Survival following transplants from different donor types with or without conditioning in (c) – patients without active infection at time of transplant, and (d) – patients with active infection at time of transplant. Source: Pai et al., Transplantation outcomes for severe combined immunodeficiency. *N Engl J Med* 371:434–46, 2014 Copyright © 2014 Massachusetts Medical Society. Reproduced with permission of Massachusetts Medical Society.

For most patients with SCID who receive an HLA-matched sibling graft, including those with functional NK cells and "leaky SCID" patients with a marginal in vitro T-cell proliferative response to phytohemagglutinin (PHA), pretransplant cytoreduction is not necessary to ensure engraftment. An important example of this is ADA-deficient SCID. A recent EBMT analysis of 54 patients with ADA-deficient SCID ($T^-B^-NK^+$) who received either a matched sibling (n = 42) or matched related graft (n = 12), almost always without conditioning, recorded 10-year DFS rates of 83% and 81%, respectively [92]. However, in patients with reticular dysgenesis (SCID with severe neutropenia and thrombocytopenia), administration of cytoreduction has been found to be required to achieve consistent engraftment of hematopoietic as well as lymphoid progenitors [90]. Nevertheless, with cytoreduction, DFS rates of 75% or more have been reported [90].

Following HLA-matched HCT for SCID, prophylaxis for GVHD is usually not given due to the low (0–25%) incidence of significant GVHD in these infants [69,89,90]. For example, of 22 consecutive patients who have received an unmodified HLA-matched sibling BMT for SCID administered without pretransplant cytoreduction at MSKCC since 1974, 18 (95%) survive with immune reconstitution; none have developed GVHD despite lack of prophylaxis and infusion of unmanipulated BM containing a median (range) of 7.18×10^8 ($2.5–10.9 \times 10^8$) total nucleated cells/kg. The low incidence of acute and chronic GVHD may in part be due to the absence of any preexisting tissue injury from cytoreduction [93] as well as the young ages of the patients and their matched siblings.

Immune reconstitution following unmodified HLA-matched related marrow transplants

Following infusion of BM from an HLA-matched sibling, homeostatic proliferation of memory T cells infused with the graft results in normal numbers of circulating T cells within 2–4 weeks post-HCT and rapid restoration of mitogen-induced and antigen-specific T-cell responses [46,89,94,95] (Figure 73.2). Circulating naïve CD45RA$^+$ T cells generated from bone marrow-derived lymphoid precursors that have undergone thymic education are usually not detected until 3–4 months post-HCT. Following an unmodified HLA-matched related HCT, specific B-cell function usually develops by 1 year post-transplant, allowing cessation of monthly intravenous gammaglobulin therapy and subsequent re-immunization [46,89,95]. We have analyzed 10 consecutive recipients of an unmodified HLA-matched sibling BMT administered without preparatory cytoreduction, between 1987 and 2005. All made specific antibody production following vaccination with routine childhood immunizations. None remain on intravenous gammaglobulin.

Alternative donor transplants for SCID

HLA-matched unmodified unrelated BMT for SCID

The first successful unrelated HCT for the treatment of SCID was performed in 1973 by O'Reilly and colleagues [84]. The stem cell source was bone marrow derived from a one-HLA class I antigen disparate female donor which was administered to a male infant with T^-B^- SCID. After multiple transplants from this donor, lymphoid engraftment and full immunologic reconstitution was achieved, complicated by the development of extensive chronic GVHD. This patient ultimately died 9 years post-HCT of metastatic squamous cell carcinoma of the skin.

Due to the lack of a bank of HLA typed normal donors, and the success of T-cell-depleted HLA haplotype disparate parental marrow grafts following their introduction in 1981, transplants of unrelated marrow were not significantly explored in the treatment of SCID until the early 1990s. By then, the National Marrow Donor Program had accrued over 1.6 million volunteer donors, and several centers had successfully employed matched unrelated grafts for hematologic malignancies [96].

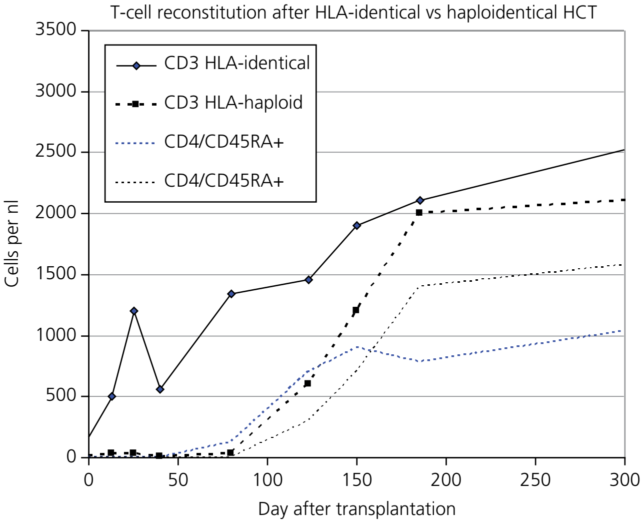

Figure 73.2 Reconstitution of total CD3$^+$ T cells and naïve CD45RA$^+$ CD3$^+$ T cells following unmodified HLA-identical sibling marrow grafts versus T-cell-depleted HLA haplotype disparate parental marrow grafts, each administered without pretransplant conditioning or post-transplant drug prophylaxis for GVHD.

In 1992 Filipovich et al. [97], reported eight SCIDs who received unrelated HCT after cytoreduction with BU/CY, and antithymocyte globulin (ATG), of whom six survived with immunologic reconstitution. An update in 1996 reported 24 SCID patients who received unrelated BMT [98]. The estimated 3-year survival was 61% with grade II–IV GVHD occurring in 17% of engrafted patients [98]. In 2006, Grunebaum et al. [91] reported that, at the University of Brescia and the Hospital for Sick Children in Toronto, 33 of 41 (80.5%) patients who received an unmodified unrelated graft survived with reconstitution of T-cell function; of whom 78% also had B-cell reconstitution. However, acute GVHD was also observed in 73% of patients. Similarly, Newcastle upon Tyne and Great Ormond Street, reported 17 SCID patients who received unmodified matched unrelated grafts after cytoreduction with treosulfan and either fludarabine or cyclophosphamide, of whom 15/17 (88%) survived with T-cell and 9/17 (53%) with B-cell reconstitution [99]. Sequential reports from the European registry Stem Cell Transplant for ImmunoDeficiencies (SCETIDE) documented a 3-year survival rate of 63% for the initial 28 SCID recipients of MUD BMT [90] and 69% for SCIDs who received MUD transplants between 1995 and 2005 [76]. In the PIDTC study, of 240 SCID patients transplanted between 2000 and 2009, 5-year survival for recipients of MUD grafts was 74% [69]. Strikingly, for 186 SCID recipients of T-cell-depleted HLA haplodisparate related grafts transplanted in the same period, 3-year survivals were 66% and 80% for patients who did or did not receive conditioning, respectively [69]. Thus, results of MUD transplants and HLA haplotype disparate related TCD grafts were equivalent and had significantly improved, but were still inferior to the 88% 3-year survival rate for recipients of matched sibling grafts (Figure 73.1b).

T-cell-depleted HLA haplotype disparate related donor transplants

The potential of T-cell-depleted (TCD) transplants was initially suggested by early studies in murine models of allogeneic HCT demonstrating that transplants of hematopoietic cells from fetal liver could reconstitute hematopoiesis in irradiated fully allogeneic recipients without GVHD [100]. In 1973, Keightly et al. [101] extended this approach to man, demonstrating the successful use of fully mismatched fetal liver cells to reconstitute a patient with ADA-deficient SCID without severe GVHD. This case and subsequent reports thus indicated that liver cells obtained from fetuses before initiation of thymic lymphopoiesis (approximately 11 weeks gestation in man) could be transplanted without risk of severe GVHD. However, in a 1983 review of 105 fetal liver transplants [102] only 22 durably engrafted and only 20% of patients survived more than 1 year post-transplant. Nevertheless, in those SCID patients who were engrafted with allogeneic fetal liver-derived hematopoietic cells, the fully allogeneic fetal T cells that developed were able to provide effective resistance against exogenous pathogens but were tolerant of cells of the patient [103].

In the early 1970s, von Boehmer et al. [104] and Reisner et al. [105] demonstrated that H-2 incompatible murine marrow and/or spleen cell transplants depleted of T cells with T-cell specific antibody or a T-cell binding lectin, peanut agglutinin, could also reconstitute lethally irradiated recipients without risk of GVHD. In 1981, our group demonstrated that HLA haplotype disparate parental marrow, depleted of T cells by agglutination with soybean lectin followed by E-rosette depletion was able to reconstitute hematopoiesis in a child with leukemia without GVHD [86]. In 1983, we reported the first successful corrections of SCID by T-cell-depleted HLA haplotype mismatched related BMTs depleted of T cells using the same technique [85]. The three infants described did not receive pretransplant cytoreduction or post-transplant GVHD prophylaxis. All three patients achieved long-term stable donor T-cell chimerism with normal T-cell function and, without acute or chronic GVHD. Similar results with haploidentical SBA$^-$E$^-$ BMT for the treatment of SCID were subsequently reported from the University of Ulm, Germany [43], the University of California Medical Center at San Francisco [106], and Duke University Medical Center, NC [89]. Transplants of TCD HLA disparate related marrow are now the most common type of transplant administered to patients lacking a matched related donor. In the most recent SCETIDE analysis, of 338 patients transplanted for SCID between 1995 and 2005, 25% received genotypically or phenotypically matched related grafts; 20% matched unrelated transplants, and 55% HLA-mismatched TCD transplants from related donors [76]. Similarly, of the 240 patients reported in the PIDTC study, 132 (55%) received HLA disparate T-cell-depleted grafts [69].

The techniques for T-cell depletion that have been utilized for transplantation of infants with SCID include soybean agglutination followed by E-rosette depletion (SBA$^-$E$^-$) [85,86], E-rosetting alone [107], antibody-mediated techniques, such as treatment of the graft with OKT3 [108] or alemtuzumab [109,110], and positive selection of CD34$^+$ progenitors from granulocyte colony-stimulating factor (G-CSF) mobilized PBMC, with or without further T-cell depletion by E-rosetting or monoclonal antibody treatment [69,111]. These techniques differ significantly in their potential to deplete T cells from marrow or cytokine mobilized progenitor cells in the peripheral blood (PBSC). SBA$^-$E$^-$ marrow grafts and CD34$^+$ PBSC selected on the CliniMACS® device reduce T cells by 3 × $\log_{10}$ and 4 $\log_{10}$, respectively [112,113] and have usually been administered without post-transplant GVH prophylaxis. In contrast, T-cell depletion by E-rosetting or treatment with murine T-cell-specific monoclonal antibodies depletes T cells by no more than 2 $\log_{10}$; and the alemtuzumab antibody reduces T-cell numbers by 2.5 $\log_{10}$ [112]. Such transplants have, in the vast majority of cases, only been administered in combination with post-transplant immunosuppressive drugs to prevent GVHD.

Rigorously T-cell-depleted transplants administered without post-transplant immunosuppressive prophylaxis have been associated with low incidences of acute and chronic GVHD and a high probability of survival with full T-cell reconstitution. For example, at our center, SBA$^-$E$^-$ HLA haplotype disparate marrow grafts have been administered to 78 consecutive children with classical or "leaky" forms of SCID since we introduced this approach in 1981, of whom 66% are surviving up to 31 years post-transplant with T-cell reconstitution in all, and B-cell reconstitution in those patients who received myeloablative conditioning (Figure 73.3). The incidence of grade II–IV acute GVHD is 10% at a median follow-up of 18 years. Three patients (6%) died of complications of GVHD. Similarly, Duke University has reported results for HLA haplotype disparate SBA$^-$E$^-$ BMT used to transplant 145 patients with SCID without conditioning pretransplant. Of these patients, 109 (75%) survive with T-cell and, 41% with B-cell reconstitution; 99 (69%) as a direct consequence of the transplant. In this series, the incidence of grade III–IV acute GVHD has also been low (10%). No patient died of GVHD [39,89].

Experience with less rigorously T-cell-depleted HLA haplotype disparate grafts administered with post-transplant administration of drugs such as cyclosporine to prevent GVHD has been associated with a somewhat higher incidence of post-transplant mortality due to GVHD and/or infections. The proportions of patients achieving long-term survival with reconstitution of at least T-cell function have ranged from 56% to 64% in single-center series [46,107,110,114]. In the SCETIDE report of children with SCID transplanted from 1983–1995, the majority of HLA haplotype disparate marrow grafts performed by centers in Europe were depleted of T cells by these less rigorous techniques. The overall survival following such grafts was 52%. The main causes of death were infections (56%), and GVHD (25%) [90].

The introduction of logistically simpler and more effective systems for T-cell depletion based on immunoadsorption to antibody-coated paramagnetic beads has permitted rapid purification of CD34$^+$ progenitor cell fractions from GCSF-mobilized PBSC. Such transplants provide high doses of CD34$^+$ progenitors (5–10 × 10^6/kg) while limiting the T-cell doses to <10^4 CD3$^+$ T-cells/kg. Such grafts can also be administered without post-transplant immunosuppression. At Ulm University, (Figure 73.4) of 71 patients with SCID transplanted with such CD34$^+$ selected, T-cell-depleted grafts fractionated on the CliniMACS device, after busulfan-based conditioning, 70% are surviving with engraftment and reconstitution of T- and B-cell function. Of 11 additional cases who were transplanted without conditioning, 72% survive with T- but not B-cell reconstitution.

In the SCETIDE report of 186 patients with SCID who have received HLA haplotype disparate grafts depleted of T cells by this approach since its introduction in 1996, most of whom also received pretransplant conditioning, 68% were surviving with at least T-cell reconstitution at a median follow-up of 3 years, a result equal to the 68% probability of survival reported by the same group for 66 recipients of marrow grafts from HLA-matched unrelated donors [76].

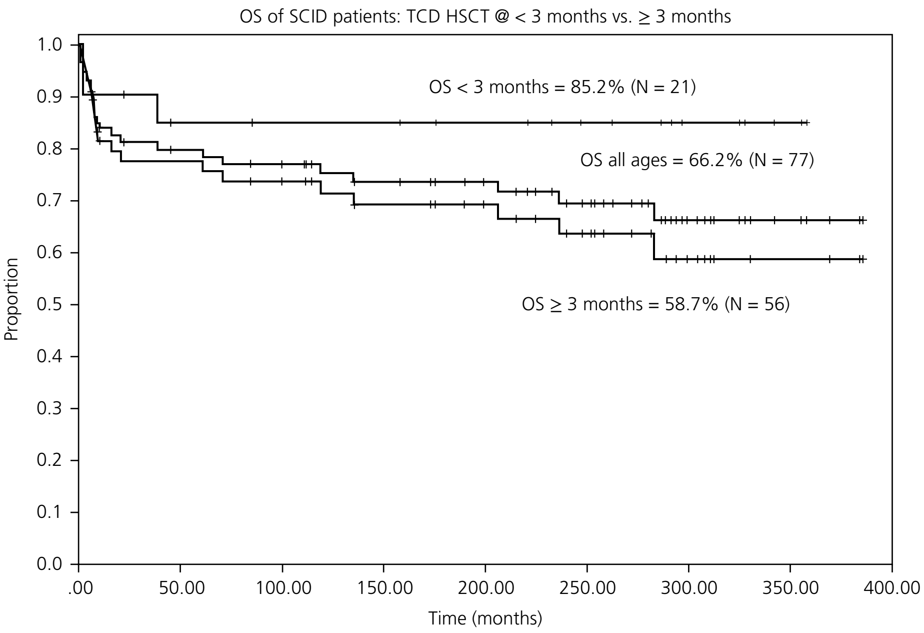

Figure 73.3 Kaplan–Meier estimates of 30-year survival for 71 consecutive patients with classical or "leaky" forms of SCID who received HLA haplotype disparate parental marrow grafts at MSKCC that were depleted of T cells by SBA lectin agglutination and E-rosette depletion. Overall survival (OS) and survivals for patients transplanted before and after 3 months of age are plotted.

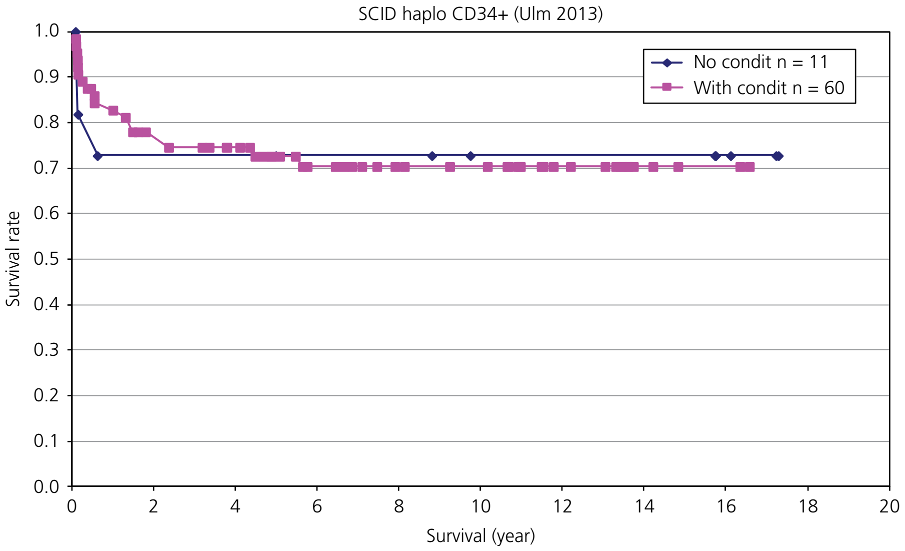

Figure 73.4 Kaplan–Meier estimates of 15-year survival for 71 consecutive patients with classical or "leaky" forms of SCID who received HLA haplotype disparate related transplants at Ulm University that were depleted by positive selection of CD34$^+$ hematopoietic precursors.

In the PIDTC study, of 137 HLA-mismatched grafts, 121 (89%) were depleted of T cells by either the lectin technique or CD34 selection. Overall 5-year survival for this group was 79% for patients who did not receive conditioning and 66% for those who did [69].

Experience with TCD HLA haplotype disparate transplants administered to children with SCID has also led to the recognition of several characteristics of the transplant host that can limit ultimate survival. The most important of these are the age and infection status at the time of transplant. In addition, phenotypic and genotypic characteristics of the patient's variant of SCID may contribute to delayed engraftment or graft failure and impaired reconstitution of lymphoid lineages [90]. Additional modifying influences that have been found to be significant include the type of T-cell depletion employed (e.g., SBA$^-$E$^-$ BMT have resulted in better outcomes than TCD BMT depleted by antibodies in T$^-$B$^-$ SCID [69,90]), the presence or absence of acute GVHD requiring treatment in the patient, and the experience of the transplant center with transplants for SCID [90].

Older patient age and the presence of infection at transplant were first identified as risk factors associated with poor transplant outcomes for patients with SCID by Stephan et al. [46] in 1993 and confirmed by Buckley et al. [89] in 1999 and most recently in a retrospective analyses of patients transplanted for SCID in Europe between 1968 and 2005 [76] and North America between 2000 and

2009 [69]. The striking advantages of a transplant administered to patients with SCID in early infancy was first demonstrated by Myers et al. [75] who reported a 95% survival rate for 22 infants with SCID transplanted within the first 3.5 months of life, 19 of whom received haplodisparate $SBA^{-}E^{-}$ BMT. Only one patient in that series developed an opportunistic infection. Similarly, Brown et al. [45] analyzed 108 patients with SCID, comparing 48 who were the first affected in the family and were not diagnosed until a median of 143 days of age with 60 siblings who were diagnosed either antenatally or at birth. Of those receiving HLA haplotype disparate TCD grafts, 87.5% of the siblings diagnosed at or before birth survived with reconstitution compared to 50% of the transplanted probands. Most of this difference was attributed to reductions in infection-related mortality in the sibling as compared to the proband group.

The relative contributions of earlier transplant and infections have recently been analyzed by the PIDTC. In this series, the age distribution of the 240 patients at transplant was bimodal, with an inflection at 3.5 months. Strikingly, 5-year survival rates for the younger infants who received HLA haplotype disparate T-cell-depleted related grafts, unmodified matched grafts from unrelated donors or cord blood transplants (95%, 100%, and 77%, respectively) did not differ significantly from that of matched sibling grafts (100%). In contrast, survival rates were significantly inferior to that of matched siblings in patients transplanted after 3.5 months of age. However, for those patients transplanted at any age who did not have an active infection or whose prior infection had resolved, 5-year survival rates were 90% and 82%, respectively [69]. These studies underscore the potential impact of early diagnosis and transplant, prior to development of infectious complications, to long-term outcome, particularly when immediately accessible HLA haplotype disparate are the donors.

The PIDTC study also demonstrated that for patients of any age with active infection at the time of transplant, pretransplant conditioning of any type prior to any transplant other than a matched sibling graft was associated with a significantly reduced 5-year survival (matched unrelated 55%; CBT 40%; TCD haplotype disparate transplants 39%) compared to matched sibling grafts and TCD mismatched grafts administered without conditioning (93% and 65%, respectively). Thus, for infants with a classical SCID phenotype [8] with active infection, an initial transplant without conditioning provides a higher likelihood of 5-year survival (Figure 73.1c vs. d).

While these findings bring into question the necessity or even the advisability of conditioning in infected patients presenting a classical SCID phenotype who are to receive transplants other than a matched sibling graft, the relatively large cumulative experience with HLA haplotype disparate TCD grafts in children with SCID has also demonstrated that "leaky" forms of SCID [8] and specific variants of SCID distinguishable by both functional phenotype and genotype, exhibit varying degrees of resistance to initial engraftment of donor hematopoietic cells and reconstitution of T cells and their function. Furthermore, if myelosuppressive conditioning is not employed, engraftment of donor B cells and reconstitution of humoral immunity is uncommon irrespective of the patient's variant of SCID.

The mechanisms contributing to resistance to engraftment of T-cell precursors in HLA disparate T-cell-depleted transplants, and to engraftment and development of B cells irrespective of transplant type in different variants of SCID, are still unclear. However, analysis of patients transplanted for these disorders as well as studies of murine models of these gene defects have provided correlative evidence implicating specific cell systems in the host as mediators of this resistance.

NK cells have been well documented to contribute to graft resistance in murine models of marrow transplantation [115]. Our group and others have also correlated the level and functional activity of host NK cells with graft failure in unconditioned SCID patients [109,116–118]. However, this correlation was not initially observed in the large series transplanted at Duke [89] although in subsequent analyses of their series, they do note that patients with the NK^{+} RAG1 and RAG2 variants of SCID have had markedly delayed reconstitution and have more frequently required secondary "booster" transplants [119]. Whether these discrepancies reflect differences in the function or maturation of NK cells in different genotypic variants of SCID, or interactions of HLA haplotype disparate donor hematopoietic cells with the genetically determined inhibitory and activating killer immunoglobulin-like receptors (KIR) expressed by host NK cells is still unclear. In an analysis of patients with NK^{+} SCID transplanted by the Duke group, donor KIR ligand absence was not significantly correlated with failure to reconstitute T-cell functions, but the group analyzed was small ($n = 22$) [120].

Residual T cells in the host may also contribute to the graft resistance observed in certain variants of SCID. For example, the oligoclonal autoreactive T cells regularly found in patients with Omenn syndrome can react against donor hematopoietic cells [121,122]. Similarly, T cells in ADA-deficient SCID patients including alloreactive T cells can be activated in the presence of ADA provided by normal blood cells [123]. Such T cells likely contribute to the enhanced resistance observed in these patients after TCD BMTs administered without cytoreductive conditioning.

Competition between host and donor thymic precursors for access to niches within the thymus essential to early thymocyte development may also prevent or impair the development and sustained engraftment of marrow progenitors from a normal donor. For example, repopulation of the thymus by wild-type marrow cells is prevented in $RAG^{-/-}$ mice in which the hosts' double negative thymocytes already occupy available sites in the thymic cortex [124]. Hypomorphic RAG1 and DNA ligase 4 mutations may also disrupt the function of epithelial cells in the thymic medulla [66] resulting in markedly reduced generation of Fox $P3^{+}$ regulatory T cells, which may result in the uncontrolled generation of oligoclonal autoaggressive T cells in Omenn syndrome. In contrast, wild-type lymphoid progenitors readily repopulate the thymus and reconstitute T-cell function in $\gamma c^{-/-}$ and $IL\text{-}7R^{-/-}$ mice [66,124]. Results in $IL\text{-}7R^{-/-}$ mice are particularly informative, since $IL\text{-}7R^{-/-}$ variants of SCID are usually NK^{+}.

The presence of transplacentally derived maternal T cells in the circulation prior to transplant could also contribute to graft failures following T-cell-depleted transplants administered without conditioning when haplotype disparate related donors other than the mother are employed. This likely explains the higher rates of engraftment observed in such patients following maternal TCD grafts [125]. Strikingly, maternal T cells can be detected prior to transplant in a proportion of patients with all classical forms of SCID, particularly those with $\gamma c^{-/-}$ variants of SCID who regularly achieve engraftment and reconstitution of donor-derived T cells without conditioning following HLA haplotype disparate maternal TCD marrow grafts, but are rarely detected in patients with Omenn syndrome [46,68,69]. Thus, transplacental engraftment of maternal T cells may provide a surrogate marker of a lack of graft resistance.

The resistance to engraftment that is displayed by certain variants of SCID has had a major impact on the engraftment and function of donor-derived T-cell precursors as well as ultimate outcomes of

HLA haplotype disparate TCD transplants administered without cytoreduction. Thus in our own and the other series 85–100% of patients with γcR$^{-/-}$ and JAK3 mutant T$^-$B$^+$NK$^-$ forms of SCID have achieved engraftment and sustained T-cell reconstitution without cytoreduction [69,89,126]. In contrast, engraftment has been less consistent in patients with the T$^-$B$^-$NK$^+$ variants of SCID if pretransplant conditioning has not been employed [116]. This has resulted in a significantly inferior outcome for these variants. In ADA-deficient SCID, of 40 patients reviewed by Hassan et al. [92], the graft failure rate was 84% for HLA haplodisparate TCD BMT. Even for recipients of unmodified grafts from HLA-matched siblings, the risk was 13%. Similarly, of the 16 ADA$^-$ SCID patients reported by the Duke group [39] who are long-term survivors following a HLA haplotype disparate TCD HSCT without cytoreduction, seven subsequently required an alternate treatment to achieve reconstitution (4 PEG ADA; 2 ADA gene therapy; 1 MUD HSCT with conditioning). Patients with deficiency of CD3δ who present with a T$^-$B$^+$NK$^+$ phenotype also fail to engraft, even after HLA-matched sibling transplants, if they do not receive myeloablative or reduced-intensity myelosuppressive conditioning. In a series of 13 cases, Marcus et al. [127] reported graft failures in 2/2 mismatched related TCD grafts and 1/2 CBT administered without conditioning, while those receiving preparatory conditioning exhibited more consistent engraftment and T-cell reconstitution. Similarly, of six reported patients with IL-7Rα$^{-/-}$ SCID, four failed to engraft after an initial transplant administered without conditioning but engrafted with reconstitution of T-cell immunity after a second transplant administered after conditioning with a busulfan- or fludarabine-containing regimen [44,89,126].

In contrast, engraftment of HLA haplotype disparate TCD marrow or PBSC has been achieved in a high proportion of patients with each of these SCID variants when they have received conditioning with myeloablative or myelosuppressive conditioning regimens including busulfan, thiotepa, or treosulfan together with cyclophosphamide or fludarabine. As a consequence, the probability of reconstitution by donor T cells and sustained normalization of T-cell immunity is markedly improved. For example, in the combined experience of MSKCC and ULM, of 13 patients with ADA$^-$ SCID who received parental SBA$^-$E$^-$ BMT after conditioning with busulfan and cyclophosphamide, all engrafted and 10 (77%) are long-term survivors with T- and B-cell reconstitution [53]. Similarly, Giliani et al. [128] reported 16 patients with IL-7Rα$^-$ SCID. Of the six patients that received cytoreduction prior to a TCD HLA haplotype disparate HCT, all six engrafted with reconstitution of T-cell immunity and long-term survival.

For patients with SCID associated with either Omenn syndrome or reticular dysgenesis, cumulative evidence indicates that at least reduced-intensity conditioning prior to transplant is indicated to secure engraftment and functional reconstitution following a TCD HLA haploidentical related HCT [109]. In patients with Omenn syndrome, early transplants administered without cytoreduction have been associated with a high incidence of graft failure [109,129]. In contrast, Mazzolari et al. [130] reported that 4/5 patients transplanted with HLA haplotype disparate TCD HCT and 3/4 recipients of unmodified marrow from matched unrelated donors transplanted after cytoreduction with busulfan and cyclophosphamide ultimately achieved full engraftment and survived with T- and B-cell reconstitution. Thus, such cytoreduction can overcome the graft resistance thought to be mediated by the small numbers of autoreactive T cells and NK cells consistently detected in these patients. Similarly, in a European compendium of patients with reticular dysgenesis, of five transplanted without high doses of busulfan and cyclophosphamide, none survived with sustained engraftment, while each of five patients treated with this high-dose combination engrafted, of whom three are long-term survivors [131].

Immune reconstitution following HLA haplotype-disparate T-cell-depleted transplants

Following an SBA$^-$E$^-$ T-cell-depleted mismatched related BMT, the majority of patients will develop normal numbers of CD3 (Figure 73.2), CD8, and CD4 T cells by 3–6 months post-HCT, irrespective of whether cytoreduction is employed [1,89]. Approximately 50% of patients will have a normal PHA response by 6 months post-transplant and 80% will have normal T-cell responses to mitogens and antigens by 6–12 months (reviewed in [1,89,90]). Myers et al. [75] also demonstrated that infants who receive an SBA$^-$E$^-$ mismatched parental BMT within 28 days of life develop higher numbers of CD45RA$^+$ and T-cell receptor excision circle (TREC) positive T cells, and more robust T-cell proliferative responses within the first 3 years post-transplant than infants transplanted at later time points. In a large European study of mismatched TCD HCT recipients using a variety of TCD techniques, the median time to develop normal T-cell proliferative responses to tetanus toxoid was 8.7 months [132]. Of the 116 patients in this study who survived at least 6 months post-HCT only 43% of those with absent T-cell function at 6 months ($n = 21$) ultimately developed normal T-cell responses, compared to 71% of those patients with normal or near-normal T-cell function 6 months post-transplant [132]. The quality and kinetics of T-cell reconstitution in the European series correlated with SCID phenotype, with a higher percentage of patients with B$^+$ SCIDs exhibiting normal T-cell function at all time points >6 months post-HCT [132] compared to patients with B$^-$ SCID. Similar findings have been reported in the PIDTC study [69].

Recent evaluations of patients with SCID 10–15 years after successful HLA haplotype disparate TCD grafts have focused attention on those characteristics of host and HCT that sustain thymic output and normal T-cell function. Initially, Patel et al. [133] showed that the average number of TREC$^+$ early thymic emigrant T cells, naïve T cells, and T-cell mitogen responses decreased with time after such transplants suggesting that the pool of thymic precursors transferred in the graft might be limited, leading to accelerated age-associated reduction in thymic output.

Borghans et al. [134] evaluated predictors of long-term T-cell reconstitution in a heterogeneous group of 19 SCID patients evaluated 5–32 years post-HCT. Unlike the patients in the study of Patel et al. [133] who received SBA$^-$E$^-$ HCT without prior conditioning, 74% of patients in this study were cytoreduced and 14 of 19 patients received transplants T-cell depleted by E-rosetting together with post-transplant GVHD prophylaxis. In 11 of 19 patients, there was no evidence of impaired T-cell reconstitution, naïve T-cell production, or reduced telomere length post-HCT when compared to age-matched controls. In the eight patients with impaired T-cell reconstitution late after transplant, these parameters were already abnormal early after HCT, suggesting that impaired engraftment rather than reduced thymic output with time was the etiology of the poor T-cell reconstitution.

In contrast to the findings of Patel et al. [133] and in accord with the findings of Borghans et al. [134], three more recent studies have shown that those patients with SCID who have received haplotype disparate TCD HCT after myeloablative conditioning and who have donor cells in their peripheral myeloid lineages or among the CD34$^+$ progenitor cells in the marrow late after transplant, also have sustained normal levels of TREC$^+$ T cells without evidence of a decline in T-cell numbers or function [114,135,136]. The study of Friedrich et al. [136] is particularly informative, since 25 of the 31 patients studied had received HLA haplotype disparate SBA$^-$E$^-$ T-cell-depleted marrow grafts, either with (n = 13) or without (n = 12) pretransplant cytoreduction. Sustained engraftment of donor type CD34$^+$ progenitor cells was regularly observed in the cytoreduced group and was directly correlated with persistently normal numbers of TREC$^+$ and CD45RA$^+$ T cells. Donor CD34$^+$ cells were not detected in the non-cytoreduced group. In contrast to those transplanted after cytoreduction, patients who had not received pretransplant cytoreduction also exhibited significant reduction of CD45RA$^+$ T cells 15 years post-transplant. Each of these studies supports the ability of the epithelium of the SCID thymus to support robust thymopoiesis in most patients [133–137], for at least two decades. Further follow-up by the Duke group suggests that after the first 10 years, TREC production and T-cell diversity stabilize [138]. However, declines in the generation of naïve thymic emigrant T cells in patients who received no cytoreduction prior to transplant remains a concern [133], and suggests that thymopoiesis may not be sustained if long-term engraftment of hematopoietic stem cells capable of continuously providing thymic precursors is not achieved [135,136]. The use of booster grafts of TCD bone marrow, without cytoreduction, to enhance T-cell function in patients with prolonged poor T-cell reconstitution has yielded mixed results. Slatter and colleagues have reported that boosts of TCD HCT following initial transplant, in the absence of cytoreduction, do not improve T-cell function [139]. However, boosts administered after reduced-intensity conditioning may provide benefit [140].

Reconstitution of specific antibody formation following an SBA$^-$E$^-$ mismatched related HCT, as with other TCD techniques, is usually observed only in those patients given pretransplant cytoreduction that includes alkylating agents that are toxic for hematopoietic stem cells, such as busulfan, treosulfan, thiotepa, or melphalan and is correlated with the degree of donor B-cell engraftment (reviewed in [1,69,99,139]). In the MSKCC series, normal antibody responses following immunization have been observed in all patients engrafted with donor B cells, compared to less than 10% of patients with exclusively host B cells (reviewed in [1]). In a retrospective analysis of 90 patients transplanted at Hôpital Necker-Enfants Malades, Neven et al. [41] also noted that following myeloablative doses of busulfan and cyclophosphamide, transplants of HLA disparate TCD marrow induced both mixed myeloid and B-cell chimerism in a high proportion of cases, with reconstitution of both B- and T-cell immunity. Similarly, Mazzolari et al. [114], who analyzed 40 patients with SCID who received matched related or unrelated unmodified or HLA disparate related TCD grafts, found that only 1/11 patients who received either no conditioning or conditioning with ATG alone engrafted with donor B cells compared to 26/29 patients who had been conditioned with ablative doses of busulfan and/or thiotepa or melphalan. However, in a single-center study by Haddad et al. [141] from the Hôpital Necker-Enfants Maladies, donor B-cell engraftment was documented in only 1/9 recipients of TCD mismatched related BMT administered after cytoreduction with lower dose busulfan (8 mg/kg) and 200 mg/kg cyclophosphamide, compared with 2/8 who did not receive conditioning. Thus, myeloablative doses of busulfan, melphalan, thiotepa, or treosulfan have been required to induce more consistent engraftment of donor B cells and reconstitution of humoral immunity in most recipients of partially matched unrelated and HLA haplodisparate TCD grafts.

The requirement for engraftment of donor B cells and/or their precursors to achieve reconstitution of humoral immunity is far from absolute, however. The argument that endogenous B cells detected in several variants of SCID are intrinsically defective and need to be replaced is not supported by the fact that antibody production is usually reconstituted following HLA-matched grafts without conditioning, even though the B cells are often of host type. This has recently also been demonstrated for a significant proportion of patients transplanted with HLA haplodisparate TCD marrow without conditioning to correct IL-7R$\alpha^{-/-}$, CD3$^{-/-}$, or ADA$^-$ variants of SCID [42,142]. Furthermore, Haddad et al. [141] have shown, by allele typing, that the antibodies detected in such cases are, indeed, of host type. In contrast, reconstitution of antibody production by host B cells is rare in $\gamma c^{-/-}$ or JAK3$^{-/-}$ SCID [42], and not seen in T$^-$B$^-$ forms of SCID associated with mutations affecting V-D-J recombination such as mutations in RAG1, RAG2, Artemis, and DNA ligase IV. Nevertheless, these instances of host B-cell-mediated reconstitution of humoral immunity have spawned an ongoing debate regarding the need, or even the advisability of myeloablative conditioning in many cases. This is a particular concern for infected patients but is also a concern for those diagnosed and transplanted in the newborn period, when myeloablation may, on the one hand, be less effective in securing donor B-cell precursor engraftment because of the resilience of host hematopoietic cells at this age and, on the other, be most likely to induce long-term damage to other developing tissues and organs, including the CNS.

Major challenges for the future thus include: (1) development of better techniques with which to define those patients whose B cells by virtue of their genetic and functional characteristics or their stage of development, can effectively cooperate with donor-derived T cells; (2) exploration of alternative transplant strategies, such as tandem transplants in which an initial transplant is given in the newborn period without cytoreduction to initially reconstitute T-cell function, and a secondary transplant is administered after myelosuppressive treatment at 18–24 months of age when brain development is complete and the patient is otherwise healthy; and (3) the development of less toxic, more targeted approaches to create a niche and/or secure engraftment of donor-derived hematopoietic stem cells and/or B-cell precursors capable of providing long-term reconstitution of humoral immunity.

Many variants of SCID are also associated with deficiencies of NK cells and their functions. Prominent among those are the $\gamma c^{-/-}$ or JAK3$^{-/-}$ variants of SCID. Without pretransplant conditioning, such patients transplanted with HLA disparate TCD grafts or unmodified grafts from matched unrelated donors usually remain NK deficient. Although conditioning with busulfan-containing regimens prior to transplant secures reconstitution of donor-derived NK cells and their function in most patients, it is not achieved in 13–30% of patients with $\gamma c^{-/-}$ or JAK3$^{-/-}$ SCID [41,114,141]. The significance of persistent NK cell deficiencies is not clear. The possibility that continuing deficiency of NK cells observed in patients with JAK3$^{-/-}$ or $\gamma c^{-/-}$ SCID patients post-transplant could contribute to the high incidence of severe HPV-associated warts, has been considered, but this complication has also been observed in patients with those variants of SCID who do achieve reconstitution of NK-cell function [41].

Umbilical cord blood cell (CBC) transplants for SCID

Umbilical cord blood transplants (CBT) have only recently been explored as an alternative source of allogeneic hematopoietic cells for transplantation of children with SCID and other immune deficiencies. However, experience with CBT in leukemia and marrow failure syndromes has demonstrated their potential to provide long-term hematopoietic and lymphoid reconstitution and has also underscored several potential advantages over unmodified unrelated marrow or PBSC transplants from adults, including a significantly reduced potential to induce severe GVHD in recipients of comparable HLA disparity, their immediate accessibility, and the broader applicability of CBTs from current, selectively recruited cord blood banks to patients from minorities underrepresented in international marrow donor registries.

In 2000, Knutsen and Wall [143] described eight infants with severe T-cell immunodeficiency disease who received an unrelated CBC transplant, seven of whom engrafted. Despite significant HLA disparity between cord blood donor and recipient, only one patient developed severe GVHD. T- and B-cell immunity recovered 60–100-days post-transplant. Similarly, Buckley et al. [89] used unrelated CBTs with BU/CY conditioning to rescue two children with X-linked SCID who rejected a TCD mismatched related BMT administered without cytoreduction. More recently, a retrospective analysis conducted by two European registries, Eurocord and Stem Cell Transplant for Immunodeficiencies in Europe (SCETIDE) compared results of CBT and HLA-mismatched related donor-derived T-cell-depleted transplants performed between 1995 and 2005 [144]. In the CBT group ($n = 74$), 67% of the CBT were matched or 1 HLA allele disparate. In contrast, 68% of the MMRD grafts were mismatched for a full haplotype. Although the proportion of patients in the MMRD group who received no or reduced-intensity conditioning was significantly higher, the proportions of patients achieving engraftment were not significantly different (CBT: 86% vs. 78% MMRD). However, the proportion of patients with both myeloid chimerism and independence of IVIG supplementation, was higher in the CBT group, consistent with the higher intensity of conditioning administered to all but 10% of the CBT recipients. Despite the greater level of HLA disparity in the MMRD group, the incidence of grade II–IV acute GVHD was significantly lower (22% in the MMRD group vs. 34% in the CBT group). The overall 5-year survival rate for the CBT group was 57% versus 62% for the MMRD group (NS). However, these good results principally reflect survival of recipients of HLA-matched or 5/6 HLA allele-matched grafts (76% and 62%, respectively). For recipients of CBT that were mismatched for ≥2 HLA alleles, 5-year survival was only 35%. In the recent PIDTC study [69], 43 patients received CBT from HLA-matched or ≥4/6 allele-matched donors. Overall 5-year survival was 58%; 77% for patients transplanted without active infection and 40% for patients with infection at time of transplant (Figure 73.1c, d).

In utero HCT for SCID

In 1996, Flake et al. reported the first successful correction of SCID by a T-cell-depleted haploidentical parental marrow graft administered in utero [145]. This patient had X-linked SCID that was diagnosed during the first trimester. The patient received three infusions of TCD BM cells containing 114.0, 8.9, and 6.2×10^6/kg $CD34^+$ cells and $<5.2\ 10^5$ $CD3^+$ cells/kg fetal weight. The cells were injected into the abdomen of the fetus between 15.0 and 18.5 weeks gestation. Circulating donor T cells were observed at birth, at 3 and 6 months thereafter. However, B cells, NK cells, monocytes, and granulocytes remained of host origin. The engrafted paternal T cells were tolerant to the patient and demonstrated normal in vitro T-cell proliferative responses. Subsequent analysis of this patient has demonstrated persistent engraftment of donor T cells with specific T-cell function and antibody production 4 years post-HCT.

In 2005, Muench et al. [146] summarized the published results of in utero transplants in 10 patients performed between 1996 and 2005, to correct γc- ($n = 5$) or IL-7Rα-deficient SCID ($n = 1$), Omenn syndrome ($n = 1$), or genetically uncharacterized T^-B^+ ($n = 1$), T^-B^- ($n = 1$), $T^-B^+NK^+$ ($n = 1$) SCIDs. Of the 10 patients with SCID, 9 received parental, TCD BM, or PBSC. Engraftment of T lymphocytes with or without donor NK cells was observed in eight patients, of whom seven achieved full functional reconstitution with donor T cells and, where evaluated, NK cells. As shown by Piroveno et al. [147], who had transplanted 5 of these 10 cases, T-cell engraftment and functional reconstitution was consistently observed in patients with the T^-B^+, $\gamma c^{-/-}$, and IL-$7R\alpha^{-/-}$ variants, but was either not detected or remained at low levels in two patients with T^-B^- SCID, of whom one had a RAG2-deficient Omenn syndrome. Furthermore, B-cell reconstitution has been detected in only two patients [145]. Nevertheless, no patients with T-cell engraftment had evidence of GVHD at birth, nor did they develop GVHD thereafter. Furthermore, engrafted and functional paternal graft-derived T cells have exhibited no alloreactivity against host-derived tissues or hematopoietic cells. Thus, although experience with intrauterine transplants for SCID remains very limited, these transplants reiterate both the potential and the limitations of HLA disparate T-cell-depleted transplants administered postnatally. They also indicate that the constraints to engraftment and, particularly, B-cell reconstitution reflect limitations to the development of normal donor-derived lymphoid progenitor cells that are already operative by 20–24 weeks gestation when the transplants have usually been administered.

Long-term complications in patients transplanted for SCID

Experience with transplants for SCID includes many patients followed for over 40 years, and has thus permitted copious assessments of long-term efficacy, the durability, and function of the transplants, and the late complications attributable to these diseases and/or their treatment. As reflected by the 85–100% rates of survival recorded for these patients at intervals of 10, 20, and 30 years post-transplant [39,41,114], recipients of HLA-matched related transplants are, with rare exceptions, in good health and leading normal lives, with stable engraftment of at least donor T cells, and, in most cases, stable reconstitution of both T- and B-cell immunity. Recurrent or severe infections or autoimmune disorders are rare in these patients after the first year post-transplant, by which time immune reconstitution has been achieved [39,41,114]. However, up to 10% of these patients have been reported in one series to exhibit subnormal height and/or weight [114]. This has usually been ascribed to prolonged steroid treatment for GVHD.

Among recipients of HLA-matched unmodified unrelated grafts or T-cell-depleted HLA disparate related transplants, late complications have been more frequent. These have included autoimmune disorders such as hemolytic anemias, autoimmune myositis, thyroiditis, and hepatitis in 2 to 15% of cases [39,41,114], recurrent or chronic sinopulmonary infections in up to 20% of patients [39], and recurrent intermittent gastrointestinal disorders, including diarrhea and GERD not related to GVHD in 16–20% of cases. Each of these complications has occurred more commonly in patients who were transplanted at ≥3.5 months of age [39], and particularly those who still had subnormal numbers and function of T cells at 1-year post-transplant especially those transplanted for T^-B^- variants of SCID, such as the $RAG1^{-/-}$ and Artemis variants [41]. It is also noteworthy that recurrent or protracted sinopulmonary infections are particularly observed among patients requiring supplemental IVIG. Moderate to severe HPV infections involving hands and feet have also been recorded in up to 26% of patients, particularly among patients transplanted for the $\gamma c^{-/-}$ and JAK-3$^{-/-}$ variants of SCID [39,41,114] and have occurred in these patients irrespective of the type of transplant administered.

Certain late complications may also be induced by preparatory myeloablative conditioning, including short stature, sterility, and abnormally formed permanent teeth. However, reliable estimates of these complications are not yet available. Other late abnormalities may be sequelae of the variants of SCID treated, such as the impaired neurocognitive development observed in a proportion of patients transplanted for ADA$^-$ SCID [53], or the result of severe infections, including encephalitis, occurring prior to or in the immediate post-transplant period [41,114].

Transplantation for other combined immunodeficiency syndromes (CID) and life-threatening genetic disorders of lymphocyte development or function

In addition to the hypomorphic or “leaky” genetic variants of SCID, a series of combined immune deficiencies (CIDs) have been identified and recently genetically characterized that affect, to varying degrees, T- and B-cell populations and their functions. These disorders include syndromes associated with profound deficiencies in response to either a broad or narrow range of exogenous pathogens. However, patients with these disorders usually retain a capacity to mount T-cell responses against allogeneic cells. As a consequence, immunoablative and myelosuppressive conditioning is usually required to consistently secure engraftment.

A consensus list of recognized CIDs has recently been reported [148] and those for which transplants have been repeatedly administered are summarized in Table 73.2, along with the distinguishing immunologic and clinical features of each disorder. These CIDs can be roughly divided into seven categories: (1) disorders of nucleotide metabolism, (2) defects of DNA recombination and repair, (3) defects in intracellular signaling molecules, (4) defects in cytokine generation, (5) defects of cytolytic effector function, (6) defects of cytoskeleton plasticity or lymphocyte effector function, and (7) defects of immune regulation.

Each of these disorders can be and has been corrected by an allogeneic HCT from a normal donor. Early results for transplants applied to patients with these disorders were, in aggregate, inferior to those reported for transplants applied to patients with SCID or Wiskott–Aldrich syndrome. For example, in the European experience of HCT for non-SCID patients with T-cell immunodeficiency performed between 1968 and 1993, 3-year overall survival was 63% for 47 recipients of an HLA-matched sibling graft and only 35% in 72 recipients of an HLA-mismatched T-cell-depleted graft [90]. In comparison, survival rates for patients with SCID receiving grafts at the same centers were 81% and 54%, respectively. However, many of these disorders are so rare that experience with allogeneic HCTs as treatment was limited to 1–3 patients at any given center. Recently, however, analyses of the cumulative experiences at collaborating centers specializing in the transplantation of patients with several of these diseases have been reported, permitting better estimates of transplant efficacy in their treatment. The results of such analyses for specific disorders can be briefly summarized.

Table 73.2 Combined immune deficiencies repeatedly corrected by transplantation

Disease	Circulating T cells	Circulating B cells	Serum Ig	Associated features	Inheritance	Genetic defects/presumed pathogenesis
METABOLIC DISORDERS						
(a) Purine nucleoside phosphorylase deficiency (PNP)	Progressive decrease	Normal	Normal or decreased	Autoimmune hemolytic anemia; neurologic impairment	AR	Absent PNP, T-cell and neurologic defects from elevated toxic metabolites (e.g., dGTP)
DISORDERS ASSOCIATED WITH DEFECTS OF DNA REPAIR						
(a) Cernunnos/ XLF deficiency	Decreased	Decreased	Decreased	Microcephaly; in utero growth retardation; radiation sensitivity	AR	Cernunnos defect resulting in impaired NHEJ
(b) DNA ligase IV	Decreased	Decreased	Decreased	Microcephaly; facial dystrophy; radiation sensitivity	AR	DNA ligase IV defect resulting in impaired non-homologous end joining (NHEJ)
(c) Nijmegen breakage syndrome	Progressive decrease	Normal	Often decreased IgA, IgE, and IgG subclasses; increased IgM monomers; antibodies variably decreased	Microcephaly; bird-like face; lymphomas; ionizing radiation sensitivity; chromosomal instability	AR	Hypomorphic mutation in NBS1 (Nibrin); disorder of cell cycle checkpoint and of DNA double- strand break repair
DISORDERS OF INTRACELLULAR AND INTERCELLULAR SIGNALING						
(a) Cartilage–hair hypoplasia	Decreased or normal	Normal	Normal or reduced. Antibodies variably decreased	Short-limbed dwarfism with metaphyseal dysostosis; sparse hair; anemia; neutropenia; susceptibility to lymphoma and other cancers; impaired spermatogenesis; neuronal dysplasia of the intestine	AR	Mutation in *RMRP* encoding the RNA segment of the mitochondrial RNA processing endoribonuclease which, with TERT, modulates cell proliferation
(b) Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID)	Impaired generation of memory T-cells	Present	Impaired antibody responses	Anhidrotic ectodermal dysplasia + specific antibody deficiency (lack of Ab response to polysaccharides); various infections (mycobacteria and pyogens)	XL	Mutations of *NEMO (IKBKG)*, resulting in impaired NF-κB activation
(c) Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID)				Anhidrotic ectodermal dysplasia + T-cell defect + various infections	AD	Gain-of-function mutation of *IKBA,* resulting in impaired activation of NF-κB

(*Continued*)

Table 73.2 (*Continued*)

Disease	Circulating T cells	Circulating B cells	Serum Ig	Associated features	Inheritance	Genetic defects/presumed pathogenesis
(d) Dock 8 deficiency-associated autosomal recessive hyper IgE syndrome	Normal	Normal	Elevated IgE	Susceptibility to bacterial, viral and fungal infections; eczema; vasculitis; CNS hemorrhage. No skeletal or connective tissue abnormalities	AR	Dock8 mutations
(e) CD40 ligand deficiency	Normal	IgM and IgD B memory cells present, but others absent	IgM increased or normal, other isotypes decreased	Neutropenia; thrombocytopenia; hemolytic anemia; biliary tract and liver disease; opportunistic infections	XL	Defects in CD40 ligand (CD40L); defective B and dendritic cell signaling
(f) MHC class I deficiency	Decreased CD8, normal CD4	Normal	Normal	Vasculitis	AR	Mutations in *TAP1*, *TAP2* or *TAPBP* (tapasin) genes giving MHC class I deficiency
(g) MHC class II deficiency	Normal number, decreased CD4 cells	Normal	Normal or decreased		AR	Mutation in transcription factors for MHC class II proteins (*C2TA*, *RFX5*, *RFXAP*, *RFXANK* genes)
DISORDERS OF CYTOLYTIC EFFECTOR FUNCTION						
(a) Familial hemophagocytic lymphohistiocytosis (FHL) syndromes						
(i) Perforin deficiency	Normal	Normal	Normal	Severe inflammation; fever; decreased NK and CTL activities	AR	Defects in *PRF1*; perforin, a major cytolytic protein
(ii) Munc 13-D deficiency	Normal	Normal	Normal	Severe inflammation; fever; decreased NK and CTL activities	AR	Defects in *MUNC13D* required to prime vesicles for fusion
(iii) Syntaxin 11 deficiency	Normal	Normal	Normal	Severe inflammation; fever; decreased NK and CTL activities	AR	Defects in *STX11*, involved in vesicle trafficking and fusion
(b) Immunodeficiency with hypopigmentation						
(i) Chediak-Higashi syndrome	Normal	Normal	Normal	Partial albinism; giant lysosomes; low NK and CTL activities; heightened acute-phase reaction; encephalopathic accelerated phase	AR	Defects in *LYST*, impaired lysosomal trafficking
(ii) Griscelli syndrome, type 2	Normal	Normal	Normal	Partial albinism; low NK and CTL activities; heightened acute phase reaction; encephalopathy in some patients	AR	Defects in *RAB27A* encoding a GTPase in secretory vesicles
(iii) Hermansky–Pudlak syndrome, type 2	Normal	Normal	Normal	Partial albinism; neutropenia; low NK and CTL activity; increased bleeding	AR	Mutations of AP3B1 gene, encoding for the β subunit of the AP-3 complex
(c) X-linked lymphoproliferative syndrome (XLP)						
(i) XLP1	Normal	Normal or reduced	Normal or low immunoglobulins	Clinical and immunologic abnormalities triggered by EBV infection, including hepatitis, aplastic anemia, lymphoma	XL	Defects in *SH2D1A* encoding an adaptor protein regulating intracellular signals
(ii) XLP2	Normal	Normal or reduced	Normal or low immunoglobulins	Clinical and immunologic abnormalities triggered by EBV infection, including splenomegaly, hepatitis, hemophagocytic syndrome, lymphoma	XL	Defects in *XIAP* encoding an inhibitor of apoptosis
DISORDERS OF CYTOSKELETON PLASTICITY OR LYMPHOCYTE EFFECTOR FUNCTION						
(a) Wiskott–Aldrich syndrome (WAS)	Progressive decrease	Normal	Decreased IgM: antibody to polysaccharides particularly decreased; often increased IgA and IgE	Thrombocytopenia with small platelets; eczema; lymphomas; autoimmune disease; IgA nephropathy; bacterial and viral infections. XL thrombocytopenia is a mild form of WAS, and XL neutropenia is caused by missense mutations in the GTPase binding domain of *WASP*	XL	Mutations in *WASP*; cytoskeletal defect affecting hematopoietic stem cell derivatives
DISORDERS OF IMMUNE REGULATION						
(a) Autoimmune lymphoproliferative syndrome (ALPS)						
(i) CD95 (Fas) defects, ALPS type 1a	Increased double-negative (CD4− CD8−) T cells	Normal	Normal or increased	Splenomegaly; adenopathy; autoimmune blood cytopenias; defective lymphocyte apoptosis increased lymphoma risk	AD (rare severe AR cases)	Defects in *TNFRSF6*, cell surface apoptosis receptor; in addition to germline mutations, somatic mutations cause similar phenotype, ALPS 1a (somatic)

Table 73.2 (*Continued*)

Disease	Circulating T cells	Circulating B cells	Serum Ig	Associated features	Inheritance	Genetic defects/presumed pathogenesis
(ii) CD95L (Fas ligand) defects, ALPS type 1b	Increased double-negative (CD4– CD8–) T cells	Normal	Normal	Splenomegaly; adenopathy; autoimmune blood cytopenias; defective lymphocyte apoptosis; lupus	AD AR	Defects in *TNFSF6*, ligand for CD95 apoptosis receptor
(iii) Caspase-10 defects, ALPS type 2a	Increased CD4– CD8– T cells	Normal	Normal	Adenopathy; splenomegaly; autoimmune disease; defective lymphocyte apoptosis	AD	Defects in *CASP10*, intracellular apoptosis pathway
(iv) Caspase-8 defects, ALPS type 2b	Slightly increased CD4– CD8– T cells	Normal	Normal or decreased	Adenopathy; splenomegaly; recurrent bacterial and viral infections; defective lymphocyte apoptosis and activation	AD	Defects in *CASP8*, intracellular apoptosis and activation pathways
(v) Activating N-Ras defect, N-Ras ALPS	Increased CD4– CD8– T cells	Elevation of CD5 B cells	Normal	Adenopathy; splenomegaly; leukemia; lymphoma; defective lymphocyte apoptosis following IL-2 withdrawal	AD	Defect in *NRAS* encoding a GTP binding protein with diverse signaling functions, activating mutations impair mitochondrial apoptosis
(vi) IPEX, immune dysregulation, polyendocrinopathy, enteropathy (X-linked)	Lack of CD4+ CD25+ FOXP3+ regulatory T cells	Normal	Elevated IgA, IgE	Autoimmune diarrhea; early onset diabetes; thyroiditis; hemolytic anemia; thrombocytopenia; eczema	XL	Defects in *FOXP3*, encoding a T-cell transcription factor

Purine nucleotide phosphorylase (PNP) deficiency

This rare autosomal recessive disorder, which affects the purine salvage pathway and prevents normal conversion of inosine to hypoxanthine and guanosine to guanine, results in intracellular accumulations of deoxyGTP that are particularly toxic to T cells as well as distinctively low levels of uric acid in blood and urine [149]. Although T-cell function may be normal at birth, profound T lymphopenia soon develops. These patients may present with severe infections, including varicella pneumonia, and their T cells may exhibit no response to PHA or antigens consistent with $T^-B^+NK^+$ SCID, or, in some cases, a less profound and sometimes fluctuating combined immunodeficiency. They also exhibit developmental delay, hypotonia, and spasticity.

Early experience with HLA-matched related grafts was largely unsuccessful due to failure of engraftment when pretransplant conditioning was not administered [149]. However, since 1996, several patients have been successfully transplanted with matched related or unrelated HCT or cord blood grafts after myeloablative conditioning with busulfan and cyclophosphamide [150] or fludarabine [151].

However, it is important to note that although transplants of HLA-matched related and unrelated marrow as well as HLA haplotype disparate TCD grafts and cord blood grafts can correct the immunologic deficits of NP deficiency, the neurologic deficits, while potentially stabilized, are not reversed.

Combined immune deficiencies associated with defective DNA repair

Generation of T-cell receptors, immunoglobulin class switching and antibody diversity all require somatic DNA rearrangements of Variable (V) Diversity (D) and Joining (J) segments, a process which entails DNA double-strand breaks and repair by non-homologous end joining (NHEJ). As previously noted, while null mutations of recombinases 1 or 2 (RAG1/2) and Artemis induce classical SCID, hypomorphic mutations may permit limited rearrangements of T-cell receptors and class switching, resulting in Omenn syndrome or, for Artemis, common variable immune deficiencies. Recently, a series of other genes have been identified that play a critical role in NHEJ that may also induce progressively severe defects in T- and B-cell development and function, resulting in life-threatening susceptibility to infections, autoimmune cytopenias and/or EBV-induced malignancies in the first decade of life. These include null mutations in the DNA-phosphokinase CS that activates Artemis [152], mutations in the Cernunnos (or XLF) gene which encodes a protein essential for initiation of NHEJ [12], hypomorphic mutations of DNA ligase IV [13], and mutations of the NBS1 gene responsible for the Nijmegen breakage syndrome [153]. Each of these diseases is also associated with microcephaly and increased sensitivity to radiation.

Experience with transplants for these disorders is very limited. However, with reduced-intensity, fludarabine rather than alkylator-based regimens, transplants of matched related and unrelated grafts have induced full chimerism, immune reconstitution and thus far, freedom from autoimmune sequelae and lymphoid malignancies. Three patients transplanted for Cernunnos deficiency have been reported. One patient, at age 9, received an HLA-C disparate graft after conditioning with fludarabine, cyclophosphamide and ATG, and did well [154]. Two other patients who were of consanguineous parents received grafts at age 15 months from an HLA-matched sibling and achieved engraftment and reconstitution without preparative cytoreduction [155]. Of five reported patients transplanted for DNA ligase IV deficiency, three were successfully transplanted while in stable condition after fludarabine, cyclophosphamide and ATG conditioning [156]. Two others who presented with recurrent infection and a SCID-like immunodeficiency died early after transplant of EBVLPD and SOS, respectively [13]. In contrast, of six patients transplanted for immunodeficiency or a lymphoid malignancy associated with the Nijmegen breakage syndrome, five survive with reconstitution [157]. In 4/5 patients, use of a non-myeloablative regimen and a matched donor (n = 4) was associated with success. In addition, one of these patients was also successfully grafted after myeloablative conditioning with melphalan, thiotepa, and fludarabine and a T-cell-depleted HLA haplotype disparate graft. Taken together, these results indicate that disorders in this class of combined immune deficiency can be corrected particularly when fludarabine-based RIC regimens and suitably matched transplants are employed.

Cartilage–hair hypoplasia (CHH)

CHH is an autosomal recessive disorder presenting with short stature, chondrodysplasia, thin hair, and variably severe immunodeficiency. CHH is caused by mutations in the RMRP gene encoding the RNA segment of the mitochondrial RNA processing endoribonuclease [59]. RMRP, in complex with the telomerase reverse transcriptase (TERT), modulates cell proliferation. Severe deficiency results in impaired growth and maturation of T cells, and can result in a SCID-like combined immunodeficiency. Less severe variants present later in life particularly with severe varicella infection, autoimmune cytopenia, and EBV-associated lymphoid malignancies [58]. The immune deficits can be reversed by an allogeneic HCT [57]. In a European survey, HLA-matched related and unrelated marrow grafts administered after myeloablative conditioning with busulfan or melphalan and fludarabine or cyclophosphamide have induced full chimerism, correction of immune deficits, and long-term survival in 4/5 and 6/8 of the cases, respectively [158]. In contrast, three patients who received HLA haplotype disparate grafts that were administered after such myeloablative conditioning coupled with alemtuzumab fared poorly, due either to graft rejection or alloimmune cytopenias resulting from selective engraftment of donor T cells [158].

Combined immunodeficiencies with ectodermal dysplasia

These disorders are caused by X-linked mutations in the gene encoding nuclear factor κβ essential modulator (NEMO or IKK-γ) or autosomal dominant gain of function mutations in Iκβα [159,160]. These defects result in impaired activation of NF-κB in response to signals from TLRs and receptors for the cytokines IL-1, IL-6, IL-18, and TNF. This inhibits T-cell proliferation in response to exogenous pathogens and prevents the generation of memory T cells. Patients with these disorders usually present in the first 6–12 months of age with recurrent bacterial and DNA virus infections. They are also distinctively susceptible to atypical mycobacterial infections as well as chronic colitis. These patients also present with ectodermal dysplasia marked by abnormal sweat glands resulting in hypo or anhidrosis, sparse hair, and distinctive conical teeth. Eight patients with severe immune deficiencies caused by NEMO ($n = 6$) or Iκβα mutations ($n = 2$) have received transplants of HLA-matched related or unrelated marrow or PBSC, or HLA disparate cord blood or T-cell-depleted parental marrow after myeloablative conditioning with busulfan or melphalan together with cyclophosphamide or fludarabine, of whom five survive with hematopoietic and immune reconstitution [161,162]. However, unlike the CID patients with disorders of DNA repair, these patients did not regularly engraft. Even with matched transplants, three required secondary transplants after additional cytoreduction to achieve durable donor chimerism [161,162]. Furthermore, while reconstitution of T-, B-, and NK-cell function reverses their abnormal susceptibility to infection, chronic colitis, not related to GVHD, usually persists [161,162] as do the other features of ectodermal dysplasia.

Dock 8-deficient hyper IgE syndrome

The hyper IgE syndromes are a series of genetic disorders with the shared features of severe eczema and atopy, sinopulmonary infections, and elevated IgE [148]. They include Job syndrome, an autosomal disorder resulting from dominant negative mutations in STAT3, with its distinctive feature of recurrent severe staphylococcal skin abscesses [148], TYK2 deficiency, an autosomal recessive disorder with its distinctive sensitivity to disseminated BCG infections [148], and an autosomal recessive disorder caused by large deletions or mutations in the dedicator of cytokinesis 8 (Dock 8) gene [148,163,164] which result in profound defects of CD4 and CD8 T-cell activation, and impairment of differentiation of Th17 cells as well as deficits in humoral immunity [163,164]. Dock 8 deficiency is marked by a progressive combined T- and B-cell deficiency, hyper IgE, and a distinctive susceptibility to chronic and often mutilating cutaneous infections caused by molluscum contagiosum, herpes simplex, and papilloma viruses and early susceptibility to squamous cell carcinomas, cutaneous T-cell leukemia/lymphoma and HPV- and EBV-associated malignancies [163,164]. Hyper IgE syndromes associated with STAT3 and TYK2 mutations can often be controlled by antibiotics and conservative therapy. In rare cases with recurrent severe infections affecting the lung, HLA-matched allografts have been successfully applied [165].

In contrast, the prognosis for patients with Dock 8 deficiency is poor [163]. Indeed, HSCT constitutes the only approach capable of controlling or curing the disorder. To date, eight patients with Dock 8 deficiency have been reported who have received HCT from HLA-matched related or unrelated donors [166]. All but one [167] received myeloablative conditioning with busulfan/cyclophosphamide, thiotepa/melphalan/fludarabine or mel/flu and ^{90}Y anti-CD66 providing 1600 cGy to the marrow. Of these eight, one died of bacterial sepsis early on. However, five of the seven survivors are engrafted with full chimerism, immune reconstitution and clearance of viral infection and eczema. The other surviving patient who received non-ablative conditioning with 400 cGy TBI/fludarabine and cyclophosphamide is a mixed chimera whose eczema and viral infections have resolved but who still has persisting atopy and mildly elevated IgE. Strikingly, despite extensive cutaneous abnormalities pretransplant, only one patient developed GVHD which was limited to the skin and cleared rapidly with systemic steroids. Thus, HLA-matched related or unrelated HSCT can be successful in a high proportion of these patients.

CD40 ligand deficiency (X-linked hyper IgM syndrome)

One of the most extensively studied and treated CID is the X-linked hyper IgM syndrome. This disorder is caused by null mutations in the gene encoding CD40 ligand, which is essential for T cells to provide help so that B cells can form germinal centers and initiate isotype switching to generate antibodies other than IgM. Absence of CD40 ligand also compromises macrophage activation and deletion of autoreactive T cells [168]. Likely, as a consequence, these patients are susceptible to chronic infections due to viruses and intracellular microbes such as *Cryptosporidium*. They are also prone to autoimmune cytopenias [169].

Results of transplants applied to patients with hyper IgM syndrome caused by CD40 ligand deficiency have improved significantly over the last decade. For example, Gennery et al. [170] reported the European experience of transplants administered after myeloablative conditioning for X-linked hyper IgM ($n = 38$) performed from 1993 through 2002. Donors included an HLA-identical sibling ($n = 14$), a phenotypic HLA-matched family member ($n = 4$), or a matched unrelated donor ($n = 22$). Of 38 patients, 34 engrafted, and 26 patients survived, of whom 22 developed normal T-cell function and 18 generated specific antibody following vaccination. One additional patient engrafted but has poor T-cell immune reconstitution. Twelve patients (32%) died from infection which was associated with grade II–IV GVHD in six. Poor prognostic factors in this study included pre-existing liver and pulmonary disease. Reduced-intensity conditioning is now being increasingly explored. The first two patients with hyper IgM syndrome and chronic liver disease who received an unmodified HLA-matched sibling HCT following cytoreduction with low-dose busulfan, fludarabine, ATG, and CyA [171] survive >2 years post-HCT (one a mixed chimera, the other a full chimera) with normal T-cell function off supplemental IVIG.

The bare lymphocyte syndromes (BLS)

The bare lymphocyte syndromes are a set of combined immune deficiencies resulting from mutations that inhibit appropriate expression of HLA proteins on the cell surface and thereby prevent the presentation of antigenic peptides required for T-cell sensitization and both T- and NK-cell recognition of antigenic targets. Type 1 BLS is caused by mutations in the TAP1 or 2 transporter genes essential to peptide presentation by class I HLA alleles [172]; type 2 BLS are caused by mutations in genes encoding components of regulatory factor X (RFXANK, REXAP, or RFX-5) that activate the promotor controlling the transcription of HLA class II alleles [173]. The more common class II BLS are associated with CD4 lymphopenia and the absence of antigen-specific T-cell responses or the T-cell help required to generate antibodies. All patients with BLS are at high risk of severe and recurrent viral, bacterial, and fungal infections [174]. In the 1980s and 1990s HLA-matched transplants administered to patients with the bare lymphocyte syndromes were associated with high rates of transplant-related mortality due to viral infections and graft rejection with overall survival rates of 50% for matched, and 0–20% for mismatched grafts. Early series recorded graft failure rates exceeding 20–30% for HLA-matched related grafts and higher for HLA disparate transplants [175]. Recent studies that have consistently employed myeloablative conditioning with busulfan and cyclophosphamide together with ATG or alemtuzumab to deplete chemotherapy-resistant host T cells that are capable of responding to HLA$^+$ donor cells, have recorded engraftment rates of 80–100% and survival with reconstitution for 60–76% of HLA-matched related graft recipients [176]. Experience with single HLA allele disparate related and unrelated marrow and cord blood transplants has also improved. While graft failure rates for these transplants remain high, a significant proportion of these patients have engrafted following secondary transplants. In the recent multicenter experience reported by Small et al. [177] 67% of such patients were long-term survivors with immune reconstitution, including 4/7 T-cell-depleted HLA haplotype disparate graft recipients. While viral infections remain a significant cause of mortality, the proportion of patients succumbing to CMV and adenoviral infections in recent series is lower, reflecting both the introduction of more effective antiviral drugs and improved supportive care.

Familial hemophagocytic lymphohistiocytosis (HLH)

The HLH syndromes include a series of genetically determined and virus-induced disorders principally characterized by inflammatory responses involving multiple organs induced by ineffectively controlled macrophage activation, expansion of T-cell populations and abnormal cytokine production, resulting in fever, severe cytopenias secondary to hemophagocytosis, adenopathy, splenomegaly, lymphoid and histiocytic infiltration and damage to organs, particularly the liver and central nervous system, and in certain types, uncontrolled lymphoproliferative diseases [178]. Diagnostic criteria that have been established include these clinical features together with laboratory evidence of hemophagocytosis, increased levels of ferritin and soluble IL-2 receptor α or deficient NK-cell function, and metabolic disturbances such as hypofibrinogenemia, hyponatremia, and hypertriglyceridemia [178]. Five genetic forms of HLH identified to date are caused by autosomal recessive loss of function mutations in genes essential to the capacity of NK cells, CD56$^+$ NK T cells or antigen-specific T cells to form functional cytolytic synapses with target cells. These include mutations in perforin (FHL-2), and the MUNC-1 (FHL-3) and syntaxin 11 (FHL-4) proteins essential to degranulation as well as mutations affecting lysosome granule trafficking (the Chediak-Higachi syndrome gene LYST) and granule exocytosis (the Griscelli syndromes types 1–4 and the Hermansky–Pudlak syndrome) (Table 73.2) [178].

Following the initial reports in 1986 of corrections of familial HLH by Fisher et al. [179], several case reports attested to the therapeutic potential of HLA-compatible HCT in each of these disorders. Subsequently, analyses of cumulative experiences with transplants of matched related or unrelated mismatched T-cell-depleted grafts administered after myeloablative conditioning with busulfan and cyclophosphamide +/− etoposide and ATG demonstrated survival rates with disease correction in 45–58% of cases [180]. The multinational compendium of Horne et al. [181] and the AIEOP study reported by Cesaro et al. [182] detailed results for groups of 86 and 61 patients respectively that had received a standard chemotherapy induction protocol (HLH-94) with dexamethasone and etoposide prior to cytoreduction and transplant. Despite induction therapy, 43% and 60% of the patients in the two studies had active disease at time of transplant. Nevertheless, Horne et al. [181] reported 3-year survival rates of 71 and 70% for recipients of matched related and unrelated grafts, 54% for partially mismatched unrelated transplants, and 50% for haplodisparate T-cell-depleted transplants. In the AIEOP study, the 8-year survival rate for the whole group was 58% and 63% for recipients of matched unrelated or mismatched related grafts after 1999 [182]. In the large single-center experience from Paris [183], an overall 5-year survival rate of 70% was observed in recipients of unmodified HLA-matched related or unrelated grafts versus 50% for recipients of T-cell-depleted HLA haplotype disparate transplants. Engraftment rates following each type of transplant were equivalent. For recipients of haplodisparate TCD grafts transplanted in remission, the 5-year survival rate was excellent (80%). However, for patients with active disease, recipients of haplotype disparate grafts fared poorly (15% 5-year survival) particularly because of graft failure. Neurologic evidence of HLH involving CNS was also found to be a portent of poor outcome [183]. However, a more recent study suggests that transplants, including haplodisparate TCD BMT, can be effective in patients with active systemic or CNS disease, if administered early in the course of relapse [184]. In each of these studies, results for patients with defined genetic causes of HLH were equivalent to those for patients with non-familial or acquired forms of HLH.

Because of the high rates of veno-occlusive disease and toxicity-associated TRM in most series of patients transplanted for HLH after myeloablative conditioning, several groups have recently explored reduced-intensity and non-myeloablative conditioning regimens. Initial results have been very promising. Cooper et al. [185] have reported 25 patients with HLH (21 in CR) including 17 with defined genetic variants who received transplants after reduced-intensity conditioning with either melphalan (140 mg/m^2), fludarabine (150 mg/m^2 total) and alemtuzumab (for HLA-matched related or unrelated grafts), or melphalan fludarabine together with busulfan (4 mg/kg daily for 2 days) and ATG for HLA non-identical grafts. All patients engrafted; 21/25 (88%) survive in remission at a median of 3 years, including 9/11 (82%) recipients of HLA disparate grafts. Similarly, Marsh et al. [186] employed melphalan and fludarabine at the same doses together with alemtuzumab to prepare 26 patients with HLH (61% in CR) for HLA-matched or 1 allele disparate marrow grafts. All patients engrafted, although 14 patients subsequently received DLI to enhance chimerism. Nevertheless, of 26 patients, 22 (89%) were surviving in remission at a median of 654 days. Thus, this reduced-intensity conditioning is sufficient to secure consistent engraftment and control of HLH while reducing TRM and improving outcome.

X-linked lymphoproliferative diseases (X-LP)

The X-linked lymphoproliferative syndromes are caused by hemizygous mutations in the SLAM-associated protein SAP or SH2DI-A (XLP-1) or the X-linked inhibitor of apoptosis, XIAP (XLP-II) which impair cytotoxic NK cell and particularly NKT and T-cell interactions with EBV transformed cells. These defects may also augment antigen-induced apoptosis of EBV antigen-activated T cells [187,188]. This likely limits expansion of populations of virus-specific T cells resulting in susceptibility to lymphoproliferative disease.

The potential of a marrow transplant to correct XLP was first demonstrated by Filipovich et al. in 1986 [189]. Since then, most patients with XLP1 have received transplants to treat or prevent fulminant EBV infection, EBV-associated lymphomas, or other lymphoid malignancies. While experience with transplants is still limited, of 14 reported who received HLA-matched related or unrelated marrow or cord blood grafts, after myeloablative conditioning (in all but one case), 10 survive with engraftment and clearance of XLP-associated disease (reviewed in [190]).

Autoimmune lymphoproliferative syndromes (ALPS)

ALPS constitute a set of genetic disorders caused by mutations in Fas, Fas ligand, or caspase-10 or -8 that cause defective Fas-mediated apoptosis and result in lymphoproliferative disorders marked by prominent adenopathy, hepatosplenomegaly, chronic or recurrent autoimmune cytopenias and, less commonly, autoimmune disorders of other organs including the joints, kidney, liver, intestinal tract, and CNS, as well as a markedly increased risk of lymphoma and other malignancies [191,192]. These patients also have increased numbers of $CD4^-$, $CD8^-$ $TCR\alpha/\beta^+$ double negative T cells in the circulation [191,192]. The clinical disorders associated with these genetic diseases vary markedly in their severity, and are often controlled by steroids alone or in combination with mycophenolate mofetil or the MTOR inhibitor, sirolimus [192]. However, allogeneic HCTs have been used to treat lymphomas developing in these patients. Experience with transplants for the different types of ALPS is still limited. However, transplants of matched unrelated marrow administered after myeloablative and, more recently, reduced-intensity conditioning have been effective in inducing full donor chimerism and long-term remissions of disease [193]. Replacement of diseased lymphoid cells of the host by those from the normal donor also corrects the abnormalities of Fas-mediated apoptosis and reverses ALPS-associated autoimmune manifestation.

X-linked immune dysregulation, polyendocrinopathy and enteropathy syndrome (IPEX)

IPEX is a life-threatening disorder caused by loss of function mutations in Fox P3 that induce in a marked deficiency of regulatory T cells and result in uncontrolled autoimmune enteropathy, diabetes, and dysfunctions of other endocrine glands, ichthyosiform dermatitis, and autoimmune cytopenias [194]. The disorder requires sustained treatment with steroids and other immunosuppressive drugs and is often difficult to arrest. Most affected patients succumb to the disease or associated infection within the first 1–3 years of life. Early reports of HLA-matched related or unrelated HCT administered after myeloablative conditioning, including TBI or high-dose busulfan, documented a high rate of mortality due to organ toxicity or infectious complications [194,195]. However, such transplants administered after reduced-intensity conditioning such as with fludarabine, melphalan, and alemtuzumab or 400TBI and fludarabine, have achieved consistent full or sustained mixed chimerism with reconstitution of Fox $P3^+$ regulatory T-cell populations of donor origin and durable resolution of IPEX-associated autoimmune enteropathy, dermatitis, and cytopenias [196,197]. While many endocrinopathies stabilize, diabetic states antedating transplant have not improved. Nevertheless, the consistent success and long-term survival of the patients receiving HLA-compatible grafts after these reduced-intensity regimens underscore the good prognosis and curative potential of such transplants in this disease.

Wiskott–Aldrich syndrome (WAS)

Wiskott–Aldrich syndrome (WAS) is a rare X-linked disorder characterized by thrombocytopenia, immunodeficiency and eczema estimated to occur in 1 in 10 million live male births [198]. Affected individuals also have an increased risk of lymphomas, particularly EBV-LPD and autoimmune disorders [199]. The mutated gene responsible for WAS and the milder disorders of X-linked thrombocytopenia and X-linked neutropenia was discovered in 1994 by Derry and colleagues [199]. The normal gene, which maps to Xp11.22-p11.23, encodes a 501 amino acid, 53 kDa intracellular protein (WAS protein or WASp), expressed in most hematopoietic cells [199]. WASp is involved in cytoskeletal organization (reviewed in [200]) and plays an essential role in T- and NK-cell activation [198,200,201]. Over 150 unique mutations in more than 300 families worldwide have now been identified [198,202].

Although all patients with WAS are thrombocytopenic, the level of T- and B-cell immunodeficiency, and subsequent risk of autoimmune phenomena (nephropathy, vasculitis) and lymphoid malignancies can be quite variable. Zhu et al. [203] first demonstrated that the clinical heterogeneity observed in WAS patients is due to differences in the expression of WASp in lymphoid cells. Currently, patients are divided into five clinical groups on the basis of WASp expression ranging from group 1, patients with mild clinical disease associated with low levels of WASp in peripheral blood mononuclear cells to group 5 patients who lack WASp expression and have severe disease [198]. This scoring of patients, based on the pattern of WASp expression in individual patients is now used to determine which patients lacking an HLA-matched related donor should be treated with supportive care alone, and which should be early candidates for HCT from alternative donors [198]. Several other studies have also shown a strong phenotype association with genotype in this disorder. For example, Jin et al. [204] evaluated the correlation of genotype with phenotype in 262 patients from 227 unrelated families with WAS/XLT followed in two centers, one in Seattle, the other in Italy. The best predictor of clinical manifestations was determined by WASp expression. The majority of patients in this study who had no detectable WASp in lymphoid cells had deletions or insertions which resulted in frameshift, non-sense, or splice site mutations. In contrast, 74% of patients with XLT had missense mutations, and low levels of WASp.

HCT for Wiskott–Aldrich syndrome (WAS)

In 1968, Bach et al. [83], reported correction of the immunologic abnormalities associated with WAS following HLA-matched sibling transplantation. However, despite high-dose CY, this patient remained thrombocytopenic. In 1978, Parkman et al. [205] corrected both the immunologic and hematologic defects in a child with WAS by administering an HLA-matched marrow graft after myeloablative cytoreduction with TBI, procarbazine, and ATG, and hypothesized that myeloablation as well as immunosuppression were required to provide "space" for hematopoietic progenitor cells to develop. Subsequently, Kapoor et al. [206] demonstrated that conditioning with BU (8 mg/kg) and CTX (200 mg/kg) also induced full hematopoietic chimerism, correcting both the lymphoid and platelet defects in a series of children with WAS. Subsequent single-center studies of HLA-identical related BMT [207–210] have recorded long-term survival rates ranging from 80–100%. Of 65 children with WAS reported between 1968 and 1997, 57 (87%) were surviving long term after HLA-matched BMT [211].

For patients without an HLA-matched sibling, transplants from closely matched unrelated donors currently also provide excellent results. In a series of 30 patients with WAS who received an unrelated unmodified BMT prior to July 1994, Filipovich reported an overall actuarial survival of 67% [98]. Analysis of these transplantation results demonstrated that children <2 years of age at the time of transplant had a much better prognosis with 21 of 23 surviving (actuarial survival at 3 years of 91%). Acute grade II–IV GVHD occurred in 50% of patients, with the highest incidence and severity observed in patients >5 years of age at HCT. In 2001, Filipovich et al. [212] summarized the results of 170 transplants reported to the International Bone Marrow Transplant Registry and/or the National Marrow Donor Registry (1968–1996) and compared 58 patients transplanted from HLA-matched siblings, 48 from other relatives, and 67 patients from unrelated donors. Transplant outcome differed according to donor type with the probability of 5-year survival of 87% (74–93%) with an HLA-identical sibling donor, 52% (37–65%) with other related donors, and 71% (58–80%) with unrelated donors. For recipients of unrelated HCT, the best outcomes were again observed in patients transplanted at less than 5 years of age.

Recent results of unrelated grafts have significantly improved, likely reflecting the introduction of improved DNA sequence-based HLA matching, broader access to donors, less toxic and better monitored myeloablative conditioning, and better supportive care. Pai et al. reviewed 16 unrelated HCT for WAS performed at the University of Brescia from 1990 through 2005 [209] of whom 13 survive (81.2%) a median (range) of 6 (0.6–9) years post-HCT. Similarly, Kobayashi et al. [210] reported that 80% of WAS patients in Japan who received an unrelated HCT ($n = 21$) survive and 75% are disease-free [210]. Prognosis was again poorer in patients who were transplanted >5 years of age and those who received cytoreduction with a regimen other than BU/CY +/− ATG.

In a recent multinational review of the results of transplants for Wiskott–Aldrich syndrome between 1980 and 2009, 91 received matched unrelated grafts, almost all after Bu/CTX+ATG. For patients grafted between 1980 and 1999 versus ≥2000, survival rates at ≥5 years were 77% versus 90% [213]. Of those transplanted at ≤5 years of age, 90% are long-term survivors compared to 73% of those transplanted at >5 years of age. Nevertheless, the improvement in results for older patients is striking when compared to survival rates reported in earlier transplant series [98,210,212].

Cord blood transplants have been only recently introduced to the treatment of patients with Wiskott–Aldrich syndrome. However, since 2003, several centers have explored this approach [210,214]. In the compendium of patients transplanted for WAS in Japan, 12/15 (80%) recipients of unrelated cord blood grafts administered after Bu/CTX +/− ATG were surviving at >5 years [210]. Similarly, in the multinational analysis reported by Moratto et al. [213], of 24 patients treated with UCB since 2000, 82% were long-term survivors.

Results of HLA haplotype disparate TCD grafts from related donors which have been applied to patients lacking an HLA-compatible related or unrelated donor have also markedly improved over the last 10 years. Early experiences with such transplants were marked by unacceptably high rates of graft failure and mortality due to GVHD and infection. As a result, long-term survival rates in early single and multicenter series ranged from 16–55% [90,207,210–212,215]. However, with the introduction of methods for adequately depleting T cells from PBSC, high doses of progenitor cells can be given without need of post-transplant immune suppression to prevent GVHD. As a result, consistent engraftment can now be achieved. Furthermore, the introduction of drugs and immunotherapies has provided increasingly effective approaches to prevent or treat serious EBV, CMV, and adenoviral infections in the post-transplant period. Reflecting these advances, 5-year survival rates reported in the recent international collaborative study reported by Moratto et al. [213] have significantly improved from the 52.2% rate observed for patients transplanted from 1980–1999 to 91.7% for patients transplanted between 2000 and 2009. Indeed, in this report, results of HLA haplotype disparate TCD grafts administered between 2000 and 2009 were equivalent to those achieved with unmodified transplants from matched unrelated donors.

Late complications in patients transplanted for Wiskott–Aldrich syndrome

Experience with allogeneic HCT for WAS dates to 1968, permitting an assessment of the influence of a successful graft on the long-term complications of WAS, particularly their susceptibility to infections by polysaccharide encapsulated bacteria, hemorrhagic EBV lymphomas, and other neoplasias and autoimmune disorders.

Results of single and multicenter trials indicate that immune reconstitution with donor cells post-transplant has reduced the risk of bacterial infections to that of normal peers in most patients transplanted for WAS. An exception, however, is the patient splenectomized prior to transplant. Such patients remain at risk of life-threatening pneumococcal, meningococcal, or other pyogenic infection if they do not adhere to penicillin prophylaxis [213,216]. While patients cytoreduced with myeloablative regimens usually experience sustained correction of thrombocytopenia, 9% of patients, specifically those who achieved only mixed chimerism, have remained thrombocytopenic, and may ultimately require a second graft [208,213]. While patients with WAS are at risk for EBV-LPD early post-transplant, long-term risk of malignancies is approximately 5%, a rate comparable to that of individuals receiving HSCT for other indications [208,213].

Conclusion

Allogeneic HCT from a normal matched related donor remains the treatment of choice for each of the different genetic variants of SCID. Currently, HLA-matched unmodified grafts from unrelated donors administered with post-transplant drug prophylaxis to prevent GVHD and rigorously T-cell-depleted HLA-haplotype disparate-related transplants administered without post-transplant GVH prophylaxis also yield good results, with long-term disease-free survival rates of approximately 70–74% overall and over 90% for patients transplanted in the first 3 months of life. The relative advantages of each approach will require careful prospective analyses of large series. Certain types of SCID, particularly, ADA-deficient SCID and NK^+ variants as well as "leaky" forms of SCID, including Omenn syndrome exhibit graft resistance and may require preparative cytoreduction, particularly when the donors are other than HLA-matched siblings. Inclusion of myelosuppressive alkylating agents such as busulfan, treosulfan, thiotepa, or melphalan is also preferable to secure consistent engraftment of donor B cells and NK cells as well as T cells in these variants and thereby achieve reconstitution of both cell-mediated and humoral immunity.

For patients with other combined immunodeficiency diseases and Wiskott–Aldrich syndrome, preparative cytoreduction with a myelosuppressive agent together with immunoablation with cyclophosphamide or fludarabine is required to achieve consistent engraftment and reconstitution. For these indications, transplants of unmodified HLA-matched related and unrelated HCT have thus far been associated with better outcomes than T-cell-depleted HLA-disparate grafts. Certain of these disorders, particularly the bare lymphocyte syndrome associated with HLA class II deficiency, exhibit excessive rates of graft failure despite use of these regimens. For the future, development of well-tolerated preparative regimens and transplant approaches that secure consistent engraftment and full chimerism is a high priority. Introduction of selected cytokines or specific cell therapies at the time of transplant may also foster full engraftment and enhance recovery of immune function

A separate chapter on gene therapy details approaches currently being evaluated in clinical trials to transduce hematopoietic stem calls with vectors encoding normal genes to correct several life-threatening disorders of immunity and hematopoiesis. Treatment with genetically modified autologous hematopoietic stem cells has recently shown great promise in the treatment of children with ADA-deficient SCID when administered after myelosuppression with busulfan. However, the proportion of patients achieving full reconstitution is thus far not different from that currently achievable with matched unrelated or T-cell-depleted HLA non-identical grafts [69]. Longer follow-up will be required to determine whether and to what degree gene therapy will supplant HCT as a treatment of choice for specific variants of SCID such as ADA deficiency. For patients with X-linked IL-2R γc-deficient SCID and Wiskott–Aldrich syndrome, gene-modified autologous HCT can reconstitute T- and, in some cases B-cell immunity. Furthermore, this approach can induce production of platelets in patients with Wiskott–Aldrich syndrome that are normal in number and function. However, the risk of leukemic transformation of genetically corrected cells has been prohibitively high. In the future, alternative vectors and strategies permitting site-specific genetic corrections may eliminate the risk of late leukemic transformation of transduced cells, and secure a role for gene therapy in these patients.

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CHAPTER 74

Hematopoietic Cell Transplantation for Storage Diseases

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Introduction

Lysosomal storage disorders (LSDs) are a large group of about 50 rare and genetically inherited metabolic disorders arising from single-gene defects that often involve a lysosomal hydrolytic enzyme or vital peroxisomal function, causing devastating systemic diseases that can affect multiple organs (Tables 74.1–74.3). These conditions may be caused by defective membrane proteins, errors in enzyme targeting or defective function of enzyme activators. These changes cause pathologic accumulation of undegraded macromolecules inside lysosomes, which represents the typical hallmark of these diseases and results in complex clinical phenotypes. Owing to the housekeeping nature and ubiquitous expression of lysosomal hydrolases, these pathologies are multiorgan, multisystemic, and usually life-threatening. Clinical manifestations tend to worsen as more waste substrate accumulates over time, with symptoms varying in severity from relatively mild to severe somatic and rapidly progressing neurologic manifestations [1,2]. The precise mechanisms by which storage materials disturb cellular functions are not fully understood; alterations occurring in many cellular processes, such as autophagy, intracellular calcium homoeostasis, signaling pathways, trafficking, inflammation and oxidative stress response, have been found to contribute to the pathologic progression. Although individually rare and with a low incidence in most populations, taken together LSDs account for about one patient in every 5000–9000 live births.

For the past three decades, hematopoietic cell transplantation (HCT) has been used as effective therapy for selected inborn errors of metabolism (IEM), mainly lysosomal storage diseases and peroxisomal disorders. The main rationale for HCT in IEM is based on the provision of correcting enzymes by donor cells within and outside the intravascular compartment. The ultimate goal of HCT is to achieve normal or near-normal quality of life by preventing further neurologic and developmental deterioration, which characterize these conditions. Detailed studies suggesting that HCT is indicated for carefully selected cases are available only for Hurler syndrome, X-linked adrenoleukodystrophy (X-ALD), and infantile Krabbe disease. Improvement of transplantation techniques and alternative therapies may change the recommended indications for HCT in IEM. A recent example of emerging transplantation techniques is the fast availability of unrelated cord blood transplants (UCBT). UCBT [3–5] makes HCT feasible in patients with rapidly progressive neurologic diseases. The rapid and easy access to umbilical cord blood units has improved the ability to move forward with HCT, shortly after diagnosis and before irreversible damage has occurred. Conversely, the success of enzyme replacement therapy (ERT) in patients with a moderate/good clinical condition generated an interest in employing ERT prior to or during HCT in patients with poor clinical condition [6,7]. Autologous HCT with genetically modified cells and mesenchymal stem cell infusions constitute emerging techniques that may, potentially, halt the progression of the disorder after successful cell therapy. Improvement in HCT techniques and novel stem cell sources will continue to impact the safety and efficacy of this therapy as well as expand the list of indications. A good functioning worldwide registry would be necessary to measure the effects of the procedures performed in more detail.

HCT for storage diseases

Why does HCT work?

Lysosomes are cellular organelles that contain hydrolytic enzymes; peroxisomes are subcellular organelles catalyzing metabolic functions primarily related to lipid metabolism. In 1968, Fratantoni and Neufeld established the concept of transferable lysosomal enzymes through metabolic cross-correction of defects in co-cultured fibroblasts of patients with Hurler and Hunter syndromes [8]. Metabolic correction of lysosomal storage diseases is due to the mannose-6-phosphate receptor-mediated endocytosis of secreted enzyme, or by direct transfer of enzyme from adjacent cells [1]. It is likely that both mechanisms occur following HCT (Figure 74.1). Variability in receptor-mediated enzyme endocytosis in various cells and tissues could affect hydrolase uptake after HCT. For example, monocytes and macrophages have receptors for *N*-acetylglucosamine and mannose; glial cells have receptors for sialic acid. As an example, X-ALD is a peroxisomal disorder of very long chain fatty acid (VLCFA) β-oxidation. The likely mechanism by which HCT halts cerebral demyelination is perhaps threefold: immunosuppression, replacement with metabolically normal cell populations leading to decreased perivascular inflammation, and metabolic correction. The impact of normalizing VLCFAs in plasma is unclear.

A critical question in HCT for storage diseases with central nervous system (CNS) involvement is whether donor-derived cells enter the CNS and achieve metabolic correction or halt inflammation. Microglia are mononuclear phagocytes in the CNS, which are hematopoietically

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Table 74.1 The mucopolysaccharidoses and mucolipidoses: mode of inheritance, enzyme/protein deficiency, incidence and carrier frequencies, treatment options, and comments

Disorder	Genetics	Enzyme/protein	Incidence in thousands/ carrier frequency (estimates)	Treatment options: HCT/ERT/SD/GT	Comments
Mucopolysaccharidoses (MPS)					
MPS I	Autosomal recessive	α-L-Iduronidase	111/148		
Hurler (MPS IH)				+ / + / – / –	HCT or HCT + ERT ERT is considered the standard therapy
Hurler-Scheie (MPS IH/S)				+ / + / – / –	
Scheie (MPS IS)				+ / + / – / –	
Hunter MPS II	X-linked	Iduronate-2-sulfatase	162/136,000		
Severe (MPS IIA)				– / + / – / –	ERT is currently offered to patients with Hunter
Attenuated (MPS IIB)				– / + / – / –	
Sanfilippo (MPS III)	Autosomal recessive				
MPS IIIA		Heparan-*N*-sulfatase	128/169	– / – / – / –	No evidence of neurocognitive efficacy following successful, timely HCT
MPS IIIB		*N*-acetylglucosaminidase	235/230	– / – / – / –	
MPS IIIC		AcetylCoA : *N*-acetyltransferase	1407/593	– / – / – / –	
MPS IIID		*N*-acetylglucosamine 6-sulfatase	1056/514	– / – / – / –	
Morquio (MPS IV)	Autosomal recessive		201/206		
MPS IV A		Galactose 6-sulfatase		– / – / – / –	Severe skeletal deformities preclude HCT
MPS IV B		β-Galactosidase		– / – / – / –	
Maroteaux-Lamy (MPS VI)	Autosomal recessive	Arylsulfatase B	248/242	+ / + / – / –	ERT is the preferred therapy; however, HCT can be considered
Sly (MPS VII)	Autosomal recessive	β-Glucuronidase	2111/726	+ / – / – / –	HCT in a very limited number of a cases
Mucolipidoses (ML)	Autosomal recessive	Phosphotransferase	422/285	+ / – / – / –	Significant clinical challenges to performing HCT primarily due to airway and pulmonary issues
ML-II (I-cell)					
ML-III (pseudo-Hurler polydystrophy)					

ERT, enzyme replacement therapy; GT, gene therapy; HCT, hematopoietic cell transplantation; SD, substrate depletion.
Data from [147].

Table 74.2 The leukodystrophies: mode of inheritance, enzyme/protein deficiency, incidence and carrier frequencies, treatment options, and comments [147]

Disorder	Genetics	Enzyme/protein	Incidence in thousands/ carrier frequency (estimates)	Treatment options: HCT/ERT/SD/GT	Comments
Leukodystrophies and other white matter diseases X-ALD phenotypes Childhood-onset cerebral X-ALD Adolescent-onset cerebral X-ALD Adult-onset cerebral X-ALD AMN with or without cerebral X-ALD Addison disease only	X-linked	ALD protein (ALDP)	19/19,000	+ / – / – / + + / – / – / – + / – / – / – ? / – / – / ? – / – / – / –	HCT is the only effective long-term therapy offering the possibility of stabilization of the cerebral disease course. Lorenzo's oil appears to decrease the likelihood of developing cerebral disease in selected boys with biochemically diagnosed X-ALD
Globoid-cell leukodystrophy	Autosomal recessive	Galactocerebrosidase	201/188	+ / – / – / –	Long-term follow-up of infants transplanted with UCB is needed
Metachromatic leukodystrophy	Autosomal recessive	Arylsulfatase-A	121/152	+ / + / – / –	The extended time to CNS stabilization and the inability of HCT to favorably affect the PNS dampens enthusiasm regarding quality of life outcomes in late-onset patients. ERT may be effective for treating the PNS; however, further study is needed
Alexander	Autosomal recessive		?/?	– / – / – / –	
Pelizaeus-Merzbacher disease	X-linked	Proteolipid protein	?/?	– / – / – / –	
Vanishing white matter disease	Autosomal recessive	Mutations in translation initiation factor eIF2B and possibly others	?/?	– / – / – / –	
Zellweger syndrome	Autosomal recessive	Several, including accumulation of VLCFA, marked deficiency of plasmalogens	?/?	– / – / – / –	

ERT, Enzyme replacement therapy; GT, gene therapy; PNS, peripheral nervous system; SD, substrate depletion; ?, unknown. See text for other abbreviations. Data from [147].

Table 74.3 The glycoprotein disorders and miscellaneous lysosomal storage diseases and inborn errors of metabolism: mode of inheritance, enzyme/protein deficiency, incidence and carrier frequencies, treatment options, and comments [147]

Disorder	Genetics	Enzyme/protein	Incidence in thousands/carrier frequency (estimates)	Treatment options: HCT/ERT/SD/GT	Comments
Fucosidosis	Autosomal recessive	Fucosidase		+ / − / − / −	
Gaucher disease I (non-neuronopathic) II (acute neuronopathic) III (subacute neuronopathic)	Autosomal recessive	Glucocerebrosidase	59/119	+ / + / + / + − / + / + / − + / + / + / −	ERT and/or SD: preferred therapies Long-term favorable outcomes have been seen with HCT, particularly in patients with Norrbottnian form
α-Mannosidosis	Autosomal recessive	α-Mannosidase	1056/514	+ / − / − / −	Impressive, favorable results with HCT
Aspartylglucosaminuria	Autosomal recessive	Aspartylglucosaminidase	2111/726	+ / − / − / −	
Cerebrotendinous xanthomatosis	Autosomal recessive	27-Hydroxylase		− / − / − / −	
Fabry disease	X-linked	α-Galactosidase	117/117,000	− / + / + / −	ERT is the preferred therapy
Farber lipogranulomatous disease	Autosomal recessive	Ceramidase	?/?	+ / − / − / −	
Gangliosidoses (GM1 and GM2 including Tay-Sachs, Sandhoff, GM2 activator deficiency) GM1 gangliosidoses GM2 gangliosidoses • Tay-Sachs • Sandhoff • GM2 activator deficiency	Autosomal recessive	β-Galactosidase Hexosaminidase A Hexosaminidase A and B	422/310 222/224 422/310	? / − / − / − − / − / + / −	No clear evidence of efficacy for HCT in any of these disorders
Glycogen storage disease II (Pompe)	Autosomal recessive	α-Glucosidase	201/191	− / + / − / −	ERT: preferred therapy
Neuronal ceroid lipofuscinosis NCL1 NCL2	Autosomal recessive	Palmitoyl protein thioesterase Tripeptidyl peptidase I	?/?	+ / − / − / −	Very limited clinical use of HCT and rapid disease course make it difficult to reach a conclusion
Niemann-Pick disease A B C	Autosomal recessive	Acid sphingomyelinase Acid sphingomyelinase NPC1 (cholesterol trafficking)	A and B: 264/249 211/230	− / + / − / − + / + / − / − + / − / − / −	ERT may have a role in type A and B patients. HCT may be effective in type B and C patients; however, experience remains very limited
Wolman syndrome	Autosomal recessive	Acid lipase	704/363	+ / − / − / −	HCT: extremely high morbidity and mortality
Canavan disease	Autosomal recessive	Aspartoacylase		− / − / − / −	Increased in Ashkenazi Jews
Cystinosis	Autosomal recessive	Cystine transporter	281/219	− / − / + / −	
Sialic acid storage disease	Autosomal recessive	Sialic acid transporter	603/363	? / − / − / −	HCT: role is unknown
Sialidosis	Autosomal recessive	Neuraminidase	4222/1027	? / − / − / −	HCT: role is unknown
Multiple sulfatase deficiency	Autosomal recessive	Multiple sulfatase factor, all sulfatases	1407/593	+ / − / − / −	HCT: extremely limited clinical experience
Hypophosphatasia	Autosomal recessive or autosomal dominant	Tissue nonspecific alkaline phosphatase		+ / − / − / −	HCT: extremely limited clinical experience

ERT, enzyme replacement therapy; GT, gene therapy; HCT, hematopoietic cell transplantation; SD, substrate depletion; ?, unknown. Data from [147].

derived. They account for 5–10% of non-neuronal cells in brain. Activated microglia participate in antigen presentation, inflammatory responses, infection, and CNS injury. In humans, repopulation with donor-derived microglia can take a year. The kinetics of microglial replacement after HCT is slower than that of other tissue macrophages (e.g., alveolar macrophages or Kupffer cells). This observation explains, in part, the inability of HCT to stabilize effectively the CNS and neurologic aspects of a rapidly progressing disorder.

History

On April 8, 1980, a 9-month-old boy received the first bone marrow transplant for Hurler syndrome [9]. Despite genotype analysis revealing homozygous W402X mutations indicative of severe Hurler syndrome (mucopolysaccharidosis IH [MPS IH]) phenotype, the patient had fully engrafted with maternal marrow, and has stable, normal intelligence [9]. In 1982, the first child with Maroteaux-Lamy syndrome, a 13-year-old girl, was successfully transplanted from her human leukocyte antigen (HLA)-matched, enzymatically normal sister, and experienced resolution of hepatosplenomegaly and normalization of cardiopulmonary function [10]; she survives and functions independently. In the late 1980s and into the 1990s, successful transplants were performed for each of the leukodystrophies, including cerebral X-ALD [11], globoid cell leukodystrophy (GLD) [12], and metachromatic leukodystrophy (MLD) [13]. In the mid-1990s to the mid-2000s, the first large multicenter reports for HCT were published on Hurler syndrome [14–16] and cerebral

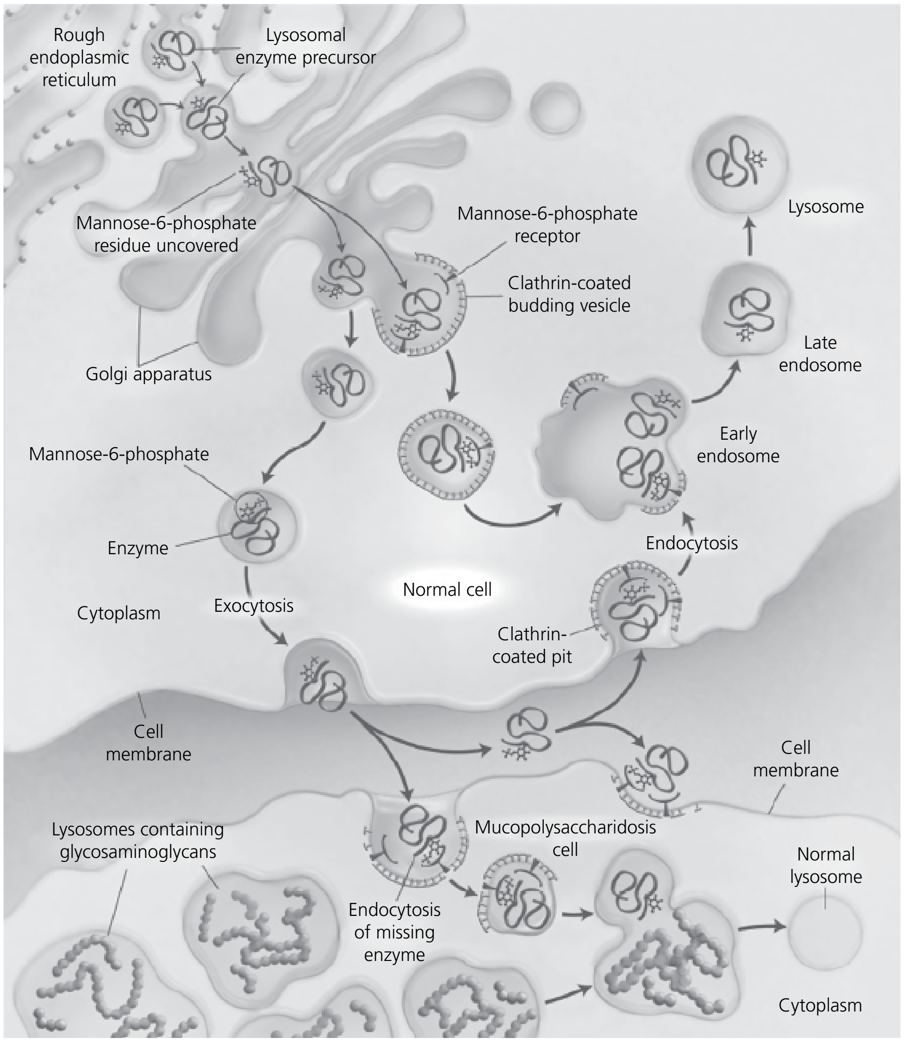
Rough endoplasmic reticulum
Lysosomal enzyme precursor
Mannose-6-phosphate residue uncovered
Mannose-6-phosphate receptor
Clathrin-coated budding vesicle
Lysosome
Late endosome
Golgi apparatus
Early endosome
Mannose-6-phosphate
Enzyme
Endocytosis
Normal cell
Cytoplasm
Exocytosis
Clathrin-coated pit
Cell membrane
Cell membrane
Lysosomes containing glycosaminoglycans
Mucopolysaccharidosis cell
Normal lysosome
Endocytosis of missing enzyme
Cytoplasm

Figure 74.1 Transport of a lysosomal enzyme in a normal cell and correction of storage in the cell of a patient with mucopolysaccharidosis. The mannose-6-phosphate-recognition signal is added to the lysosomal enzyme precursor in the late Golgi compartments, where enzyme modified by mannose-6-phosphate binds to mannose-6-phosphate receptors. The enzyme–receptor complex is packaged into a transport carrier vesicle and delivered to early endosomes, in which low pH promotes the dissociation of the enzyme from the receptor. The enzyme is then delivered to the mature lysosome, and the mannose-6-phosphate receptor is recycled to the Golgi apparatus. A small amount of the mannose-6-phosphate-modified enzyme escapes capture by the mannose-6-phosphate receptors and is released into the extracellular space. This enzyme can be recaptured by binding to a mannose-6-phosphate receptor in a clathrin-coated pit on the cell surface. In a patient who has undergone hematopoietic stem cell transplantation, enzyme released from a donor-derived cell can be taken up by a mucopolysaccharidosis cell, which corrects aberrant glycosaminoglycan storage. Source: Muenzer et al. 2004 [146]. Reproduced with permission of the Massachusetts Medical Society.

X-ALD [17]. Sources of hematopoietic cells for transplantation included bone marrow [12,13,17], peripheral blood [18], and umbilical cord blood (UCB) [17,19,20]. In addition to high-dose regimens, reduced-intensity conditioning regimens were employed effectively [18]. Successful use of combination therapy, ERT, and HCT for patients with Hurler syndrome has been described [6,7]. Finally, participants in the field have recognized the importance of a comprehensive approach to caring for these patients, which is described later.

General considerations

The following elements are critical to the success of HCT in treating patients with storage disorders: a multidisciplinary and multispecialty approach to evaluation and follow-up; careful attention to optimizing HLA typing, hematopoietic cell source selection; the risks and benefits of high-dose versus reduced-intensity conditioning regimens, supportive care measures, and long-term post-HCT evaluations. Several sets of evaluation and follow-up guidelines are provided later in this chapter (Tables 74.4 and 74.5).

Mucopolysacharidoses (MPS) and mucolipidoses (ML)

In this section, MPS disorders (Hurler, Hurler-Scheie, Scheie, Hunter, Sanfilippo, Morquio, Maroteaux-Lamy, and Sly) and mucolipidoses (ML; I-cell disease and pseudo-Hurler polydystrophy) will be presented and discussed (Table 74.1).

The MPS diseases are a group of lysosomal storage disorders caused by deficiency of degradative enzymes of glycosaminoglycans

Table 74.4 Guidelines for the pre-hematopoietic cell transplantation (HCT) evaluation (section A) and post-HCT (section B) follow-up of patients with mucopolysaccharide, mucolipidosis, and glycoprotein metabolic disorders

Section A	**Pre-HCT**
Neurology	Examination including assessment for signs or symptoms of hydrocephalus in patients with Hurler syndrome
Neuropsychology	Test of cognitive ability according to age and developmental level, e.g., Mullen Scales of Early Learning to obtain Early Learning composite or WISC III Language assessment to obtain receptive and expressive language scores Vineland Adaptive Behavior Scales
Neuroradiology	MRI of brain and upper cervical spine with attention to the odontoid process in patients with Hurler syndrome
Cardiology	Echocardiogram, EKG
Pulmonology	Chest X-ray (posteroanterior and lateral) Polysomnogram
Audiology	ENT as needed for placement of tubes Brainstem auditory evoked response if requested by audiology department
Ophthalmology	Routine evaluation and if possible ocular pressures in patients with Hurler syndrome
Genetic counseling	
Neurophysiology	Electrophysiology studies (EMG) with surface electrodes to evaluate for carpal tunnel syndrome if clinically indicated in patients with Hurler syndrome
Occupational, physical, and speech and language therapy	Assessment and therapies as needed, of particular importance in children with Hurler syndrome
Donor studies	Donor enzyme activity level, if possible, in addition to infectious disease markers
Molecular biology studies	On admission to hospital, consider obtaining blood samples for DNA (e.g., mutation analysis, pharmacogenomics)
Lumbar puncture	If there are clinical concerns about possible, significant increased intracranial pressure, a lumbar puncture with determination of opening and closing pressures may be performed with determinations of cerebrospinal fluid glucose and protein in patients with Hurler syndrome
Section B	**Post-HCT**
Blood and marrow transplant (BMT) team	BMT physician, neurologist, neuropsychologist or developmental pediatrician, cardiologist, pulmonologist/sleep disorder specialist, endocrinologist, audiologist, ophthalmologist, occupational and physical therapist, speech and language therapist, advanced practice nurse, nurse coordinator, social worker; others as needed, including occupational and physical therapists
Neurology	Examination
Neuropsychology	Test of cognitive ability according to age and developmental level, i.e., Mullen Scales of Early Learning (Early Learning Composite), or Stanford Binet Intelligence Scale (Composite Score), or Wechsler Preschool and Primary Scale of Intelligence (Full Scale IQ) Language assessment to obtain receptive and expressive language scores Vineland Adaptive Behavior Scales
Neuroradiology	MRI of brain and upper cervical spine (attention to odontoid process)
Cardiology	Echocardiogram EKG
Pulmonology	Chest X-ray Consider follow-up polysomnogram as clinically indicated
Audiology	ENT as needed for placement of tubes Brainstem auditory evoked response if requested by audiology department
Ophthalmology	Electroretinogram
Orthopedic surgery	Orthopedic evaluation of spine, hips, and knees, and hand surgery assessment for carpal tunnel syndrome
Endocrinology	Growth hormone stimulation test Free thyroxine Thyroid-stimulating hormone levels Dual-energy X-ray absorptiometry scan Bone age X-ray
Neurophysiology	Electrophysiology studies (EMG) with surface electrodes to evaluate for carpal tunnel syndrome
Occupational, physical, and speech and language therapy	Assessment and therapies as needed
Engraftment and chimerism studies	Variable number of tandem repeat or XY fluorescence in situ hybridization studies and enzyme level (days +21–30, +60, +100, +6 months, +1 year, and yearly post-HCT thereafter as needed

EKG, electrocardiogram; EMG, electromyelogram; ENT, ear, nose, and throat; MRI, magnetic resonance imaging.

(GAGs) [1], which include heparan, dermatan, keratan, and chondroitin sulfates, that individually or in combination accumulate intracellularly, leading to cellular dysfunction. These GAGs are usually excreted in urine and can be detected by initial diagnostic screening tests. Genes and their complementary DNAs (cDNAs) encoding most of the enzymes have been cloned, leading to characterization of their primary structures, production of recombinant enzymes, and elucidation of disease-causing mutations. MPS disorders, their genetics and respective enzymes, incidence and carrier frequencies, and treatment options are presented in Table 74.1. All of the MPS and ML disorders are autosomal recessive in their inheritance except for Hunter syndrome (X-linked). ML II (I-cell) and ML III (pseudo-Hurler polydystrophy) are related, rare genetic diseases in which there is abnormal lysosomal enzyme transport in cells of mesenchymal origin. Newly synthesized lysosomal enzymes are secreted into the extracellular medium rather than being targeted correctly to lysosomes. Affected cells show dense storage material-filled inclusions; lysosomal enzymes are present at elevated levels in serum and body fluids of affected patients [21].

Shared clinical features among the MPS disorders include progressive, multiorgan system involvement, organomegaly, dysostosis multiplex, and facial dysmorphia. Hearing, vision, respiratory, and cardiovascular function are often affected. Severe cognitive

Table 74.5 Guidelines for the pre-hematopoietic cell transplantation (HCT) evaluation (section A) and post-HCT (section B) follow-up of patients with leukodystrophies

Section A	**Pre-HCT**
Neurology	Examination
Neuropsychology	Age-appropriate battery of neuropsychologic tests, i.e., Wechsler Preschool and Primary Scale of Intelligence (WPPSI-revised) and Wechsler Intelligence Scale for Children III (WISC-III) Wechsler Adult Intelligence Scale III to obtain: • Full Scale IQ Score • Verbal IQ Score • Performance IQ Score Other testing to measure school achievement, language, visual perception, attention, memory, motor function, executive function. and behavior Vineland Adaptive Behavior Scales
Neuroradiology – cerebral X-ALD	MRI of brain including contrast agent (gadolinium) with severity score determinations (Loes Score [63]); Magnetic resonance spectroscopy if available and deemed to be potentially informative
Neuroradiology – GLD [12] and metachromatic leukodystrophy	MRI of brain
CSF	Examination of CSF (including CSD protein): may be informative in patients with advanced disease at HCT to evaluate status of blood–brain barrier disruptions (i.e., degree of CSF protein elevation; e.g., in infants with GLD, CSF protein levels of 500–600 mg/dL can be seen)
Pulmonology	Chest X-ray
Ophthalmology	Assessment as needed
Endocrine	To assess treatment of adrenal insufficiency (i.e., dosing of hydrocortisone) in boys with X-ALD; routine assessment of all patients for thyroid function, progress toward puberty, growth, and development
Neurophysiology	Electrophysiology studies (EMG): a long-term evaluation in boys transplanted for cerebral X-ALD who are reaching the age at which they are at risk for developing AMN, i.e., approximately ≥20 years of age. The question of whether such men will develop AMN is unanswered
Occupational, physical, and speech and language therapy	Assessment and therapies as needed
Section B	**Post-HCT**
Blood and marrow transplant (BMT) team	BMT physician, neurologist, advanced practice nurse, nurse coordinator, social worker; others as needed, including occupational and physical therapists
Neurology	Examination
Neuropsychology	Age appropriate battery of neuropsychologic tests, i.e., Wechsler Preschool and Primary Scale of Intelligence (WPPSI-revised) and Wechsler Intelligence Scale for Children III (WISC-III) Wechsler Adult Intelligence Scale III to obtain: • Full Scale IQ Score • Verbal IQ Score • Performance IQ Score Other testing to measure school achievement, language, visual perception, attention, memory, motor function, executive function, and behavior Vineland Adaptive Behavior Scales
Neuroradiology – cerebral X-ALD	MRI of brain including contrast agent (gadolinium) with severity score determinations (MRI severity or Loes Score [63]); recommended minimum number of examinations – approximately 2–3 months post-HCT to evaluate for resolution of gadolinium enhancement (hallmark of active demyelination in cerebral X-ALD) and at 1 year post-HCT to evaluate severity score at time when stabilization has been achieved [64]. Subsequent brain MRIs as clinically indicated Magnetic resonance spectroscopy if available and deemed to be potentially informative
Neuroradiology – GLD and MLD	MRI of brain at 1 year post-HCT and annually, if clinically indicated, until stable myelination is demonstrated
CSF	Examination of CSF (including CSD protein): may be informative in patients with advanced disease at HCT to evaluate status of blood–brain barrier disruptions (i.e., degree of CSF protein elevation; e.g., in infants with GLD, CSF protein levels of 500–600 mg/dL can be seen)
Pulmonology	Chest X-ray
Ophthalmology	Assessment as needed
Endocrine	To assess treatment of adrenal insufficiency (i.e., dosing of hydrocortisone) in boys with X-ALD; routine assessment of all patients for thyroid function, progress toward puberty, growth, and development
Neurophysiology	Electrophysiology studies (EMG): a long-term evaluation in boys transplanted for cerebral X-ALD who are reaching the age at which they are at risk for developing AMN, i.e., approximately ≥20 years of age. The question of whether such men will develop AMN is unanswered
Engraftment and chimerism studies	Patient's variable number of tandem repeats and appropriate enzyme levels (days +21–30, +60, +100, +6 months, +1 year, and yearly post-HCT thereafter); follow-up evaluation of VLCFA has been discontinued as published studies showed a 55% reduction in these levels after HCT but not complete normalization [66]. VLCFA levels do not appear to have any clinical significance or correlation with disease activity or clinical status

AMN, adrenomyeloneuropathy; CSF, cerebrospinal fluid; EMG, electromyogram; GLD, globoid cell leukodystrophy; ML, metachromatic leukodystrophy; MRI, magnetic resonance imaging; VLCFA, very long chain fatty acid; X-ALD, X-linked adrenoleukodystrophy.

deficits with developmental delay are observed in Hurler (MPS IH), the severe phenotype of Hunter (MPS IIA), and Sanfilippo syndromes (MPS III) [1].

HCT represents an effective therapy with the longest clinical experience for selected MPS disorders. MPS and ML disorders that can benefit from HCT include Hurler (MPS IH), Maroteaux-Lamy (MPS VI), and Sly (MPS VII) syndromes, as well as I-cell disease (ML II). On the other hand, either limited or lack of effectiveness of HCT in benefiting the CNS and/or the skeletal system has significantly dampened enthusiasm for transplant in patients with Hunter (MPS II), Sanfilippo (MPS III), and Morquio (MPS IV) syndromes (Table 74.1).

MPS DISORDERS FOR WHICH HCT CAN BE BENEFICIAL: MPS I, MPS VI, AND MPS VII

Hurler (MPS IH), Hurler-Scheie (MPS IH/S), and Scheie syndromes (MPS IS)

Epidemiology, molecular biology, and etiology

MPS I is an autosomal recessive lysosomal storage disorder that is caused by deficient enzyme activity of α-L-iduronidase (IDUA), leading to lysosomal accumulation of GAGs in multiple tissues throughout the body. With an overall prevalence of 1 in 100,000 live births [22] and significant variability in presentation, diagnosis of MPS I in all its forms poses a true challenge. The initial diagnosis is primarily based on physician recognition of signs and symptoms. Deficient IDUA activity and excess urinary GAG excretion are seen in all patients, but do not accurately predict disease severity or form [22]. Most known disease-causing mutations (over 100 to date) are individually unique ("private") and uncharacterized.

Clinical description

MPS I is a chronic, progressive disease affecting the heart, eyes, bones, joints, respiratory system, facial appearance, viscera, and often the CNS. It has historically been classified clinically into three syndromes based on age of onset, rapidity of progression, and presence and degree of cognitive involvement [1]. Hurler syndrome, also known as MPS IH, describes patients with the most severe form of MPS I, with signs and symptoms typically appearing in infancy and a median age of death of 6.8 years when untreated. Patients with Scheie syndrome, MPS IS, present later in childhood and demonstrate slower symptom progression with preservation of cognition and survival into adulthood. Hurler-Scheie, MPS IH/S, describes an intermediate form with no or mild cognitive impairment and death usually occurring in adolescence or early adulthood when untreated. Patients with Hurler-Scheie and Scheie syndromes have also been referred to as having attenuated MPS I. Collectively, these forms represent different degrees of severity along a disease spectrum without strict clinical, biochemical, or molecular diagnostic criteria in place to differentiate them.

Hematopoietic cell transplantation for MPS I

Since the first report of successful reversal of clinical features of Hurler syndrome and improvement in biologic markers after HCT in one infant with Hurler syndrome [9], hundreds of patients with MPS IH and MPS IH/S have been transplanted. Large HCT experiences with allografts from related, unrelated and cord blood units from both related and unrelated donors [15,22,23] have provided guidance for patient selection and timing of HCT, and a realistic assessment of the effects of HCT on various organs and tissues in MPS IH and MPS IH/S, as well as neurocognitive function (Figure 74.2). While it is difficult to provide a precise estimate of long-term engrafted survival for Hurler HCT patients, the likelihood is as high as 80–85% using bone marrow or UCB (Figure 74.3) [19,23].

The decision on whether to proceed with HCT for patients with Hurler syndrome is a complex one. Factors include the child's neurocognitive and developmental status with emphasis on the potential for growth, the presence or absence of hydrocephalus with or without a ventriculoperitoneal shunt, and a history of pulmonary and airway complications such as frequent pneumonias, chronic hypoxia, sleep apnea, and cardiac failure. It is imperative that a comprehensive pre-HCT evaluation be performed and that detailed information be provided to families with particular attention to disease aspects that are typically resistant to the beneficial effects of HCT, such as skeletal abnormalities, some ocular and auditory features, and cardiac aspects. Long-term, coordinated, comprehensive monitoring not only for late effects from HCT (see Chapter 105), but also for disease-specific aspects, should be performed in close collaboration with educators and therapists (e.g., physical, occupational, speech, and language) (Table 74.4).

With successful donor-derived engraftment, MPS IH, Hurler syndrome, patients can experience favorable long-term HCT

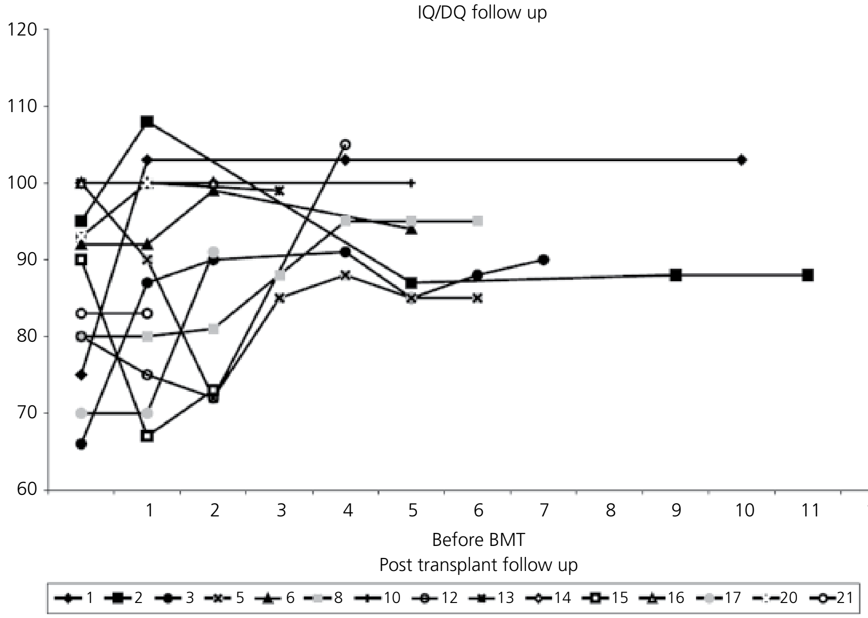

Figure 74.2 Neurocognitive function as measured by intelligence or developmental quotients (IQ or DQ) in 15 patients with Hurler syndrome before and after hematopoietic cell transplantation. BMT, bone marrow transplantation. Source: Souillet et al. 2003 [23]. Reproduced with permission of Nature Publishing Group.

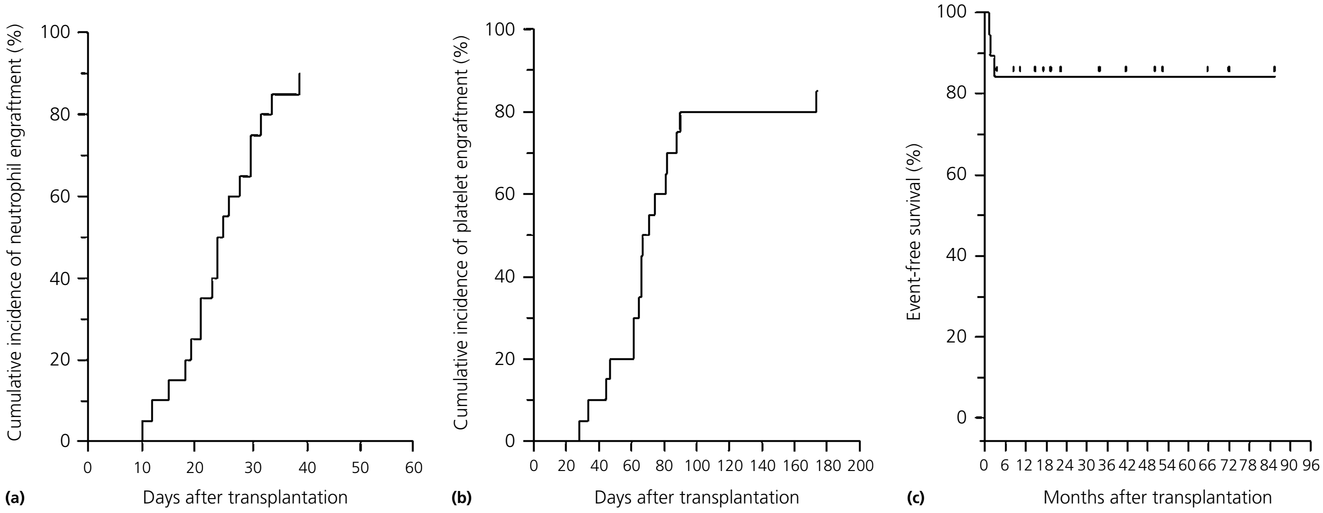

Figure 74.3 Cumulative incidence of neutrophil (a) and platelet (b) engraftment after cord blood transplant, and Kaplan–Meier estimates of the probability of event-free survival (c). In (a), myeloid engraftment was defined as an absolute neutrophil count of at least $500/\text{mm}^3$ on three consecutive days. The one patient who remained aplastic after the first cord blood transplant received a second cord blood transplant on day +63. Myeloid engraftment occurred on day +26 after the second transplant. In (b), platelet engraftment was defined by a platelet count of at least $50{,}000/\text{mm}^3$ without need for transfusion for at least 7 consecutive days. In (c), event-free survival was defined by survival with full (>99%) donor chimerism. Tick marks indicate the most recent follow-up visit for each patient. Source: Staba et al. 2004 [19]. Reproduced with permission of the Massachusetts Medical Society.

outcomes. These outcomes include stabilization of the CNS with preservation of neurocognitive function, and motor development within the normal range and capacity for independence in activities of daily living. Careful neuropsychologic and neuroradiologic assessments are needed [24–26].

When performed early in the disease course, HCT can improve metabolism and clearance of GAGs in the highly perfused visceral organs, such as the liver, spleen, adenoid, tonsils, heart, and lungs. A major reduction of the hepatosplenomegaly as well as relief of the obstructive airway symptoms, including persistent rhinorrhea and obstruction of the upper airways, is seen in all patients within the first 3–6 months after HCT [16,23,27]. Myocardial muscle function is stabilized or improved, and coronary artery patency has been demonstrated up to 14 years after HCT [28]. The long-term outcome of cardiac valvular thickening and insufficiency require continued monitoring. Furthermore, HCT helps preserve intellectual function and prevent manifestations of the classic severe Hurler phenotype with a relatively low risk of progressive cognitive deterioration. This has been achieved only when HCT is performed early in life and before cerebral damage has occurred [14–16,23,27,29]. Significant correlations, between the age at HCT and the slope of the cognitive development after HCT (−0.64; $p < 0.03$ and −0.51; $p = 0.01$), as well as the baseline Mental Development Index (MDI) and slope of the cognitive development after HCT (0.76; $p < 0.05$ and 0.77; $p = 0.0002$), have been documented in MPS IH patients [14,15] indicating that both a younger age at HCT and a higher baseline MDI are associated with better cognitive trajectories after HCT. Therefore, some authorities had suggested that HCT should only be indicated in MPS IH patients under the age of 2 years and with no more than minimal cognitive impairment (MDI $\geq$ 0.70). However, as the neurocognitive outcome of MPS IH patients older than 2 years at HCT or with a baseline MDI below 70 varies considerably [14–16,26], these contraindications must be used with caution [16]. The neurocognitive situation before HCT may be difficult to evaluate, especially in younger patients or when hearing loss is present. In addition, HCT may still be indicated after 2 years of age in those patients who present with a slightly milder phenotype. Therefore, the decision about SCT must be made on an individual basis.

Some disease features show less favorable responses due to poor penetration of laronidase into the relevant tissue. Principal among these are skeletal abnormalities also known as dysostosis multiplex. Successfully transplanted children often require major orthopedic surgical procedures for genu valgum, acetabular hip dysplasia, kyphoscoliosis, carpal tunnel syndrome, and trigger digits by 6 years of age. Orthopedic aspects of MPS disorders were the subject of a consensus conference held in Manchester, United Kingdom, in 2003 (Table 74.6). Interestingly, upper cervical spine stabilization has occurred long term in many donor-engrafted Hurler patients [30].

Cell source for HCT in patients with MPS I

Since the first bone marrow transplantation in a child with MPS IH in 1981, approximately 500 MPS patients have been transplanted using a variety of donor sources, including bone marrow (BM) or peripheral blood stem cells (PBSC) from related non-carrier and carrier donors, BM or PBSCs from unrelated donors, and unrelated partially matched umbilical cord blood units. The Mendelian inheritance of human leukocyte antigen (HLA) and MPS genes restricts the chances of finding a non-carrier matched related BM donor to only about one in 16 of patients [5]. Thus, the majority of patients who may benefit from HCT will need alternate donor sources. In general, there are more published reports of UCBT in the last few years [3,4,19,22,31]. Results of a multinational MPS I registry showed a continuous trend in the proportion of patients receiving stem cells from cord blood or PBSC rather than BM increasing from 26 out of 158 patients (16.5%) before 2003 to 33 out of 65 patients (50.7%) in 2003–2005 and 39 out of 64 patients (60.9%) in 2006–2009 [22]. In various studies, UCBT as the stem cell source in MPS IH patients was associated with higher rates of successful sustained engraftment, compared to bone marrow and peripheral blood (84–85% vs. 26–75%) [14,15,19,23,31]. However, the results obtained using UCBT mainly involved HCTs performed

Table 74.6 Consensus statement prepared by facilitator Ms Jean Mossman on the orthopedic management of mucopolysaccharidosis (MPS) patients who have had a hematopoietic cell transplant (HCT). Consensus position statements arising from conference held October 3–4, 2003 in Manchester, UK

A. General session

Speakers and contributors to this session and their affiliations: Peters, C. currently: The Children's Mercy Hospital, Kansas City, USA, previously: University of Minnesota, Minneapolis, USA; Wynn, R. Royal Manchester Children's Hospital, Manchester, UK; Steward, CG. Bone Marrow Transplant Unit, Royal Hospital for Children, Bristol, UK; Wraith, E. Willink Biochemical Genetics Unity, Royal Manchester Children's Hospital, Manchester, UK; Guffon, N. Centre de Référence des Maladies Héréditaires du Métabolisme, Hôpital Edouard Herriot, Lyon, France.

- As a result of HCT and other emerging treatments for MPS diseases, the management of skeletal abnormalities has become increasingly important
- HCT has favorably altered the natural history of some MPS diseases and can partially correct the skeletal abnormalities
- Multiple lines of accumulating evidence in patients and animals provide compelling evidence that early HCT has a positive impact on skeletal disease, in particular carpal tunnel syndrome and trigger finger
- HCT improves the soft tissue abnormalities associated with MPS diseases
- HCT is expected to be more effective as prophylaxis than treatment
- Skeletal disease varies between types of MPS disease
- The etiology of skeletal disease is uncertain: it is not clear how glycosaminoglycan accumulation leads to the skeletal dysplasia
- At present, it is not possible accurately to predict which children will develop skeletal abnormalities of sufficient severity to require intervention, and ongoing review of the likely orthopedic problems is essential
- The outcome of orthopedic surgery for skeletal and soft tissue problems (genu valgum, hip dislocation, trigger finger, and carpal tunnel syndrome) in the group of patients for whom these symptoms are a problem is encouraging
- Most parents of children who have had surgery have been pleased with the results
- Assessing the long-term outcome of surgical intervention in children who have received bone marrow transplants is important and requires follow-up into adulthood
- An international registry of both treated and untreated patients, including procedures and outcomes, is needed as a matter of urgency
- Data fields collected by registries should have input from patients and families to ensure that the data collected are relevant to the decisions future parents will make regarding treatment for their children

B. Knee surgery session

Speakers and contributors to this session and their affiliations: Ogilvie, JW, currently: Shriners Hospital for Children, Salt Lake City, USA, previously: University of Minnesota, Minneapolis, USA; Meadows, T. Royal Manchester Children's Hospital, Manchester, UK.

- >50% of engrafted patients develop knock knees
- Good correction can be achieved with both stapling and osteotomy
- Changes with growth can result in failure of both procedures, and more than one procedure may be necessary
- Stapling can fail as a result of permanent growth failure of the growth zone on the other side of the knee
- Assessment for knee surgery: in children where there is clinical suspicion of genu valgum, a posteroanterior standing X-ray of the entire lower limbs is indicated

Stapling

- Epiphyseal stapling should be considered when the tibiofemoral angle >~15° *and*
- Epiphysis is ossified enough to hold staple
- Age >4–5 years may be optimal *and*
- There is an expectation of a further 1–2 years of growth remaining
- The staple should be removed when angle is reduced to 0–5°. Two staples, of a design that provides adequate anchorage but which can be removed, should be used

Osteotomy

Osteotomy should be considered when:

- Degree of deformity >~20°
- Stapling has failed to correct the problem
- The deformity is not at the knee
- The patient is too old and therefore there is not enough growing time left
- Acute correction versus graduated correction: Patient preference, in the light of a clear explanation and open discussion of the risks and benefits of both approaches, should contribute to the decision about which approach to use
- Patient groups have an important role to play in producing information setting out the risks and benefits

Osteotomy	Stapling
More morbidity	Less morbidity
Several days in hospital	Outpatient or short stay if not done alongside other procedures
Days or weeks to become ambulatory, depending on procedure	Ambulatory within days

C. Hip surgery session

Speakers and contributors to this session and their affiliations: Meadows, T. Royal Manchester Children's Hospital, Manchester, UK; Ogilvie, JW, currently: Shriners Hospital for Children, Salt Lake City, USA, previously: University of Minnesota, Minneapolis, USA; Garin, C. Hôpital Edouard Herriot, Lyon, France.

- The same basic principles for indication for surgery apply as in non-MPS hip dysplasia:
  - Shallow acetabulum
  - Shenton's line broken
  - Patients for whom longevity is assumed
  - If the degree of dysplasia is likely to lead to
    - early or premature arthritis
    - adverse effect on quality of life is likely

The aim of surgery is to:

- Reduce the hip
- Provide cover
- Early rehabilitation

Hip surgery should be undertaken before the ability to remodel the acetabulum is lost

- Assessment
  - – Anteroposterior X-ray of the pelvis
  - – Consider magnetic resonance imaging

Surgery may be to the pelvis, femur or both

(*Continued*)

Table 74.6 (*Continued*)

The operation technique will depend on:
- Age
- Severity of problem
- Surgical preference

Both hips may be operated on at the same time, depending on the technique used, the general wellbeing of the patient and the preference of the patient/family and the surgeon
One technique which has been used successfully:
1) Single stage
   a) bilateral femoral osteotomy
   b) bilateral iliac osteotomy
      Femoral varus with or without rotation osteotomy
      Degas-type iliac osteotomy
2) 6 weeks in a cast
   a) progressive ambulation
   b) gentle physical therapy

D. Carpal tunnel syndrome session
Speakers and contributors to this session and their affiliations: Van Heest, A and Khanna, G. University of Minnesota, Minneapolis, USA.
- Screening testing for all children with MPS using nerve conduction velocity is essential. Children do not complain of pain, numbness or weakness until the carpal tunnel syndrome is severe and neurologic damage may be permanent. Nerve conduction velocity testing can diagnose carpal tunnel in its milder form, when median nerve compression is reversible
- Nerve conduction velocity with decreased velocity or decreased amplitude or increased latency in median nerve function compared with ulnar nerve function over the same carpal distance is diagnostic of carpal tunnel syndrome
- Untreated carpal tunnel syndrome leads to loss of thenar muscle function and loss of sensation in the thumb, index, long, and part of the ring finger
- Carpal tunnel syndrome in MPS diseases is due to dysplasia of the carpal bones, leading to a narrower bony carpal tunnel, combined with glycosaminoglycan deposits on the flexor tendons within the carpal tunnel, resulting in increased pressure on the median nerve
- Studies have demonstrated that carpal tunnel release surgery improves median nerve function
- With improved blood and marrow transplant techniques, treating patient with MPS IH, approximately 50% of children acquire carpal tunnel syndrome. Decreased risk of developing carpal tunnel syndrome exists for patients transplanted at a younger age and in patients with normal or carrier levels of enzyme post-transplant

E. Trigger finger session
Speakers and contributors to this session and their affiliations: Van Heest, A and Khanna, G. University of Minnesota, Minneapolis, USA.
- Untreated locked trigger finger can lead to permanent hand joint contractures
- Surgical release of the A1 and A3 pulleys with possible resection of half the flexor digitorum superficialis tendon may lead to prevention of contractures if locked digits exist. Stretching exercises for the fingers and night-time splinting do not resolve the underlying cause
- Screening for trigger finger includes palpation of the flexor tendons for nodules as well as documentation of active and passive range of motion of each finger joint

F. Spinal surgery for kyphosis session
Speakers and contributors to this session and their affiliations: Garin, C. Hôpital Edouard Herriot, Lyon, France; Ogilvie, JW, currently: Shriners Hospital for Children, Salt Lake City, USA, previously: University of Minnesota, Minneapolis, USA; Williamson, B. Royal Manchester Children's Hospital, Manchester, UK.
- Purpose of spinal surgery is:
  - to keep the spine balanced
  - to protect neural elements
  - to maximize mobility and function by minimizing the deformity
  - to retain quality of life
- Spinal surgery should only be undertaken after a surgeon with expertise in operating on children with MPS diseases has clinically reviewed the case
- Indications for surgery:
  - these may be different from the indications that would apply to non-MPS children
  - consistent deterioration on serial X-rays
  - clinical need as judged by expert opinion
  - some surgeons operate only when the degree of curvature is in the 60–70° region
- There is a role for patient organizations in educating patients and their families on the need to monitor change in the spine and to be alert to neurologic changes that might arise from spinal changes:
  - Weakness
  - Pain
  - Decreasing activity tolerance
  - Deteriorating bowel or bladder function
- Assessment: standing 2 m posteroanterior and lateral X-ray
- Surgical approach
  - Anterior column support
  - Anterior posterior surgery
- The role of braces
  - There is no evidence that bracing alters the natural history of spinal changes prior to surgery
  - On this basis, if families can tolerate no bracing, it is an acceptable approach to take
- Post-surgical management
  - A cast should be applied in the operating room at the end of surgery and should remain in place for between 6 weeks and 3 months
  - Subsequently, the patient should wear a brace until the bones have healed/united/fused
  - Physiotherapy should only be undertaken with a treatment plan agreed in advance with the surgeon
  - Normal activity can be resumed at 12 months, although this does not include contact sports and other potentially dangerous activities such as tumbling

G. Spinal surgery for scoliosis session
Speakers and contributors to this session and their affiliations: Garin, C. Hôpital Edouard Herriot, Lyon, France; Ogilvie, JW, currently: Shriners Hospital for Children, Salt Lake City, USA, previously: University of Minnesota, Minneapolis, USA; Williamson, B. Royal Manchester Children's Hospital, Manchester, UK.
- Purpose of spinal surgery is:
  - to keep the spine balanced
  - to protect neural elements
  - to maximize mobility
  - to retain quality of life
- Indication for surgery: apply same principles as for non-MPS children
- Spinal surgery for scoliosis should only be undertaken after a surgeon with expertise in operating on children with MPS diseases has clinically reviewed the case

during the last two decades, whereas many of the studies using BM or PBSC have been accomplished over the last several decades. Therefore, these higher rates of successful engraftment in UCB recipients might have been caused by other factors, including changes in conditioning regimen and supportive care. This is supported by a retrospective report by the European Group for Blood and Marrow Transplantation (EBMT) study, analyzing risk factors for graft failure in 146 MPS IH patients [32]. There was no significant difference in engraftment between the use of UCBT, BM, and PBSC stem cells. Although no difference in survival or graft failure could be demonstrated between the recipients of the various stem cell sources, the authors did show that more patients receiving a UCBT achieved full donor chimerism (93%), compared to other stem cell sources (BM and PBSC, 66%: OR 9.31; $p = 0.044$). Furthermore, all patients receiving a UCBT obtained normal enzyme levels, compared to 60% in the other group [32]. Higher incidences of full donor chimerism and normal enzyme levels after UCBT in MPS IH patients was documented by other studies [3,19,31]. This was documented to occur in other forms of inherited errors of metabolism [3,20].

Theoretically, in addition to achieving normal enzyme levels and higher levels of donor engraftment the use of UCB for HCT has several other potential advantages, including:

1. CBUs are readily available, significantly reducing the time between diagnosis and HCT [19,20]. This is particularly important for the rapidly progressive CNS involvement in MPS IH patients.
2. Donors with human leukocyte antigen (HLA) disparities can be used, increasing the possibility of finding a suitable donor [19].
3. Lower rates of acute and chronic graft-versus-host disease (aGVHD, cGVHD) have been reported despite higher levels of HLA incompatibility [3,19].
4. Although highly speculative, there are suggestions of transdifferentiation into cell types such as osteoblasts, chondroblasts, and neurons, because of the more primitive stem cell population in UCB [33].

The theoretical disadvantages of using UCB as a stem cell source are:

1. A protracted duration to engraftment, due to the lower amount of stem cells in UCB.
2. The availability of only one donation per donor.
3. Less adoptive immunity that may increase the likelihood of life-threatening viral reactivations.

These disadvantages are, however, less applicable to MPS IH patients due to their typical young age and small size. These patients require small doses of stem cells in order to obtain successful engraftment and as relatively low graft failure rates are reported after CBT, a single UCB is sufficient in the majority of cases.

In conclusion, UCBT might be the preferential stem cell source for HCT in MPS IH patients as full donor chimerism and higher enzyme levels have been suggested to positively influence the neurocognitive outcome after HCT.

Enzyme replacement therapy and HCT for MPS I

Enzyme replacement therapy is an etiology-specific treatment that seeks to address the underlying pathophysiology of MPS I by delivering sufficient α-L-iduronidase (IDUA laronidase) activity to reverse and prevent GAG accumulation. ERT with recombinant human IDUA, laronidase, has shown efficacy with low toxicity in a randomized, double-blinded, placebo-controlled, multinational phase 3 study [34].

Since the 1980s HCT has proved to be the only known curative measure for the severe form of MPS IH [9]; MPS IS (Scheie syndrome) and MPS IH/S (Hurler-Scheie syndrome), both demonstrating less aggressive phenotypes compared to MPS IH, with normal to mildly delayed neurocognitive development, and survival beyond the first decade and often into the third decade [1]. While HCT can successfully treat MPS IH/S or MPS IS, the significant toxicities associated with HCT and the availability of ERT has made its application in these less severe forms difficult to justify; therefore the use of HCT in MPS IH/S and MPS IS has progressively declined and use of recombinant IDUA increased in treatment of these patients [22,34].

ERT has become a mainstay of therapy for patients with MPS IH/S and MPS IS, the attenuated forms of IDUA deficiency that do not have CNS manifestations. As the intravenous administration of the enzyme has been shown to not effectively cross the blood–brain barrier, the treatment of severe MPS I deficiency with enzyme replacement alone has not been accepted; in the MPS IH (Hurler syndrome) population, HCT remains the standard of care. However, one major consequence of the success of ERT in MPS IH/S and MPS IS has been the potential benefits of adding ERT either prior or after HCT [35,36]. The use of ERT in association with HCT resulted in enhanced cognitive outcomes, providing new evidence that ERT is a valuable addition to standard HCT. Although the mechanism responsible for this improved outcome is unknown, it is possible that both direct and indirect effects may be involved [36].

Outcome of HCT in patients with MPS I

Generally, with successful HCT, children with MPS IH can achieve normal neurodevelopment [19] and do not require ventriculoperitoneal shunting [27]. While age and cardiopulmonary and neurodevelopmental status are important determinants of outcome in patients with MPS IH following HCT, other contributing factors include quality and quantity of developmental services and therapies (e.g., speech and language therapy) as well as the number and severity of specific post-HCT complications such as GVHD, infections, and organ toxicities [14,15,19,23,27,29,37,38]. On occasions when primary or secondary graft failure has occurred, successful long-term engrafted survival with a favorable quality of life has been achieved following a second HCT [39]. Considerable attention has been paid to issues related to quality of life, including musculoskeletal, motor, and adaptive function in Hurler patients after HCT [24,25,40], as well as to optimizing patient safety during and after HCT. ERT using laronidase prior to, during, and after HCT has been administered to reduce the total body burden of GAGs, and theoretically to reduce the likelihood of an MPS-related peritransplant complication [6,7].

Maroteaux-Lamy syndrome (MPS VI)

Epidemiology, molecular biology, and etiology

Mucopolysaccharidosis VI (MPS VI, Maroteaux-Lamy syndrome) is characterized by an absence or deficiency of N-acetylgalactosamine 4-sulfatase (arylsulfatase B) resulting in accumulation of dermatan sulfate. The principal clinical features of children with MPS VI in a survey of 121 untreated patients with MPS VI older than 4 years of age revealed that while MPS VI patients are not known to have primary cognitive impairment due to central nervous system GAG storage, they suffer a broad range of symptoms including short stature, large head with coarse facial features, flat nasal bridge, enlarged tongue, several skeletal and joint abnormalities, valvular heart disease, hernias, weakness, hepatosplenomegaly, airway obstruction, chronic ear and respiratory infections, carpal tunnel syndrome, corneal clouding, poor vision, and blindness [41].

Consequently, patients born with rapidly progressing MPS VI usually suffer severe disability and diminished life expectancy.

Hematopoietic cell transplantation for MPS VI

In the absence of ERT or HCT, life expectancy for rapidly progressing patients is limited to the 2nd or 3rd decades, while slowly progressing patients have a life expectancy into the 5th or 6th decade of life [42]. Following engraftment of HCT, increased arylsulfatase B activity can be demonstrated and there is evidence for overall clinical improvement in the condition of these patients. Long-term improvements in facial dysmorphism, hepatosplenomegaly, joint mobility, and cardiac manifestations has been demonstrated in MPS VI patients following HCT [43]; however, skeletal disease known as dysostosis multiplex tends to persist or progress despite HCT, and visual manifestations show only limited beneficial effects [44].

Prior to the availability of ERT, the clinical management of MPS VI was limited to supportive care and allogeneic HCT; however, due to the rarity of this disease, little is known about the long-term outcomes of HCT for MPS VI.

Outcome of HCT in patients with MPS VI

HCT has been used successfully to treat MPS VI for nearly 25 years [10,43], with resolution of hepatosplenomegaly, airway obstruction, and sleep apnea, prevention of further cardiopulmonary deterioration, and improved joint mobility. Visual acuity improved in some cases [10,43], although corneal haze does not necessarily resolve. As in other MPS disorders, HCT has not treated effectively skeletal abnormalities (Table 74.6). An analysis using aggregate data gathered by the Center for International Blood and Marrow Transplant Research (CIBMTR) between 1982 and 2007 to determine survival probability for patients with MPS VI following allogeneic HCT identified 45 MPS VI patients with a median age of 5 years (range, 1–22 years) at the time they received an allogeneic HSCT. Cumulative incidence (95% CI) of aGVHD at 100 days was 36% (21–53%). Probability of survival was 78% (65–89%) at 100 days and 66% (52–79%) at 1 and 3 years. While these data are based upon small numbers of recipients, they represent the largest series to date and may help clinicians assess the relative risks and benefits of currently available therapies [45].

Enzyme replacement therapy and HCT for MPS VI

A recombinant human arylsulfatase B (rhASB) treatment of MPS type VI is available and has been investigated since 2004. Results of a phase 3 study showed that rhASB was well tolerated and did not result in discontinuation of the study drug or in serious sequelae for any patient. The improvement in functional tests presented for 48 weeks of this phase 3 study confirmed the effectiveness of weekly administration of 1.0 mg/kg rhASB on endurance and urinary GAG excretion and demonstrated a favorable safety profile for this compound in patients with MPS VI. Finally, the improvements in efficacy and safety were confirmed in the follow-on 24-week open-label extension period [46]. With the availability of an effective ERT, the future role of HCT in treatment of patients with MPS VI is unclear (Table 74.6).

Sly syndrome (MPS VII)

Mucopolysaccharidosis type VII (MPS VII; β-glucuronidase deficiency) is a rare autosomal recessive genetic disorder due to an inherited defect in the lysosomal hydrolase, β-glucuronidase, which in turn causes accumulation of undegraded glycosaminoglycans in the lysosomes. The use of HCT for MPS VII is limited by the rarity of the disorder and its predilection toward hydrops fetalis, the neonatal form is one of the few lysosomal storage diseases with clinical manifestations in utero or at birth, although attenuated forms do exist [47]. MPS VII, in certain circumstances, can be ameliorated by HCT provided the neurodevelopmental and clinical status of the patient is satisfactory at the time of HCT [47]. In this case report, the 12-year-old MPS VII patient experienced major improvements in motor and pulmonary function with diminished upper respiratory tract and middle ear infections, leading to an improved quality of life.

Mucolipidoses (ML) types II and III (I-cell disease and pseudo-Hurler polydystrophy)

I-cell disease (ML II) and pseudo-Hurler polydystrophy (ML III) are lysosomal storage diseases caused by abnormal lysosomal enzyme phosphorylation and localization. In both diseases, newly synthesized lysosomal enzymes are secreted into the extracellular medium instead of being targeted correctly to lysosomes. All cells and tissues of affected patients are deficient in UDP-N-acetylglucosamine: lysosomal enzyme N-acetylglucosamine-1-phosphotransferase activity. ML II is characterized by severe psychomotor retardation and shares many clinical and radiologic features with Hurler syndrome. However, clinical differentiation is possible due to earlier onset of signs and symptoms, absence of GAGs in urine, and the more rapidly progressive course, leading to death between 5 and 8 years of age. Neonates with I-cell disease usually show characteristic facial features, craniofacial abnormalities, and restricted joint movement despite generalized hypotonia. Other presenting features may include striking gingival hyperplasia, congenital hip dislocation, fractures, hernias, and bilateral talipes equinovarus. The clinical course includes progressive failure to thrive and developmental delay, usually evident by age 6 months; linear growth decelerates during the first year and stops during the second. Joint immobility progresses, with claw hand deformity and kyphoscoliosis ensuing. Hepatomegaly is prominent while splenomegaly is minimal. Respiratory infections are frequent, as is otitis media. Cardiomegaly and murmurs are common, and aortic insufficiency is not infrequent [21].

Historical experience with HCT for ML II (I-cell disease) existed initially only in patients with end-stage cardiopulmonary disease [48]. Over the past decade, transplant experience with a small cohort of patients ranging in age from 0.3 to 1.7 years has demonstrated that long-term engrafted survival can be achieved in selected patients who continue to make developmental progress, albeit at slower than normal rates [49]. Obstructive sleep apnea may require tracheostomy prior to HCT as well as continuous positive airway pressure or bilevel positive airway pressure support. Further, careful long-term monitoring is required as airway complications and pulmonary hypertension can occur.

MPS DISORDERS FOR WHICH HCT IS NOT BENEFICIAL: MPS II, MPS III, MPS IV

Hunter syndrome (MPS II)

Epidemiology, molecular biology, and etiology

Mucopolysaccharidosis II (MPS II or Hunter syndrome) is a rare genetic disease caused by deficiency of the lysosomal enzyme iduronate-2-sulfatase (IDS). MPS II has an incidence of approximately 0.31 to 0.71 per 100,000 live births and it is found almost exclusively in young males as it is an X-linked condition. Affected females with a clinical picture in many cases similar to that of the young males have been described [50]. MPS II is a chronic, progressive disease with a clinical picture similar in certain aspects to that of MPS I: there is great variability in the clinical manifestations, including CNS involvement, and it can therefore be classified into a severe or "neuropathic" form and an attenuated or "non-neuropathic" form. The more common severe form presents before the age of 3 years with profound neurocognitive and developmental delay, and shares clinical features with MPS IH, including facial dysmorphia, progressive conductive and sensorineural hearing loss, upper airway obstruction with sleep apnea, cardiopulmonary dysfunction, hepatosplenomegaly, joint stiffness, and short stature [51,52]. Dysostosis multiplex is less severe and corneal haze is absent in Hunter syndrome. The attenuated form of MPS II is associated with less neurocognitive impairment and, in some cases, normal intelligence; survival can extend into the fifth and sixth decades, in contrast to MPS IIA (severe Hunter syndrome) with its shortened life expectancy of one to two decades [51,52].

Hematopoietic cell transplantation for MPS II

While HCT seems to help patients with MPS IIA and MPS IIB (attenuated Hunter syndrome) with respect to organomegaly, airway obstruction, and cardiac function, boys with MPS IIA still have demonstrated profound neurocognitive disabilities despite early, successful HCT [26,48,53]. The failure of HCT to favorably alter the long-term neurocognitive outcome in MPS IIA suggests that iduronate-2-sulfatase is not being effectively delivered to essential components of the CNS. In cases of transplanted MPS IIB patients, there have been somatic benefits, and intellectual function has remained intact as expected based upon the natural history of the disorder. Ethical issues continue to arise in the face of limited somatic benefit and minimal-to-absent neurocognitive gains in patients with MPS IIB undergoing HCT [54]. In conclusion, there is little evidence to support the use of HCT in MPS II, Hunter syndrome. To date, only small case series have been reported. These indicate that HCT results in stabilization or improvement in some somatic manifestations of Hunter syndrome, as seen with recombinant ERT, but little or no cognitive benefit has been demonstrated [5,54].

Enzyme replacement therapy and HCT for MPS II

ERT with recombinant human idursulfase (IDS, Elaprase®) has been shown to be a safe and effective therapy across a wide range of ages and disease severity; improving respiratory function, joint mobility, walking ability, and quality of life [55,56]. However, similar to HCT, ERT for Hunter syndrome has not been demonstrated to and is not predicted to cross the blood–brain barrier. Nearly all published experience with ERT with IDS has therefore been in patients without cognitive impairment (attenuated phenotype). Little formal guidance is available on the issues surrounding ERT in cognitively impaired patients with the severe phenotype. The clinical experience of an expert panel convened to provide guidelines based on experience with 66 patients suggests that somatic improvements (e.g., reduction in liver volume, increased mobility, and reduction in frequency of respiratory infections) may occur in most severe patients. Cognitive benefits have not been seen. It was agreed that patients with the severe phenotype are candidates for at least a 6–12-month trial of ERT, excluding patients with a severe neurologic impairment, those in a vegetative state, or those who have a condition that may lead to near-term death. The decision to initiate ERT in severe Hunter syndrome should be made by the physician and parents and must be based on realistic expectations of benefits and risks, with the understanding that ERT may be withdrawn in the absence of demonstrable benefits [57].

Sanfilippo syndrome (MPS III)

Mucopolysaccharidosis III (Sanfilippo syndrome, MPS III) is a lysosomal storage disorder in which there is impaired degradation of heparan sulfate. Though clinically similar, there are four subtypes of MPS III, A–D, each with a unique enzyme deficiency. Neurobehavioral manifestations are the hallmarks of all four types of MPS III [1]. Characteristic findings include extreme hyperactivity, aggressive behaviors, attention deficits, and progressive neurocognitive delay often associated with expressive language disabilities. Unlike other mucopolysaccharidosis, patients with MPS III have early and severe involvement of the CNS with few somatic manifestations. Consequently the typical coarse facial features, organomegaly, and dysostosis multiplex seen in other mucopolysaccharidosis are less prominent in these patients. Cognitive deterioration, however, typically begins between 2 and 6 years of age, and by age 10 years many have profound impairment [1]. As in MPS II, HCT is able to effectively treat the various somatic aspects of MPS III; however, the uniformly poor neurocognitive and developmental outcomes despite fully donor-engrafted marrow transplants performed early in the disease course (Figure 74.4) [58,59] have been attributed to reduced efficiency of uptake of MPS III-specific hydrolases, leading to diminished clearance of substrate. Evidence that the use of umbilical cord blood has a more potent effect on gaining entrance into the brain and being more effective than other sources of donor cells for these and other patients is still

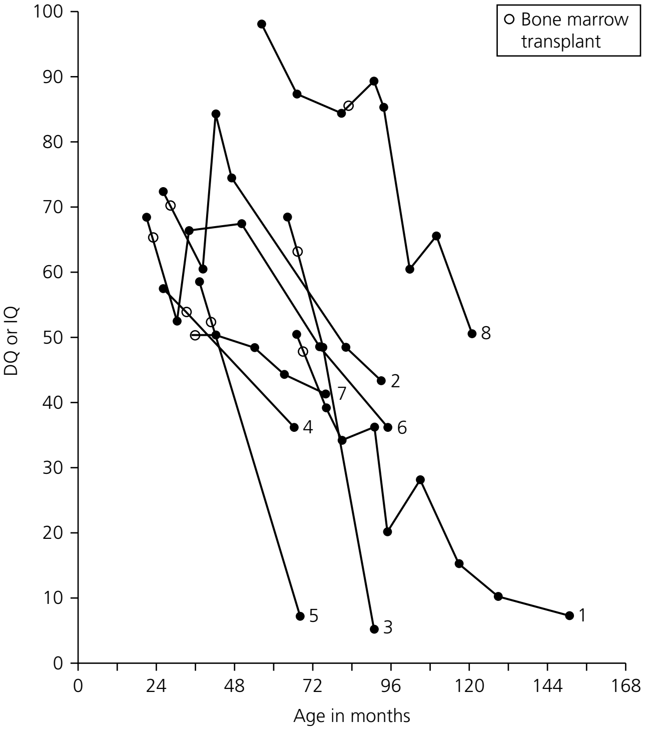

Figure 74.4 Decline in intellectual function (developmental quotient or intelligence quotient) in eight children with mucopolysaccharidosis III (Sanfilippo syndrome). Source: Klein et al. 1995. *Bone Marrow Transplant* **15**(Suppl. 1):S176–181. Reproduced with permission of Nature Publishing Group.

lacking at present. Alternative therapies, including enzyme replacement, are being investigated but are not yet available for any of the MPS type III subtypes. Although clinical efficacy has not yet been fully demonstrated for HCT or any other therapy, it is likely that future developments will lead to disease-modifying treatment for this devastating condition.

Morquio syndrome (MPS IV)

The two distinct biochemical forms of MPS IV have similar clinical features, including severe dysostosis multiplex, dwarfism, atlanto-axial instability, short trunk, and hyperextensible joints with ligamentous laxity. Corneal haze is mild, hepatosplenomegaly moderate, cardiac abnormalities unusual, and intelligence preserved [26]. HCT is incapable of ameliorating the severe skeletal deformities. Therefore, this shortcoming of HCT and the MPS IV clinical features make HCT a suboptimal intervention for this disease.

Leukodystrophies and other white matter diseases

Leukodystrophies are a group of disorders characterized by myelin dysfunction, either at the level of myelin formation or maintenance, resulting either in its impaired development (dysmyelination) or in its destruction (demyelination). In some leukodystrophies, the peripheral nervous system (PNS) may also be affected and the presence of abnormal PNS myelin can be observed. In dysmyelination, myelin formation does not proceed appropriately culminating in myelin with defects in structure and function, which may further increase the susceptibility to myelin damage and loss. On the other hand, demyelination is characterized by a normal synthesis and formation of myelin, which is followed by a decrease or loss of myelin. There are several factors contributing to myelin loss, but as commonly observed in peroxisomal and lysosomal disorders, the accumulation or defect of a given metabolite is generally the major underlying cause. The leukodystrophies are generally rare, but they have a significant impact in the society with an estimated overall incidence of 1 in 7500 live births. Currently, there is no cure for leukodystrophies and the development of effective treatments remains challenging.

In this section, leukodystrophies (X-ALD, GLD, and MLD) will be presented and discussed in detail (see later and Table 74.2); other white matter diseases, such as Alexander disease, Pelizaeus-Merzbacher disease (PMD), vanishing white matter disease (VWMD), and Zellweger syndrome will be presented and discussed in a limited manner (Table 74.2).

X-linked adrenoleukodystrophy (X-ALD)

Epidemiology, molecular biology, and etiology

X-linked adrenoleukodystrophy is a peroxisomal disorder characterized by cerebral demyelination, adrenal insufficiency, and progressive neurologic deterioration. The de novo mutation rate in X-ALD is 7.8% of cases, and in 42% of index cases the first recognized mutation occurs in the mother. The *ALD* gene (located at Xq28) encodes the ALD protein (ALDP, ABCD1), which is a member of the ATP-binding cassette transport protein family (ABC transporters) and is likely involved in transport of fatty acyl coenzyme A substrates or their cofactors into peroxisomes. Its metabolic hallmark is the accumulation of very long chain fatty acids (VLCFA) in tissues and plasma, due to impaired transport and β-oxidation of these fatty acids in peroxisomes. The contribution to cerebral and adrenal dysfunction is poorly understood, significant efforts are directed at achieving a better understanding of the underlying pathophysiology.

X-ALD has a minimum frequency of 1 : 16,800 in the total population and approximately 1 : 20,000 males (Table 74.2) [60]. It predominantly affects males, although 40% or more of female heterozygous carriers exhibit mild-to-moderate non-cerebral signs of the disease. X-ALD is an inflammatory demyelinating disorder that in its most severe form affects children with a rapid and progressive demyelination. The inflammatory demyelination may also affect neurons and their axons [61].

Clinical description

There is no genotype–phenotype correlation in X-ALD; that is, six distinct clinical phenotypes exist independent of VLCFA abnormality or ALDP expression, mutation, or family history. Genotypic analysis definitively identifies the 20% of female heterozygous carriers who can have normal levels of fasting plasma VLCFAs. The clinical phenotypes and their relative percentages are as follows [62]:

- childhood cerebral (30–40%);
- adolescent cerebral (5–10%);
- adult cerebral (~5%);
- adrenomyeloneuropathy (AMN; 40–50%);
- AMN with cerebral involvement (25% of the total AMN cases);
- Addison only (variable percentage, dependent on age).

Childhood-onset cerebral disease, with its median age of onset of 7 years, encompasses adrenal insufficiency in 90% of affected boys.

The predominant pattern of demyelination seen by brain MRI is posterior (i.e., parieto-occipital lobes) in 80–85% of cases. Frontal lobe and pyramidal tract involvement are also seen in 10–15% and approximately 5% of cases, respectively [63–65]. Neurologic deterioration occurs in all boys.

Neurologic deterioration occurs in all boys and can include vision impairment, cortical blindness, hearing, auditory processing problems, aphasia, apraxia, swallowing difficulty, gait and motor difficulties, fine motor deficits, bowel and/or bladder dysfunction, seizures, sparse hair, adrenal insufficiency, and hypogonadism manifested by low testosterone and high luteinizing hormone levels. The MRI of the brain shows characteristic ascending involvement of the pyramidal tracts from the pons to the internal capsules. Of note, men can develop cerebral demyelination that is similar to that seen in childhood cases. Disability, dementia and death can occur within a few months to several years from the onset of clinical symptoms.

A complete evaluation of a boy with cerebral X-ALD includes a thorough neurologic examination; comprehensive neuropsychologic assessment with particular attention to the performance IQ (PIQ), which is a sensitive indicator of deficits in visual perceptual and spatial processing, speed and efficiency of task completion, and novel problem solving; and an MRI of the brain with and without contrast (gadolinium) with an MRI severity score determination, referred to as the Loes score [63].

Boys diagnosed with X-ALD due to a family history merit careful, complete serial monitoring beginning at age 3 years and continuing into adolescence, with the objective being to detect cerebral demyelination at a very early stage and to intervene with HCT in a timely and presumably effective manner. To date, MRI scanning of the brain with gadolinium is the most effective clinical tool to perform serial monitoring. It is strongly recommended that scans be performed at least every 6 months, and more often if there is evidence of the earliest stages of dysmyelination. This is particularly relevant for boys under 10 years of age with X-ALD who have the highest risk for developing childhood-onset cerebral demyelination that is rapidly progressive. However, in no circumstances should HCT be performed in the absence of cerebral disease (see below).

Hematopoietic cell transplantation

The first transplant for childhood cerebral X-ALD was performed in 1982 in a boy with advanced-stage disease; despite a successful graft, he died of progressive X-ALD [84]. However, Aubourg and colleagues in Paris successfully transplanted a boy with early-stage cerebral X-ALD and published their findings in 1990 [14], thus beginning the modern era of successful transplantation for this disorder. HCT is accepted as an effective therapy for the early stages of X-ALD. Shapiro et al. described the long-term results (5–10 years) of transplant in 12 boys [66]. They described the prospects for achieving stability in the extent of MRI demyelination and the changes in neuropsychologic function, with a propensity for deterioration before ultimate stabilization in visual processing as evidenced by PIQ score in boys with a posterior pattern. Interestingly, VLCFA concentrations decreased by 55% but remained elevated in the long term, suggesting that VLCFA levels are not an optimal surrogate marker for this disease. A large, multinational registry of patients with X-ALD showed that only boys with early-stage disease benefit from HCT [17]. The overall 5-year survival was 56% (95% CI, 44–68%). Boys transplanted for early-stage cerebral disease (zero or one neurologic deficit and MRI severity score <9, had a 92% survival at 5 years; Figure 74.5). This report also provided insight into the probability of neurologic deterioration following HCT (Table 74.7). Specifically, in boys with no neurologic deficits before HCT, there was still a 44% likelihood that the neurologic deficit score would increase afterwards [17]. The concept of an ALD-disability rating scale (ALD-DRS) was introduced, although not yet validated (Table 74.8). This scale proved informative in examining neurologic deficits, neuropsychologic function, MRI severity, and ALD-DRS levels after HCT in patients with cerebral X-ALD according to baseline PIQ (Table 74.9). Both PIQ and ALD-DRS levels were significantly poorer after HCT in boys whose baseline PIQ was below 80. The ALD-DRS levels before and after HCT were reported and gave insight into the potential for significant worsening of the level of disability depending on the status of the disease at time of HCT (Table 74.10). Finally, the neuropsychologic functional outcomes of boys after HCT was assessed and reported based upon their MRI pattern of demyelination (Table 74.11). The largest single institution experience of allogeneic HCT for patients with X-ALD [67] showed a 5-year survival of 76% (95% CI, 64–88%). Survival differed based on the severity of cerebral disease, based on Loes score at the time of HCT. For boys with a baseline

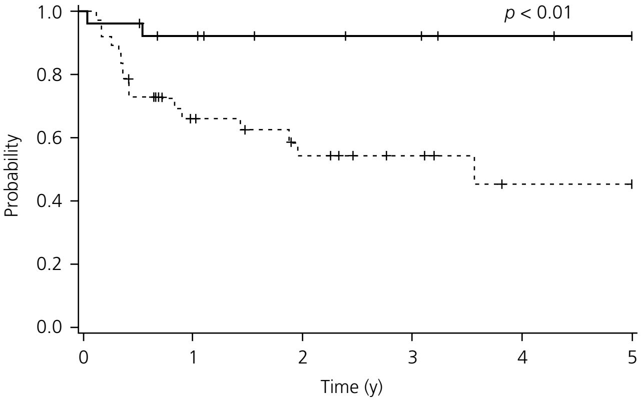

Figure 74.5 Kaplan–Meier estimate of survival for cerebral X-linked adrenoleukodystrophy following hematopoietic cell transplantation by number of neurologic deficits (e.g., limitations of vision, hearing, speech, gait, fine motor skills, and/or activities of daily living) and magnetic resonance imaging (MRI) severity score before transplantation. The solid line indicates patients with zero or one neurologic deficit and an MRI severity score less than 9 ($n = 25$). The dashed line indicates patients with two or more neurologic deficits or an MRI severity score of 9 or greater ($n = 37$). Ticks on the probability lines indicate dates of censoring at last follow-up. y, years. Source: Peters et al. 2004 [17]. Reproduced with permission of the American Society of Hematology.

Table 74.7 Neurologic deficit (e.g., impairments of vision, hearing, speech, gait, fine motor skills, and/or activities of daily living) score before and after hematopoietic cell transplantation (HCT) in 94 patients with cerebral X-adrenoleukodystrophy

		After HCT number (%)				
Before HCT	**Number**	**0**	**1**	**2**	**More than 2**	**Unknown or not available**
0	32	**18 (56)**	4 (13)	1 (3)	9 (28)	–
1	28	3 (11)	**10 (36)**	4 (14)	9 (32)	2 (7)
2	21	–	1 (5)	**6 (29)**	14 (67)	–
More than 2	9	–	–	–	**9 (100)**	–
Unknown or not available	4	–	–	–	–	**4 (100)**
Totals	94	21	15	11	41	6

–, none.
Bold text indicates patients for whom neurologic deficit scores were unchanged.

Table 74.8 X-linked adrenoleukodystrophy (ALD) Disability Rating Scale (ALD-DRS)

Scale level	Description
0	No difficulties
I	Mild learning or coordination difficulties from ALD; patient does not require support or intervention
II	Moderate learning, sensory, and/or neurologic abnormality; patient requires support or intervention in a few areas
III	Severe learning, sensory, and/or neurologic abnormality; patient requires support or intervention in many areas
IV	Loss of cognitive ability and disorientation; patient requires constant supervision

Table 74.9 Neurologic deficits (e.g., impairments of vision, hearing, speech, gait, fine motor skills, and/or activities of daily living), neuropsychologic function, magnetic resonance imaging (MRI) severity, and adrenoleukodystrophy (ALD) Disability Rating Scale (ALD-DRS) levels after hematopoietic cell transplantation in patients with cerebral X-linked ALD according to baseline performance intelligence quotient (PIQ)

	Baseline PIQ below 80	Baseline PIQ of 80 or more	*p*
Neurologic deficits			0.29
Median, number (range)	2 (0–4)	1 (0–4)	
Observations, number	32	43	
PIQ			<0.01
Median, number (range)	45 (45–63)	78.5 (45–122.5)	
Observations, number	6	26	
MRI Severity Score			0.25
Median, number (range)	12.8 (8–19)	8 (0–23.5)	
Observations, number	8	23	
ALD-DRS level			<0.01
Median, number (range)	IV (I–IV)	II (0–IV)	
Observations, number	28	36	

Table 74.10 X-adrenoleukodystrophy Disability Rating Scale (ALD-DRS) levels before and after hematopoietic cell transplantation (HCT) in 94 patients with cerebral X-ALD

		Level after HCT, number (%)					
Level before HCT	**Number**	**0**	**I**	**II**	**III**	**IV**	**No data, because of death**
0	13	8 (62)*	3 (23)	1 (7.6)	–	1 (7.6)	–
I	21	1 (4.8)	3 (28)†	4 (19)†	3 (14)	6 (28)†	1 (4.8)
II	36	1 (2.8)	1 (2.8)	10 (2.8)‡	1 (2.8)	19 (53)¶	4 (11)
III	12	–	–	–	1 (8.3)	10 (83)§	1 (8.3)
IV	1	–	–	–	–	1 (100)*	–
Missing data	11	–	–	1 (9)	–	–	5 (45)
Totals	94	10	10	16	5	37	11
Deaths	35	1	2	5	0	16	11

*1 death; †2 deaths; ‡3 deaths; §4 deaths; ¶9 deaths.

Table 74.11 Comparison of neuropsychologic functional outcomes according to magnetic resonance imaging pattern of demyelination following hematopoietic cell transplantation (HCT) for cerebral X-linked adrenoleukodystrophy

	Parietal-occipital lobes, *n* = 25	**Frontal lobes or pyramidal tracts, *n* = 7**	**Total, *n* = 32**	**No neuropsychologic function assessment after HCT because of death, *n* = 29**	***p***
*Verbal intelligence quotient**					
Baseline (range)	99 (59–27)	89 (70–107)	**98 (59–127)**	**92 (74–124)**	**NS**
After HCT	82 (46–123)	79 (57–113)	80.5 (46–123)	–	–
Mean change (95% confidence interval)	**–13.7** (–20.5 to –6.8)	**–6.4** (–17.8 to 4.9)	–12.1 (–17.1 to –7.1)	–	**0.25**
*Performance intelligence quotient**					
Baseline (range)	96 (45–122.5)	92 (61–100)	**94.5 (45–122.5)**	**77.5 (45–138)**	**<0.01**
After HCT	64 (45–122.5)	93 (54.108)	73 (45–122.5)	–	–
Mean change (95% confidence interval)	**–21.6** (–30.6 to –12.6)	**0.4** (–6.8 to 7.7)	**–16.8** (–24.6 to –8.9)	–	**0.03**

NS, not significant.
*VIQ and PIQ baseline evaluations and those after HCT: median (range).
Bold denotes statistical comparisons and corresponding *p* values.

Loes score <10, survival at 5 years was estimated at 89%, whereas for those with advanced radiographic disease (Loes score ≥ 10), 5-year survival was estimated at only 60% ($p = 0.03$). A trend toward superior survival was seen in boys with no baseline clinical evidence of cerebral disease, Estimate of 5-year survival for this clinically silent group was 91%, whereas that for boys with clinically evident brain disease was 66% ($p = 0.08$). In this study, post-HCT progression of radiographic, as well as clinical neurologic dysfunction, as measured by the functional tests and Loes scores depends significantly on baseline Loes score, functional tests, PIQ, and FSIQ. Data from this study suggest that more extensive cerebral disease at the time of HCT correlates with greater progression of cerebral disease after transplantation. Such neurologic function outcomes data are critical to shaping important decision-making when parents and clinicians are considering HCT for boys with advanced cerebral disease and may prove important in developing alternative therapies for this population [67].

The putative mechanism by which HCT halts the progress of CNS demyelination in cerebral X-ALD is unclear. It has been speculated that immunosuppression contained, together with long-term donor-derived engraftment, would halt the inflammatory process and contribute donor-derived microglia, respectively.

The neurologic benefits of HCT in X-linked adrenoleukodystrophy are mediated by the replacement of brain microglial cells derived from donor bone marrow myeloid cells. To clarify the mechanisms of HCT, intravenous injection was performed in ABCD1 mutant mice [68]. Treatment led to a significant reduction of VLCFA accumulation in the spleen and lung after transplantation, but not in the brain and spinal cord. This correlated with a higher engraftment of transplanted cells in the spleen and lung, along with detection of the ALD protein, and little engraftment in the CNS with no detection of ALD protein [68].

Gene therapy and autologous HCT for X-linked ALD

Adrenoleukodystrophy (ALD) gene transfer with lentiviral vectors in vitro has yielded biochemical correction of monocytes/macrophages derived from ALD protein-deficient human $CD34^+$ cells [9]. The transplantation in vivo of lentivirally transduced murine ALD hematopoietic cells into ALD mice resulted in the replacement of 20–25% of brain microglial cells expressing the ALD protein 12 months after transplantation [10]. On the basis of these promising preclinical data Cartier et al. initiated an HSC gene therapy trial in two X-ALD patients who had developed progressive cerebral demyelination. Both were candidates for allogeneic HCT, but had no HLA-matched donors or cord blood. Autologous $CD34^+$ cells were genetically corrected ex vivo with a lentiviral vector encoding wild-type ABCD1 cDNA, and then reinfused into the patients after a myeloablative conditioning regimen [69]. Over 3 years of follow-up, the hematopoiesis remained polyclonal in the two patients treated with 7–14% of granulocytes, monocytes, and T- and B lymphocytes expressing the lentivirally encoded ALD protein. There was no evidence of clonal dominance or skewing based on the retrieval of lentiviral insertion repertoire in different hematopoietic lineages by deep sequencing. Cerebral demyelination was arrested 14 and 16 months, respectively, in the two treated patients, without further progression up to the last follow-up, a clinical outcome that is comparable to that observed after allogeneic HCT [70]. The results of this study suggest that HSC-based gene therapy is a potential therapeutic option for X-ALD patients with progressive cerebral demyelination as HSC gene therapy abrogates the morbidity and mortality risks associated with conventional allogeneic HCT.

Cell source for HCT in patients with X-ALD

A single-center report of its experience with UCB HCT for 12 patients with X-ALD, including six with early- and very early-stage cerebral disease (Loes scores 3–8) revealed despite HLA mismatching, similar outcomes to those previously reported after HLA-matched bone marrow transplantation [17,59], even when morbidity and mortality is considered [71]. When UCBT was done in children who have not reached the age at which cerebral X-ALD typically progresses, they tolerated the procedure well; continued to have normal neurologic exams, typical development, and no additional changes in MRI scans at 4, 6, and 8 years of age [71].

In the Cord Blood Transplantation (COBLT) study, the median age of the UCB recipient was 1.8 years, and it is unclear if X-ALD patients had evidence of cerebral disease at the time of HCT [3]. In conclusion, the number of patients reported with UCBY is too low to overturn the present recommendation of the National Marrow Donor Program, International Bone Marrow Transplant Registry, and the Working Party on Inborn Errors of the European Group for Blood and Marrow Transplantation for waiting until progressive MRI changes or abnormalities in neurobehavioral testing are evident. The recommendation remains that HCT be reserved for X-ALD patients with early but definite evidence of cerebral disease by brain MRI [72].

Lessons learned and considerations to be given in each case of cerebral X-ALD include

1. the timing of HCT and disease status;
2. the optimal hematopoietic cell source (marrow, peripheral or UCB, HLA match, cell dose, and carrier status of the donor, if applicable);
3. the conditioning regimen: high dose versus reduced intensity;
4. the availability of a multispecialty, multidisciplinary team for evaluation and treatment;
5. quality of life for the patient and his family.

Based upon the long-term outcomes of HCT for cerebral X-ALD, the worldwide HCT experience for this disease, and knowledge of the natural history, the following guidelines can be promulgated: MRI severity score and gadolinium enhancement are highly predictive of the likelihood of disease progression; for example, an MRI severity score of at least 1–3 points or more, and gadolinium enhancement in boys under 10 years of age is associated with approximately 90% likelihood of severe, progressive demyelination [64–66,73]. Boys with advanced disease (i.e., very late stage) manifested by low PIQ (<80), a very sensitive neuropsychologic parameter for visual processing, neurologic deficits, and high Loes severity score [63], typically over 13 points, are poor candidates for HCT. Boys with very early-stage and early-stage cerebral disease defined by Loes severity score between 1 and 8 points who typically are without neurologic and neuropsychologic abnormalities are the best candidates for HCT.

Challenges in use of HCT in patients with X-linked ALD

Despite significant progress in the field of HCT for cerebral X-ALD, a number of limitations and unanswered questions remain:

1. the fact that, in most instances – about two-thirds of cases – HCTs have been performed in symptomatic boys with cerebral X-ALD rather than in boys with early stages of disease who had undergone serial MRI monitoring after receiving the biochemical diagnosis of ALD due to a positive family history of this X-linked disease;
2. our continuing inability to predict phenotype in a boy or an adult man;
3. the need for a measure of cerebral disease velocity;
4. the implementation of a more precise system for staging of cerebral disease prior to HCT that builds upon the following: very early stage = MRI score 1–3; early stage = MRI score 4–8; late stage = MRI score 9–13; very late stage = MRI score greater than 13;
5. the pressing need for newborn screening for X-ALD.

In the meantime, novel approaches to boys and men with very late-stage cerebral disease are also needed. Further, the field of HCT for men with adrenomyeloneuropathy (AMN) and cerebral disease is beginning to be orchestrated on an international front through centers in Germany, The Netherlands, and the United States. Clearly, the need for such cooperation and collaboration is essential, together with international databases and registries with attention to longitudinal outcomes.

Non-transplant approaches

Removal of accumulated VLCFA may, theoretically, be a possible treatment for adrenoleukodystrophy. The primary non-transplantation approach to X-ALD has been treatment with Lorenzo's oil (a mixture of glyceroltrioleate and glyceroltrierucate), which normalizes plasma VLCFA concentrations. There is evidence that, in patients with the biochemical diagnosis of X-ALD who are started on Lorenzo's oil and the special diet, are compliant, and start at an early age, there is a decreased likelihood of developing childhood-onset cerebral demyelination [74]. However, it has no effect in patients with established neurologic deficits, as brain levels of VLCFA are unchanged by the treatment. 4-Phenylbutyrate and fenofibrate are compounds known for their ability to reduce VLCFA levels in cultured cells and in the ABCD1 knockout mice, due to enhanced peroxisomal proliferation and upregulation of genes involved in β-oxidation and fatty acid transport [68]. However, the efficacy and usefulness of these compounds for X-linked adrenoleukodystrophy patients has not been established so far.

Other efforts to improve results related to HCT for cerebral X-ALD have focused on the use of *N*-acetyl-L-cysteine in patients with very late-stage cerebral disease [75], use of advanced imaging techniques such as magnetic resonance spectroscopy to evaluate the brain after HCT [76], and the use of magnetic resonance spectroscopy as well to evaluate for the earliest evidence of changes associated with demyelination [77].

Globoid cell leukodystrophy

Epidemiology, molecular biology, and etiology

Globoid-cell leukodystrophy (GLD) or Krabbe disease is a severe autosomal recessive, lysosomal neurodegenerative disorder caused by deficiency of galactocerebrosidase (GALC) [78]. Although its overall prevalence is 1 : 100,000 births in the general population, the frequency of Krabbe disease is as high as 1 : 150 in certain communities. The resultant accumulation of psychosine, a toxic metabolite derived from galactocerebroside, leads to the death of cells forming the myelin sheath of the nervous system [79,80] (Table 74.2). The condition is characterized by white matter degeneration in the CNS and peripheral nervous system, with large macrophages (globoid cells).

Clinical description

Early infantile Krabbe disease, also known as classic Krabbe disease, presents with sudden onset irritability, startle response to stimuli and developmental arrest between 3 and 6 months of age in a previously healthy infant [80]. Over the course of several months, affected infants develop hypertonicity, seizures, blindness and incapacitation, culminating in death often by age 2 years [80]. Feeding difficulties and aspiration pneumonia are frequent complications. Krabbe disease may present later in infancy, in childhood, or even adulthood. Children with late infantile Krabbe disease present after 6 months of age with symptoms similar to the early infantile form, followed by rapid disease progression and death within 2 years. Juvenile Krabbe disease usually presents acutely with ataxia or spastic paresis between 3 and 8 years of age, followed by a slowly progressive disease course [80]. Intellect, often intact at the onset of symptoms, may deteriorate or remain stable for years to decades after symptoms begin.

Hematopoietic cell transplantation

Treatment for GLD is currently limited to pre-symptomatic hematopoietic HCT [12,20]. The first definitive report on the use of HCT for GLD was published in 1998 and described five cases (four late onset, and one Krabbe disease) [12]. Hematopoietic cell sources included HLA-identical marrow in four cases and UCBT in one. In all cases, high-dose conditioning was administered; total body irradiation was given to one patient. This report provided the proof of principle for the use of HCT in patients with GLD. The four cases of late-onset GLD received their HCT between the ages of 2.6 and 11 years of age, with follow-up from 3 to 9 years. The patient with Krabbe disease underwent HCT at age 2 months, and follow-up of 14 months was reported. The outcomes may be summarized as follows: (1) GALC normalized in all patients; (2) there was normal, stable neuropsychologic function in the three late-onset disease patients; (3) MRI of the brain showed an "improvement in demyelination" based upon reduced abnormal signal intensity; (4) cerebrospinal fluid (CSF) protein normalized in all cases except for the infant with Krabbe disease who still had a CSF protein level of over 150 mg/dL at follow-up, although this was decreased from greater than 550 mg/dL at baseline.

Cell source for HCT in patients with X-ALD

A report of the use of UCBT in patients with classic Krabbe disease included 25 infants, of whom 11 were "asymptomatic" and 14 were symptomatic prior to transplant [20]. Comparison of outcomes between the two groups of patients focused on survival, engraftment/GALC, developmental and neurologic status, and MRI of the brain. The authors found the following: (1) engrafted survival among the asymptomatic infants was seen in all 11 of 11, while it occurred in six of the 14 patients who were symptomatic at HCT; (2) none of the patients achieved normalization of CSF protein; (3) among the symptomatic infants, all had major developmental and neurologic deficits after HCT; (4) significant residua were also present among those transplanted in an asymptomatic condition. Of these 12 patients, only four could be described as functioning normally. Problems included mild-to-moderate expressive language delays and mildly-to-severely impaired gross motor function.

The effect of UCBT on peripheral neuropathy, a significant clinical problem in patients with classic Krabbe disease, was studied in 12 patients with GLD, of whom nine had classic Krabbe disease and three had late-onset disease. Serial nerve conduction studies were performed in patients after HCT, although two did not have pre-HCT studies. Further studies are needed to better assess the clinical significance of the nerve conduction velocity results and how best to express these results; in the case of this report, values were expressed as age-matched standardized ratios [81].

Gene therapy for GLD

Gentner et al. found that forced overexpression of the gene encoding GALC is toxic to both mouse and human stem cells and early progenitors but not to their differentiated progeny. A potentially powerful new therapeutic tool for regulating gene expression is the use of microRNAs, which are small non-coding ribonucleic acids that can either block translation of target messenger RNAs or cause their direct degradation. Gentner et al. showed that a specific microRNA, which is found in hematopoietic stem cells but not in their differentiated progeny, could indeed downregulate GALC expression from a genetically modified construct containing the GALC-encoding gene preceded by a microRNA target sequence. By incorporating miR-126 target sequences into a GALC-expressing vector, they suppressed GALC expression in HSCs while maintaining robust expression in mature hematopoietic cells. This approach protected HSCs from GALC toxicity and allowed successful treatment of a mouse GLD model, providing a rationale to explore HSC-based gene therapy for GLD in humans [82,83].

Advancements in diagnostic and imaging tools

With the promise of pre-symptomatic HCT and the advent of assays to detect lysosomal enzyme deficiencies in newborn dried blood spots, newborn screening programs began to consider the addition of Krabbe disease. New York became the first state to screen for the disorder, using measurement of GALC enzyme activity in dried newborn blood spots, followed by enzyme activity in leukocytes/molecular analysis, when indicated [84,85]. Although diagnosis of the disease in the newborn period can be achieved, significant challenges remain to select patients who will benefit from HCT and to predict post-transplant neurodevelopmental outcome [84]. While further investigation into the long-term outcome of pre-symptomatic HSCT for Krabbe disease is needed, other benefits of newborn screening for the disorder are apparent. These include avoidance of diagnostic delay and the recognition of couples at risk who may benefit from available reproductive options. Recently, the state of Illinois also began screening for Krabbe disease. It is likely that other programs will do so in the future [83,86].

With the development of diffusion-tensor MRI (DTI), there is the promise of more sensitive means to assess myelination patterns. With DTI, the MR signal is sensitized to microscopic movement of water molecules. Water motion in white matter myelination is anisotropic (i.e., has a tendency to diffuse along one direction rather than in all directions), a feature that can be quantitatively and reproducibly measured with anisotropy maps. Another report on patients with GLD described the serial assessment of DTI anisotropy measurements in seven patients with early-onset Krabbe disease who had received UCB HCT. Fractional anisotropy ratios were reported, and all patients exhibited worsening of the white matter signal intensity by conventional MRI [87].

Challenges in use of HCT in patients with GLD

The current limitations of HCT for GLD and remaining unanswered questions relate to the following areas:

1. late-onset GLD cases are very rare, and consequently the experience with HCT is still quite limited;
2. the long-term neurologic, neurocognitive, neurodevelopmental, and quality of life outcomes of patients with classic Krabbe disease who have received UCBT in the first 1–2 months of life;
3. the impact of newborn screening for GLD and whether there is justification for its expansion;
4. international discussion, review, and planning for the future, which is urgently needed.

Non-transplant approaches

Non-transplant approaches to patients with GLD primarily fit under the broad category of supportive care measures and may be directed toward the treatment of seizures, spasticity, feeding difficulties, and constipation.

Metachromatic leukodystrophy

Epidemiology, molecular biology, and etiology

Metachromatic leukodystrophy (MLD) is an autosomal recessive lysosomal storage disorder that is caused by the inherited deficiency of the lysosomal enzyme arylsulfatase A (ASA). ASA catalyzes the desulfation of cerebroside 3-sulfate (sulfatide), an acidic sphingolipid mainly present in myelin sheaths. Deficiency of ASA causes the accumulation and massive intralysosomal deposition of this sphingolipid, which manifests itself as metachromatic staining. Metachromatic leukodystrophy is further characterized by a progressive and widespread loss of myelinating cells leading to severe dysfunction of the nervous system. Neurologic findings may include spasticity, neuropathy, dementia, and seizures. Importantly, the time of onset and severity correlate with residual ASA activity. Of absolutely critical importance is confirmation of the diagnosis by documenting elevation of urinary sulfatides. This is necessary due to the common ARSA pseudodeficiency allele in the general population. MLD caused by saposin B deficiency can present as any form of MLD caused by ARSA deficiency. Multiple sulfatase deficiency associates the signs of MLD with MPS and icthyosis. Saposin B deficiency and multiple sulfatase deficiency share with MLD increased CSF protein, slowed nerve conduction velocities, and increased urinary sulfatides.

Clinical description

The phenotypes of MLD include late-infantile MLD and late-onset forms. Late-infantile (early onset, 0.5–4 years of age) presents generally in the first or second year of life due to loss of motor milestones including gait disturbance, decreased deep tendon reflexes, abnormal speech, loss of neurocognitive skills, optic atrophy, progressive spastic quadriparesis, increased CSF protein, and slowed nerve conduction velocities, culminating in dementia and death within several years. The early juvenile form (late onset, 4–6 years) shows gait and postural abnormalities, emotional and behavioral disturbances, optic atrophy, progressive spastic quadriparesis, increased CSF protein, and slowed nerve conduction velocities. The late juvenile form (late onset, 6–16 years) exhibits behavioral abnormalities, poor school performance, language regression, gait disturbance, slowly progressive tetraparesis, increased CSF protein, and slowed nerve conduction velocities. Finally, the adult form (late onset, >16 years) is characterized by mental regression, psychiatric symptoms, incontinence, slowly progressive spastic tetraparesis, normal or increased CSF protein, and normal or slowed nerve conduction velocities.

Late-onset forms of MLD with disease manifesting during school age, adolescence or adulthood present with progressive motor signs and symptoms, including gait disturbance, clumsiness, tremor, and dysarthria. Gradual decline in neurocognitive function is also observed. Neurocognitive and neurobehavioral symptoms seem to predominate in those patients whose disease first manifests in late teenage years or in early to middle adulthood. In fact, many such cases are misdiagnosed as schizophrenia or major psychosis. New-onset attention deficit hyperactivity disorder in school-age children is typical, and they are often treated for years until mental decline is observed. Attention deficits, impulsive behavior, disinhibition, impulsivity, loss of spatial skills, memory loss, and personality changes, together with features of frontal lobe dysfunction, often progress over years to decades. Peripheral nervous system disease is variably present.

Hematopoietic cell transplantation

The experience with HCT as a therapy for MLD extends over 20 years; however, despite advances in the field, HCT for MLD remains challenging. Results of several reports show that HCT performed in late-onset pre-symptomatic patients could halt disease progression. Nevertheless, HCT is only suitable for patients with mild nervous system involvement and with results only seen in the CNS and not in the PNS [88]. Furthermore, all patients transplanted for symptomatic late-infantile MLD have done poorly in all aspects. Even in the few cases of pre-symptomatic HCT for late-infantile MLD, the results have been disappointing, although cognitive but not motor function has been preserved in some patients. All children who were transplanted prior to the development of symptoms have become wheelchair bound and totally dependent in activities of daily living.

The indolent course in adult cases, and the potential for intervening at an appropriate stage of disease for juvenile-onset cases, permits the potentially effective use of HCT for these forms of MLD. HCT can succeed in stabilizing CNS disease and function while having little to no beneficial effect on the peripheral nervous system [13,59,89,90]. However, results are mixed, in some patients neurologic deficits continue to progress, and HCT may stop major neurologic deficits progression, but not all, only in selected patients [91,92].

Challenges in use of HCT in patients with MLD

The current limitations of HCT for MLD and unanswered questions relate to:

1. the fact that it is virtually impossible to effectively treat (i.e., transplant) cases of late-infantile MLD due to the rapid rate of disease progression;
2. late-onset cases still show significant peripheral nervous system disease after HCT, and the time to achieve CNS stabilization appears to be longer than that observed in patients with cerebral X-ALD or late-onset GLD;
3. the possible role of enzymes (ERT) for the peripheral nervous system in MLD remains unknown or speculative. Important ERT experience with the mouse model of MLD has been published. Results of clinical trials with ERT in children with late-infantile MLD are still pending.

ERT, gene therapy, and cellular therapy for MLD

While ERT therapy has been successful in a number of storage disorders, it has not been efficient for the treatment of disorders with prevailing CNS pathology, mostly, because the blood–brain barrier is considered impermeable to high molecular mass proteins. Apart from this, other significant issues such as immune responses following enzyme administration, persistence of already established degeneration and poor accessibility of specific types of affected tissues, may compromise the ability to restore the enzyme deficiency. In a clinical phase I/II study children with late-infantile MLD were treated with intravenously administered rhASA doses of up to 2.5 mg/kg; no alteration in motor or neurologic dysfunction was ultimately observed. Stroobants et al. have continuously delivered rhASA for 4 weeks in a knockout mouse model, in an effort to bypass the blood–brain barrier, with the use of implanted osmotic pumps in the lateral ventricles of the brain. Their experiment led to a marked improvement of sulfatide clearance and reversal of CNS impairment with the use of a lower dose compared to that administered in intravenous ERT, thus suggesting that intracerebroventricular route of administration might be a promising treatment approach for MLD patients. However, no data regarding this treatment modality in MLD patients is currently available. In summary, ERT represents an important therapeutic regimen for the treatment of systemic and PNS pathology in MLD patients, while the obstacle of delivering the enzyme to the CNS remains a main disadvantage. This problem could probably be solved in the future with the intracerebroventricular or intrathecal enzyme delivery.

Mesenchymal stem cells (MSC) have been shown to induce prevention of loss of nerve conduction velocity. This observation led to a phase I clinical trial in six patients who had previously received matched sibling donor HCTs [93]. They received a single infusion of mesenchymal stem cells (MSCs) ($2–10 \times 10^6$ MSCs/kg of recipient body weight) from the original donor. There was no infusion-related toxicity. It was reported that four patients with MLD had “significant improvements in nerve conduction velocities after MSC infusion” (e.g., peroneal nerve 5–6 m/s and median nerve 8–9 m/s); however, no clinically apparent changes occurred in patients’ overall health or mental and physical development after MSC infusion.

Preclinical studies involving, autologous HCT with gene therapy, intracerebral gene therapy, microcapsulated recombinant cells, oligodendrocytic progenitor cells, neural progenitor cells-neurospheres, and embryonic stem cells, to mention some, are ongoing but not yet ready for the clinical arena [94].

Other white matter disorders

Alexander disease

Alexander disease is a rare, fatal disorder of the CNS that occurs primarily in infants and children. Mutations in the glial fibrillary acidic protein gene have now been shown in a number of biopsy- or autopsy-proven patients with Alexander disease [95]. Histologic demonstration of Rosenthal fibers in brain specimens is no longer required for diagnostic purposes. In infants and young children, Alexander disease causes developmental delay, psychomotor retardation, paraparesis, feeding problems, megalencephaly, seizures, and hydrocephalus. Pseudobulbar and bulbar signs predominate in juvenile cases. Characteristic MRI findings have been reported in Alexander disease. Adult cases, which can be more variable, may resemble multiple sclerosis and include palatal clonus. Although therapeutic options are being investigated, only supportive treatments are currently available [95,96] (Table 74.2).

Pelizaeus-Merzbacher disease

Pelizaeus-Merzbacher disease (PMD) disease is an X-linked disorder caused by various mutations of the X-linked proteolipid protein 1 gene (PLP1) that include copy number changes, point mutations, and insertions or deletions of a few bases. These mutations lead to a clinical spectrum from the most severe connatal PMD, to the least severe spastic paraplegia 2 (SPG2). Signs of PMD include nystagmus, hypotonia, tremors, titubation, ataxia, spasticity, athetotic movements and cognitive impairment; the major findings in SPG2 are leg weakness and spasticity. A diffuse pattern of hypomyelination is seen on MRI of PMD/SPG2 patients. A similar constellation of signs and pattern of hypomyelination lead to the autosomal recessive disease called Pelizaeus-Merzbacher-like disease 1 (PMLD1) and the less-severe spastic paraplegia 44 (SPG44), caused by mutations of the gap junction protein, gamma-2 gene (GJC2), formerly known as the gap junction protein, α-12 gene (GJA12). Magnetic resonance spectroscopy (MRS) and brainstem auditory evoked potentials (BAEP) may assist with differential clinical diagnosis of PMD and PMLD1. Supportive therapy for patients with PMD/SPG2 and PMLD1/SPG44 includes medications for seizures and spasticity; physical therapy, exercise, and orthotics for spasticity management; surgery for contractures and scoliosis; gastrostomy for severe dysphagia; proper wheelchair seating, physical therapy, and orthotics to prevent or ameliorate the effects of scoliosis; special education; and assistive communication devices. There is no rationale for the use of HCT to treat PMD (Table 74.2).

Vanishing white matter disease

Vanishing white matter (VWM) is one of the most prevalent inherited childhood leukoencephalopathies, but this may affect people of all ages, including neonates and adults. Typical cases have their onset in late infancy or early childhood, with neurologic involvement characterized by progressive spasticity and ataxia, seizures, dysarthria, and optic atrophy, in which minor stress conditions, such as fever or mild trauma, provoke major episodes of neurologic deterioration. Brain MRI shows diffuse, symmetric hypomyelination. Typical pathologic findings include increasing white matter rarefaction and cystic degeneration, oligodendrocytosis with highly characteristic foamy oligodendrocytes, meager astrogliosis with dysmorphic astrocytes, and loss of oligodendrocytes by apoptosis. VWM is caused by mutations in any of the genes encoding the five subunits of the eukaryotic translation initiation factor 2B (eIF2B), EIF2B1 through EIF2B5. eIF2B is a ubiquitously expressed protein complex that plays a crucial role in regulating the rate of protein synthesis. VWM mutations reduce the activity of eIF2B and impair its function to couple protein synthesis to the cellular demands in basal conditions and during stress. Reduced eIF2B activity leads to sustained improper activation of the unfolded protein response, resulting in concomitant expression of proliferation, prosurvival, and proapoptotic downstream effectors. Consequently, VWM cells are constitutively predisposed and hyperreactive to stress, in view of the fact that VWM genes are housekeeping genes.

There are no specific treatments for VWMD aside from avoidance of clinical situations recognized to exacerbate the disease, and there is no rationale for the use of HCT to treat VWMD (Table 74.2).

Zellweger syndrome

Zellweger syndrome is a rare autosomal recessive severe peroxisomal disorder affecting infants who demonstrate characteristic facial features, profound hypotonia, seizures, and liver and renal dysfunction. It can be diagnosed on the basis of abnormalities of VLCFAs and plasmalogens (phospholipids in which the group at a C1 of glycerol is an ether-linked alcohol rather than an ester-linked fatty acid). Brain MRI shows polymicrogyria. Treatment focuses on addressing feeding difficulties, various therapies, supportive care measures, management of liver dysfunction (e.g., supplemental vitamin K and fat-soluble vitamins) to control bleeding tendencies; ursodeoxycholic acid, adrenal hormone replacement, and docosahexaenoic acid supplementation. There is no rationale for the use of HCT to treat Zellweger syndrome (Table 74.2).

Glycoprotein metabolic and miscellaneous disorders

In this section, glycoprotein metabolic diseases [fucosidosis, Gaucher disease, α-mannosidosis, and aspartylglucosaminuria (AGU)] will be presented and discussed (see below) (Table 74.3). Miscellaneous disorders [cerebrotendinous xanthomatosis, Fabry disease, Farber lipogranulomatous disease, gangliosidoses (GM1, GM2, and Tay-Sachs and Sandhoff diseases), glycogen storage disease II (Pompe disease), neuronal ceroid lipofuscinosis (CLN1 and CLN2), Niemann-Pick disease, Wolman syndrome, Canavan disease, cystinosis, sialic acid storage disease, sialidosis, multiple sulfatase deficiency, and hypophosphatasia] will be presented and discussed in a limited fashion (Table 74.3).

Fucosidosis

Epidemiology, molecular biology, and etiology

Fucosidosis is an autosomal recessive disorder due to a deficiency of α-L-fucosidase, which results in the accumulation of fucose-containing oligosaccharides, glycolipids, and glycoproteins [97]. Clinical features include progressive cognitive and motor deterioration, seizures, coarse facial features, growth retardation, recurrent infections, angiokeratoma corporis diffusum, visceromegaly, and dysostosis multiplex. Although the disease is panethnic, the majority of patients have been from Italy or the south-western region of the United States. A high frequency of consanguinity has been noted in affected families. A summary of the glycoprotein metabolic diseases (fucosidosis, Gaucher disease, α-mannosidosis, and AGU) is presented in Table 74.3.

Clinical description

The clinical course is variable; the majority of patients have a slow deterioration over several years. In severely affected patients, onset of psychomotor retardation in the first year of life, growth retardation, coarse facies, dysostosis multiplex, and increased sweat chloride [97]. At least two clinical phenotypes have been described, though in actuality there may be a continuum with a wide spectrum of severity. Attenuated phenotypes are characterized by angiokeratoma, longer survival, and more normal levels of sweat sodium chloride. The diagnosis is confirmed by enzyme analysis in leukocytes or fibroblasts, molecular DNA testing, and specific MRI findings [97,98].

Hematopoietic cell transplantation

For fucosidosis, detailed studies of a valid animal model, namely the Springer Spaniel, include the use of HCT after total lymphoid irradiation to correct the enzymatic deficiency [99]. BMT in dogs with fucosidosis is able to increase fucosidase activity in plasma, leukocytes, and visceral tissues [99]. Importantly in the context of a leukodystrophy with a neuronal component, neural tissues also showed increased enzymatic activity. Nevertheless, because the canine model resembles closely the human disease, it has been shown that therapeutic interventions after the appearance of clinical symptoms are ineffective [100,101]. Experience with HCT in fucosidosis patients is limited [102,103]. After successful engraftment, a progressive increase in α-L-fucosidase levels was documented in multiple tissues, including plasma and cerebrospinal fluid. Concomitantly with the increase in enzymatic activity, an improvement in myelination as well as normalization of myelination on MRI examination was observed although there was a decrease in psychomotor functions. The caveat behind the knowledge gathered from the human and canine trials resides in the time of intervention. In disorders like fucosidosis, the rapid decline after the appearance of first symptoms may hinder the benefits of bone marrow transplantation, since the cellular alterations and tissue pathology cannot be rescued by donor-derived monocytes or differentiated cells. Nevertheless, these studies have highlighted the need to improve the diagnosis and functional assessment in this disease to increase the time frame available to consider cellular therapy. Due to disease variability, a definitive conclusion regarding the benefits of HCT cannot be reached at this time.

ERT, gene therapy, and cellular therapy for fucosidosis

A gene therapy trial has been reported in the canine model using retroviral gene transfer into autologous hematopoietic stem cells [104]. Despite the lack of engraftment, given the recent advances in vector design, transduction and in vitro manipulation of bone marrow cells, improved strategies should be pursued to delineate the best approach for defining a cellular therapy for fucosidosis. Kondagari et al. have reported the use of ERT in fucosidosis dogs [105]. Intracisternal delivery of recombinant canine α-L-fucosidase was able to reduce the accumulation of fucosylated glycoconjugates. Given these results and that an ERT can be initiated before the development of symptoms, combinatorial approaches including ERT and transplantation may prove to be more beneficial than a single therapeutic intervention.

Gaucher disease

Epidemiology, molecular biology, and etiology

Gaucher disease is the most common lysosomal storage disorder. It is autosomal recessive and characterized by deficient activity of the lysosomal enzyme glucocerebrosidase and as a result, the accumulation of glucocerebroside in the lysosomes. The glucocerebrosidase gene (GBA) has been mapped to chromosome one, band q21. More than 200 mutations that cause Gaucher disease have been documented. The incidence of Gaucher disease in the general population is less than 1 in 30,000–40,000. It is more prevalent in people of Eastern European (Ashkenazi) Jewish descent with a prevalence of 1 in 1000 and a carrier frequency of 1 in 14 people [106]. The pathophysiologic feature of Gaucher disease is the presence of Gaucher cells derived from the monocyte-macrophage system. Most Gaucher cells are found in the spleen, liver, bone marrow, and lymph nodes causing enlargement and dysfunction of these organs resulting in clinical manifestations.

Clinical description

Classically three types of Gaucher disease have been identified based on the presence or absence of primary CNS involvement and the severity of the disease.

Type 1 Gaucher disease is the most common form accounting for 90–95% of the cases. It is characterized by onset in adulthood and by the absence of primary CNS involvement. However, some individuals, who have severe mutations, present with an onset in early childhood. Type 2 Gaucher disease is characterized by severe neurologic involvement and an onset during infancy. Type 3 Gaucher presents by the onset of neurologic disturbances later in the first decade of life. Types 2 and 3 are very rare and occur in less than 1 in 100,000 of the population. The prototype of type 3 Gaucher disease is found with high frequency in the Norbottnian population of northern Sweden [106].

Typically the infant is normal at birth, with clinical features becoming apparent as the amount of substrate accumulating increases. Bleeding due to thrombocytopenia, anemia, and hepatosplenomegaly are the common early features. Bone involvement is common but is not always associated with symptoms. The symptoms of bone disease when present range from mild pain to severe bone crises. Growth retardation is seen in moderate to severe forms of disease. The neurologic symptoms seen in types 2 and 3 include oculomotor apraxia, opisthotonus, bulbar signs [107]. The clinical course of type 1 Gaucher disease has a broad spectrum of severity ranging from individuals who have been diagnosed in their eighth or ninth decade to children who die of complications during their first or second decade. Most infants with type 2 disease die within the first 2 years of life; and the severity of type 3 is intermediate between types 1 and 2 [108]. A confirmed diagnosis can be made by measuring the glucocerebrosidase activity of peripheral blood leucocytes or cultured skin firm diagnosis can be made by measuring the glucocerebrosidase activity of peripheral blood to establish a disease prognosis [107].

Hematopoietic cell transplantation

Since the pathophysiology of Gaucher disease is due to accumulation of lipid-laden macrophages, HCT was thought of as a logical treatment for the disease. The first successful HCT for Gaucher disease was performed by Ringdén et al. [109]. Multiple reports since that time have documented the feasibility and efficacy of this modality [110–114]. The choice of donor cells has expanded over the last few decades to include marrow from matched or mismatched family members, unrelated donors.

The outcomes of HCT are affected by numerous factors such as donor enzyme level, degree and persistence of donor chimerism, and post-transplant complications [72]. Benefit of transplantation varies between organ systems. Hematologic and physical improvement is rapid and sustained. Reticuloendothelial organs, such as the liver and spleen regress within a few months [72] and there is some evidence that the skeletal changes seen in Gaucher disease regress. The evidence for the beneficial effect of HCT on the regression of the neurologic disease in people with types 2 and 3 is conflicting [109,113,114]. The response is slow and the effectiveness appears to depend on the age of the individual, severity of the disease and its progression at the time of treatment.

ERT, gene therapy and cellular therapy for Gaucher disease

The advent of ERT for Gaucher disease has revolutionized the treatment approach to this disorder. Alglucerase, a human placental β-glucocerebrosidase was modified to expose variable numbers of mannose residues, so that it can be targeted to the macrophages. The Chinese hamster ovary cell-derived recombinant ERT imiglucerase was approved by the Food and Drug Administration (FDA) in 1994 and shortly thereafter replaced alglucerase and became the preferred form of treatment for symptomatic individuals with type 1 disease. Treatment results in significant regression of disease in these individuals and has very few side effects [115]. The main drawbacks of ERT are that weekly infusions of the enzyme are required, and the treatment is very expensive. The estimated annual cost of therapy in the United Kingdom for a 50-kg patient is around £86,000 (around US$110,000) for the medication alone [116]. Furthermore, since the enzyme does not cross the blood–brain barrier, it cannot be effective in types 2 and 3 diseases to improve neurologic symptoms [115]. Improvements in HCT technologies make it a less expensive procedure that offers the potential of permanent cure when compared to ERT. However, the procedure is associated with a mortality ranging from less than 5% to more than 10% depending on the availability of a fully matched donor. Therefore, the standard of care for Gaucher disease remains ERT.

Besides ERT and HCT there are a few new promising treatment options [117]. These include: a newer generation of therapeutic recombinant enzymes (velaglucerase alfa), which improves the delivery of the enzyme to cells and is under development, substrate reduction therapy (SRT), drugs that can cross the blood–brain barrier, pharmacologic chaperone therapy, consisting of specific small molecules that inhibit substrate synthesis, or act as chaperones to increase residual activity of glucocerebrosidase by stabilizing misfolded mutant proteins, preventing endoplasmic-reticulum-associated degradation in proteasomes [117].

α-Mannosidosis

Epidemiology, molecular biology, and etiology

α-Mannosidosis arises from deficient activity of the lysosomal enzyme α-mannosidase [97]. The gene maps to chromosome 19p13.2–q12.

Clinical description

Three clinical types have been suggested [118,119]. Type 1: mild form clinically recognized after 10 years of age, without skeletal abnormalities and very slow progression. Type 2: the attenuated (juvenile–adult, type) phenotype accounts for approximately 10–15% of cases. It is characterized by a slower rate of progression with development of ataxia at age 20–30 and survival into adulthood. Type 3: severe form, immediately recognized, with skeletal abnormalities, and rapidly progressive mental retardation, hepatosplenomegaly, severe dysostosis multiplex, and often death between ages 3 and 12 years.

Hematopoietic cell transplantation

The earliest report of HCT in a patient with α-mannosidosis demonstrated reversal of the somatic changes of the disease, but did not affect lysosomal storage within brain tissue. The conclusion was that HCT may not be a suitable treatment for α-mannosidosis [120]. However, it was noted that the lack of effect could be attributed to the fact that donor cells came from the mother who was a carrier of α-mannosidosis. The hypothesis that early HCT could prevent neurologic deterioration was tested in a cat model. Subsequently, a few reports have shown that intellectual function can be stabilized in selected patients, with improvement in adaptive skills, hearing, and verbal memory function [121].

In summary, the possible benefits of BMT in patients with α-mannosidosis type 3 must be weighed against the overall risk of procedure-related morbidity and mortality. The benefits are greater in younger patients before complications have developed. Therefore, BMT is an option in the first decade of life which makes early identification of affected patients critical.

Aspartylglucosaminuria

Epidemiology, molecular biology, and etiology

Aspartylglucosaminuria (AGU) is an autosomal recessive lysosomal storage disease caused by defective activity of the lysosomal enzyme aspartylglucosaminidase resulting in degradation of Asn-linked glycoproteins. The enzyme is necessary for hydrolysis of the protein–oligosaccharide linkage in Asn-linked glycoprotein substrates undergoing metabolic turnover. Loss of glycosylasparaginase activity leads to accumulation of the linkage unit Asn–GlcNAc in tissue lysosomes. Storage of this fragment affects the pathophysiology of neuronal cells most severely [122]. A specific AGU mutation, representing 98% of the disease mutations, is concentrated in the Finnish population with over 200 patients. The carrier frequency in Finland has been estimated to be in the range of 2.5–3% of the population. So far there are 20 other rare family AGU alleles that have been characterized at the molecular level in the world's population [122].

Clinical description

A growth spurt in infancy is the earliest sign of the disease. Delayed speech development, concentration difficulties, clumsiness and restlessness are common presenting symptoms. Aspartylglucosaminuria is characterized by global developmental delay with onset between 2 and 4 years of age. Delayed speech and motor clumsiness are often preceded by frequent upper respiratory tract infections. Patients reach developmental capacities equivalent to that of a 5- to 6-year-old around puberty and then slowly deteriorate to a severely delayed level by adulthood. Mild connective tissue changes lead to coarse facial features, thickened calvarium, and osteoporosis. Seizures are common later in the disease course, with abnormalities in the differentiation between gray and white matter as well as delayed myelination apparent on brain MRI [123].

Hematopoietic cell transplantation in aspartylglucosaminuria

Only few reports have described the use of HCT in patients with AGU [124–126]. The medical center in Helsinki, Finland, has experience with HCT in three patients with follow-up ranging from 1 to over 5 years including serial MRI and biochemical and clinical examinations [126]. MRIs of the brain in transplanted patients with at least 2 years of follow-up showed nearly normal gray–white matter relationships, and improved neuropsychologic function had also occurred [126]. However, more recently, a longer term follow-up described five AGU patients who had a successful HCT between 1991 and 1997. After long-term follow-up the first two patients were more severely mentally retarded in comparison to non-transplanted patients. In addition, while the general state of health of non-transplant patients was good, the five patients who underwent HCT had post-transplant complications. The authors concluded that HCT cannot be recommended for the treatment of patients with AGU after infancy [124].

Miscellaneous disorders

Cerebrotendinous xanthomatosis

Cerebrotendinous xanthomatosis is a rare autosomal recessive disorder due to defective activity of mitochondrial enzyme sterol 27-hydroxylase (CYP27A) [127]. The gene is found on chromosome 2. More than 300 patients have been reported worldwide and over 50 different mutations in CYP27A identified, most of which result in absences or inactive enzyme. The clinical features include diarrhea, cataracts, tendon xanthomas, and neurologic signs such as dementia, psychiatric disturbances, pyramidal and/or cerebellar issues, and seizures. Strongly suggestive cerebellar findings are noted on brain MRI. Abnormalities of bile acids in urine and serum, and elevated plasma and tissue cholestanol, are the biochemical hallmarks at diagnosis and in subsequent monitoring on treatment with chenodeoxycholic acid. There is no role for HCT in this disorder (Table 74.3).

Fabry disease

Fabry disease is an X-linked recessive inborn error of glycosphingolipid catabolism due to deficient activity of lysosomal hydrolase α-galactosidase A in affected hemizygous males. This defect leads to systemic deposition of glycosphingolipids, predominantly globotriaosylceramide in the following tissues: body fluids, endothelium, perithelium, smooth muscle cells of blood vessels, ganglia, heart, kidneys, eyes, etc. Onset is usually in childhood or adolescence with pain and paresthesias of the extremities, vessel ectasia (angiokeratoma) in skin and mucous membranes, and hypohidrosis. Corneal and lenticular opacities are often noted. With aging, proteinuria, hyposthenuria, and lymphedema appear. Severe renal impairment leads to hypertension and uremia. Causes of death are usually renal, cardiac, or cerebrovascular. Heterozygous females may have an attenuated form of the disease. The gene is located at Xq22.1. Over 150 mutations have been identified, including partial gene rearrangements, splice-junction defects, and point mutations, thereby emphasizing the heterogeneity of the molecular defects causing this disease. The primary treatment is ERT with additional supportive care measures as indicated, such as treatment for neuropathic pain, anticoagulants in patients who are prone to strokes, and hemodialysis for patients with end-stage renal disease. HCT is not considered an appropriate therapy (Table 74.3).

Farber lipogranulomatous disease

Farber disease is a rare lysosomal storage disorder caused by deficiency of acidic ceramidase. Formation of subcutaneous and periarticular ceramide-containing nodules causes painful joint swelling, progressive disability, and hoarseness. The pathophysiology whereby the defect in ceramide metabolism leads to chronic granulomatous inflammation remains to be elucidated. Most patients with Farber disease die of progressive neurologic deterioration early in infancy; however, since there is a wide spectrum of clinical severity/CNS involvement, depending on residual lysosomal ceramide, there is a broad spectrum of disease severity. Children with Farber disease without CNS involvement (Farber disease type 2/3) still have a severe disease due to chronic granulomatous inflammation with progressive joint deformities causing pain and immobility, and effects on the lower airways causing chronic respiratory failure with death before age 20 years. Allogeneic HCT has been attempted in patients with Farber disease based on the reasoning that a population of cells with normal enzyme activity could ameliorate the effects of the deficient enzyme [128,129]. In transplants of two mildly affected Farber patients (type 2/3), granulomatous infiltrations were reduced, the hoarseness disappeared, and joint mobility improved [128]. However, earlier transplants on patients with the more common type 1 Farber disease had limited success. In these studies, BMT lessened the peripheral symptoms but there was no improvement in neurologic function and patients died soon after transplant [129]. In summary, HCT performed in patients with Farber disease and CNS involvement has been unsuccessful, with patients dying of progressive neurodegeneration while peripheral symptoms including granuloma resolved [128].

Gangliosidoses (GM1, GM2, Tay-Sachs, Sandhoff)

The GM2 gangliosidoses are a group of inherited disorders caused by excessive accumulation of ganglioside GM2 and related glycolipids in lysosomes, especially in neurons. With infantile onset, the rapidly progressive neurodegenerative disease leads to death by 4 years of age (classic Tay-Sachs disease, Sandhoff disease, and GM2 activator deficiency), while later onset subacute or chronic forms show more slowly progressive neurologic conditions compatible with survival into late childhood, adolescence, or even adulthood. HCT does not appear to successfully correct these disorders (Table 74.3). Deficiency of lysosomal β-galactosidase is manifested clinically as two different diseases, GM1 gangliosidosis and Morquio B (MPS IVB, discussed previously). GM1 gangliosidosis can have its onset in infancy, childhood/adolescence or adulthood. The clinical course is generally progressive neurologic deterioration. The role of HCT for GM1 gangliosidosis remains unclear since most cases are too advanced at diagnosis or progress too rapidly for benefit to be derived from HCT. Conflicting results have been documented in studies reporting the use of HCT in patients with the various gangliosidoses [130,131]. A few of the reports suggest that HCT may be helpful in later onset cases at an early stage of the disease process.

Glycogen storage disease type II (Pompe disease)

Glycogen storage disease type II is also called acid maltase deficiency or Pompe disease. It is an autosomal recessive disorder of glycogen metabolism arising from deficiency of lysosomal hydrolase acid α-glucosidase activity. The clinical presentation encompasses a range of phenotypes, all of which include varying degrees of myopathy, but differ with respect to onset, extent of organ involvement, and rate of progression to death. Infantile-onset Pompe disease, the most severe form, is characterized by a generalized muscle weakness, hypotonia and a hypertrophic cardiomyopathy with death typically in the first year of life due to cardiorespiratory or respiratory failure. Late-onset forms of Pompe disease (juvenile and adult forms) feature progressive weakness without significant cardiomyopathy. Patients have significant morbidity and with disease progression become wheelchair-dependent and require artificial ventilation. The cause of death in late-onset Pompe disease is usually respiratory failure, which can be precipitous. The histopathology of Pompe disease includes marked lysosomal accumulation of glycogen in cardiac and skeletal muscle, which culminates with the leakage/rupture of lysosomes and cytoplasmic pooling of glycogen. GAA is normally widely expressed from a promoter with resemblance to a "housekeeping" gene [132].

The primary therapies are ERT and supportive care measures. There is not a role for HCT in this disorder (Table 74.3) [132].

Neuronal ceroid lipofuscinosis (NCL1, NCL2)

Two forms of neuronal ceroid lipofuscinosis (NCL1, infantile, and NCL2, classic late-infantile) are recognized as true lysosomal enzyme storage disorders. NCL1 is characterized by normal development until age 6–12 months, followed by psychomotor retardation, microcephaly, myoclonus, and visual deterioration. NCL2 presents between 2 and 4 years of age with severe myoclonic seizures and slow progression to blindness. Since both diseases are lysosomal, the implication is that HCT might be effective therapy [133]. However, this has not been borne out by presymptomatic patients with NCL2 who have been transplanted, or by the existing animal model studies. Additional clinical data are needed to determine whether the natural history of NCL2 is too rapidly progressive for HCT to be beneficial (Table 74.3). Current ongoing trials investigate the therapeutic utilization of cross-correction by enzyme-replacement therapy, gene therapy, and stem cell therapy, which have the potential of translating these treatments into the clinic [133].

Niemann-Pick disease types A, B, and C

Niemann-Pick disease types A and B are lysosomal storage disorders resulting from deficient acid sphingomyelinase enzyme activity. Niemann-Pick type A is a rapidly progressive disorder of infancy characterized by failure to thrive, hepatosplenomegaly, neurodegeneration, and death, often by 2–3 years of age. HCT has not been effective in preventing the inexorable neurodevelopmental decline [134]. Niemann-Pick type B is a phenotypically variable disorder that is usually diagnosed in childhood due to marked hepatosplenomegaly. HCT appears to effectively treat the somatic manifestations of Niemann-Pick type B disease [135].

Niemann-Pick type C is an autosomal recessive lipidosis resulting from a unique error in cellular trafficking of exogenous cholesterol, and is associated with lysosomal accumulation of unesterified cholesterol. A case has been reported of a 3-year-old girl with Niemann-Pick type C disease who underwent allogeneic HLA-identical sibling donor HCT who engrafted. She survived with improvement in somatic disease features including hepatosplenomegaly and marrow and lung infiltration; however, her neurologic status continued to deteriorate [136]. It is therefore unclear whether HCT has a role in the effective treatment of the CNS in Niemann-Pick type C.

Wolman syndrome

Wolman disease is an autosomal recessive disorder of infancy caused by deficiency of the soluble mannose-6-phosphate-targeted lysosomal enzyme acid lipase (AL). Deficiency of this enzyme leads to massive storage of cholesterol and cholesteryl esters. Triglycerides and cholesteryl esters are internalized by the cell through receptor-mediated endocytosis of lipoproteins and metabolized, in part, by AL. The clinical manifestations of this disorder are caused by both accumulation of cholesteryl esters and triglycerides, and toxicity from lipoprotein oxidation. Children affected with Wolman disease develop early hepatosplenomegaly, jaundice, diarrhea, severe vomiting, failure to thrive, and pathognomonic calcification of the adrenal glands. Wolman disease is uniformly lethal in early infancy unless treated with HCT [137,138]. Graft failure and the high mortality rate associated with disease-related pretransplant liver injury and post-transplant liver sinusoidal obstruction syndrome (veno-occlusive disease) have been reported as major barriers to optimal outcomes [137].

Wolman disease is rare and only a few patients underwent HCT. Wolman disease remains near-universally fatal. Curing this disease with HCT is challenging, with the majority of attempts resulting in death because of liver failure from progressive Wolman disease or from other transplant-related complications including infection, GVHD, and failure to engraft. Long-term follow-up data are limited. Tolar et al. report in addition to a lasting correction of hepatic dysfunction, and normal adrenal function that normal neurologic outcome is possible after successful HCT [139]. Their report suggests that timely HCT in patients with Wolman disease, despite management challenges, may potentially be beneficial.

In conclusion, HCT has been performed successfully in a small number of patients and may have a role in a selected group of patients treated prior to onset of permanent organ damage.

Canavan disease

The clinical features of Canavan disease include poor head control and hypotonia at 2–4 months of age, generalized seizures, opisthotonic posturing, loss of very early milestones, increased head circumference, white matter changes (leukodystrophy) on brain MRI, hypotonia that progresses to spasticity, and, late in the course, decerebrate or decorticate posturing [140]. This autosomal recessive disorder arises due to deficient aspartoacylase activity. There is no role for HCT in the treatment of Canavan disease; the primary treatments are supportive in nature (Table 74.3).

Cystinosis

Cystinosis is a rare, autosomal recessive lysosomal storage disorder due to defective carrier-mediated transport of the amino acid cystine across the lysosomal membrane [141]. The major clinical manifestation is renal failure at age 9–10 years. The gene is located on chromosome 17p13 and codes for cystinosin. Therapy includes replacement of renal losses due to Fanconi syndrome, provision of thyroxine, insulin, pancreatic enzymes, and testosterone for deficient patients, and symptomatic care for ophthalmic complaints. Although the potential of hematopoietic stem cell in preservation of kidney function in a mouse model for cystinosis has been investigated, there is no clear role for HCT in cystinosis presently (Table 74.3).

Sialic acid storage diseases

The lysosomal free sialic acid storage diseases, namely Salla disease and infantile free sialic acid storage disease, are autosomal recessive disorders with various degrees of psychomotor retardation. Dysmyelination is an invariable characteristic as seen on brain MRI. The fundamental problem is accumulation of free sialic acid in lysosomes due to an impaired carrier-mediated transport system in the lysosomal membrane. There is no clear indication that HCT is beneficial (Table 74.3).

Sialidosis

Sialidosis is an autosomal recessive disorder consisting of two clinical phenotypes. Type 1, the milder form, is characterized by development of ocular cherry-red spots and generalized myoclonus in the second or third decade, as well as seizures, hyperreflexia, and ataxia. Type 2 disease has an early age of onset, and is a severe and rapidly progressive disorder with an MPS-like phenotype with organomegaly, dysostosis multiplex, and mental retardation [97]. The gene which codes for neuraminidase is found on chromosome 6p21. There is no definitive treatment (Table 74.3).

Multiple sulfatase deficiency

Multiple sulfatase deficiency is a rare (1 : 1.4 million) autosomal recessive disorder characterized by deficiencies in all 12 known sulfatases, and leading to a clinical presentation that generally resembles late-infantile MLD. There has been very limited experience with HCT for this disease, and no definite conclusion can be drawn at this time as to its utility (Table 74.3).

Hypophosphatasia

Hypophosphatasia is an autosomal dominant or autosomal recessive disorder caused by defective osteoblasts and chondrocytes due to inactivation of tissue-non-specific alkaline phosphatase. There is limited experience to date with HCT for this disorder [142].

Future directions

ERT, substrate depletion, alternative stem cells, complementary and combination therapies, gene therapy, alternative approaches to HCT with or without stem cell selection, and newborn screening are all part of this rapidly advancing field of clinical medicine [7,18,143–145]. Alternative and complementary therapies to HCT, as well as combinations of therapies for the inherited metabolic storage diseases, are either in place or being developed. ERT has been available for patients with Gaucher disease type 1 for over 15 years [117]. ERT has made the transition from clinical trial to proven therapy for Fabry, Pompe, and Niemann-Pick type B diseases, and selected MPSs including MPS I, II, and VI. In the case of Fabry disease, ERT reduced severe neuropathic pain, stabilized renal function, and improved cardiac and vascular structure and function. In Pompe disease, a fatal cardiac and skeletal muscle disorder due to acid maltase deficiency, ERT has improved cardiac function and structure and increased overall muscle strength. Important lessons from these experiences include the variability of the clinical response to ERT depending upon the disease, the organ or tissue, the dose of enzyme, and the schedule of administration. Of particular importance is the observation that there is limited-to-no penetration by exogenous intravenous enzyme into the CNS or peripheral nervous system. In the area of substrate depletion or deprivation, a number of disorders have been treated, including Gaucher disease, GM2, Fabry disease, and cystinosis. Drugs that slow the rate of formation of accumulating glycolipids are under development as well.

Alternative stem cells, including both embryonic and adult stem cells, are being investigated in transplantation and gene transfer/therapy trials. This is important since bone marrow-derived MSCs remain host derived despite successful HCT with engraftment in patients with lysosomal and peroxisomal disorders.

The scope of gene transfer applications in therapy for human diseases continues to expand and includes genetic storage diseases. Hematopoietic stem cells have been considered appropriate targets for therapeutic gene transfer due to their ability to self-renew as well as differentiate into multiple lineages. Diseases such as Gaucher disease, Hurler syndrome, X-ALD, MLD, and other enzyme-deficiency states may be amenable to treatment by gene transfer into hematopoietic stem cells, due to enzyme production, release, and uptake leading to metabolic correction. In conclusion, enhanced interactions among the many disciplines and specialists caring for patients with these complex disorders are of paramount importance to the coordinated advancement of this very exciting field of clinical medicine and research.

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CHAPTER 75

Hematopoietic Cell Transplantation for Macrophage, Granulocyte and Osteoclast Disorders

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Introduction

The human body is equipped with a complex phagocytic and immune system to defend against a wide variety of microbes ranging from viruses to bacterial and fungal offenders. The first line of defense is a set of two systems, namely, the monocyte–phagocyte system and the granulocyte–phagocyte system. The primary cells of these two systems, monocytes and granulocytes, arise from a common committed progenitor in the bone marrow [1–3]. The pattern of the inflammatory response in the monocyte–phagocyte system is similar to that seen in the granulocyte–phagocyte system. Any quantitative or qualitative defect in the elements of these defense systems will result in an inadequate host defense against microbial infections.

The monocyte–phagocyte system is defined as a continuum from monoblasts to promonocytes to monocytes with eventual migration of the monocytes into tissues such as lungs, liver, spleen, and the brain to form fixed tissue macrophages that perform specialized functions suited for the specific location. The life span of monocytes varies between 3 and 6 days. The tissue macrophage does not usually undergo cell division and their life span is estimated to range from weeks to years. However, if the cell proliferation regulatory controls are lost or altered, uncontrolled proliferation and accumulation of macrophages may take place in multiple organs. One such example is the hemophagocytic lymphohistiocytosis (HLH) as described later in this chapter. In HLH (familial or secondary), excessive accumulation of tissue macrophages results in organ dysfunction/enlargement. The excessive "cell eating" behavior of the macrophages seen in HLH results in low blood counts. Abnormal proliferation and accumulation of macrophages at specific anatomic sites may result in signs and symptoms pertinent to those sites. For example, central nervous system (CNS) involvement may result in serious clinical focal neurologic manifestations including hypotonia or hypertonia, convulsions, hemiplegia/tetraplegia, or blindness.

The functions of the monocyte–macrophage system are manifold [3]. The mononuclear phagocytes play an important role as phagocytic and immunoregulatory cells. As phagocytic cells, macrophages secrete a variety of antimicrobial agents including lysozyme, neutral proteases, acid hydrolases, and cytokines. The macrophages, for example, secrete the cytokine interleukin-1 (IL-1). Interleukin-1, in turn, stimulates vigorous production of hydrogen peroxide and toxic oxygen metabolites not only by the macrophages but also by the neutrophils. The activation of macrophages in response to insults such as infection is central to their role in host defense. Intact chemotactic mechanisms result in the accumulation of macrophages at the site of insult. Defective mononuclear phagocytic chemotaxis may result in an inadequate host defense against infection such as seen in mucocutaneous candidiasis. The immuno-regulatory functions of the macrophages are also diverse and complex. Macrophages process and present antigens to lymphocytes and secrete cytokines important for the development of lymphocytes and the regulation of their replication. The immune responses are mediated via secretory products that have pleotropic effects affecting nearly all cell types. Macrophages also participate in removing immune complexes from the circulation.

The inflammatory cascade of the granulocyte–phagocyte system has two major components: (1) vasodilatation and increased vascular permeability to phagocyte products, and (2) leukocytic events. The leukocytic events include several sequential steps, some of them overlapping in nature: (1) margination, (2) adhesion to the endothelial surface, (3) emigration which includes "rolling and diapedesis," (4) phagocytosis and intracellular degranulation and killing, and (5) release of leukocyte products. The leukocytic events are elucidated in detail in Figure 75.1.

Initially, pro-inflammatory cytokines such as TNFα, IL-1, IL-6, and IFNγ are released by endothelium, T and B cells resulting in vasodilatation and increased vascular permeability. Immunoglobulins and the complement system activate phagocytes and enhance microbial opsonization. A redundancy of chemoattractants, such as various cytokines and complement fragments, are generated to ensure that the leukocytes will be attracted to the site of insult. The chemoattractant molecules also upregulate certain adhesion receptors on the leukocytes as well as promote recruitment of the cytoskeletal actin for movement of phagocytes.

Following margination or peripheral orientation of leukocytes in the moving blood stream, the white cells adhere in great numbers to the endothelial surface and eventually migrate to the infected or the injured site. The initial step in the emigration of white cells, often referred to as "rolling" or "tethering," is mediated by a family of glycoprotein adhesion molecules called selectins (L-, E-, and P-selectins). Some of the rolling leukocytes become firmly attached to the vascular endothelium via β integrin adhesion receptors on the leukocytes. The integrins are the glycosylated heterodimers of non-covalently linked β and α chains. The type of β integrin is determined by the type of the β subunit in the complete molecule. An important type of β integrin is the β_2 integrin. Three

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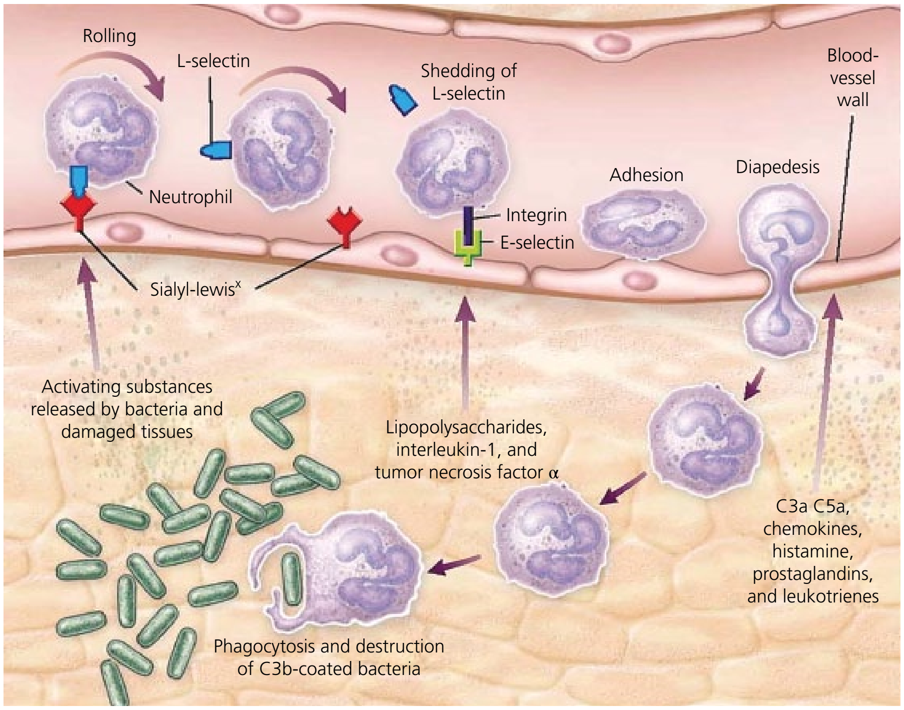
Rolling
L-selectin
Shedding of L-selectin
Blood-vessel wall
Neutrophil
Adhesion
Diapedesis
Integrin
E-selectin
Sialyl-lewis^x
Activating substances released by bacteria and damaged tissues
Lipopolysaccharides, interleukin-1, and tumor necrosis factor α
C3a C5a, chemokines, histamine, prostaglandins, and leukotrienes
Phagocytosis and destruction of C3b-coated bacteria

Figure 75.1 The acute inflammatory response. Neutrophils are among the first cells to arrive at the scene of an infection and are important contributors to the acute inflammatory response. As the neutrophil rolls along the blood-vessel wall, the L-selectin on its surface binds to carbohydrate structures such as sialyl-LewisX on the adhesion molecules on the vascular endothelium, and its progress is eventually halted. As the neutrophil becomes activated, it replaces L-selectin with other cell-surface adhesion molecules, such as integrins. These molecules bind E-selectin, which is present on the blood-vessel wall as a result of the influence of inflammatory mediators such as bacterial lipopolysaccharides and the cytokines interleukin-1 and TNFα. The activated neutrophil then enters the tissues, where it is attracted to the infection site by a number of chemoattractants. The neutrophil can then phagocytose and destroy the C3b-coated bacteria. Source: Delves PJ, Roitt IM. The immune system. *N Engl J Med* 2000; **343**(1): 37–49. Reproduced with permission of the Massachusetts Medical Society. (For a color version of this figure, please see the color plate section.)

different β_2 integrin molecules result when the same β_2 integrin subunit (CD18) is linked with different α subunits (CD11a, CD11b, and CD11c). Defective adhesion has been noted in an inherited heterogeneous defect called leukocyte adhesion deficiency type I (LAD-I). In this disorder, mutations in the common β_2 integrin subunit are the underlying cause of defective chemotaxis. Two patients have been described with the absence of neutrophil receptors for E-selectin resulting in defective adhesion, and the disorder is called LAD type II.

The emigration of the adhered or firmly attached leukocytes at the endothelial surfaces into the site of injury or infection involves the presence of a chemotactic gradient that creates the directional movement, the chemotaxis, of the cells. The phagocytes move by extending pseudopodia the interior of which consists of a branching network of actin filaments and the contractile protein, myosin. Defects in neutrophil motility involving actin have been noted in neutrophil actin deficiency syndromes. In the next step, the accumulated leukocytes recognize the opsonized targets by attaching to the latter via opsonin receptors on leukocyte surfaces. The major humoral opsonins that coat the microbes include IgM, IgG1, IgG3, C3b, and C3bi. In LAD-I, ingestion of C3bi-opsonized microbes is also impaired. Once the leukocyte–opsonin interaction has occurred, the process of engulfment is initiated.

As the final step, the engulfed material is destroyed through intracellular cytocidal and digestive activity. This process involves oxygen-independent (in the hypoxic necrotic environment of tissue injury) and the oxygen-dependent pathways of microbial killing. In the oxygen-independent pathway, numerous cationic antimicrobial proteins contained within the neutrophil azurophilic granules are utilized. These antimicrobial proteins include proteins such as defensins and serpocidins. In addition, the azurophilic and specific granules also contain other degradation enzymes such as lysozymes and hydrolases. Deficiencies in neutrophil granule proteins or defects in granule formation or degranulation are associated with recurrent bacterial infections as seen in Chediak–Higashi syndrome (CHS). The oxygen-dependent pathway of intracellular microbial killing, often referred to as “respiratory burst oxidase” or NADPH pathway, utilizes the generation of superoxide radicals through mitochondrial-based reactions. Mutations in the oxidase subunit result in the well-known disease entity of chronic granulomatous disease (CGD).

In addition to the normally functioning granulocytes, adequate numbers of granulocytes are necessary for normal host defense. Granulocytopenia due to congenital defects, malignancy, or after administration of chemotherapeutic agents results in increased susceptibility for bacterial infections.

The congenital disorders that affect the monocyte–macrophage and granulocyte system present in early childhood with recurrent, severe and often life-threatening infections. This chapter will address the various disorders of macrophages and granulocytes and the role of hematopoietic cell transplantation (HCT) as an important treatment modality (Table 75.1).

Table 75.1 Hematopoietic cell transplantation for macrophage and granulocyte disorders

Macrophage disorders
Hemophagocytic lymphohistiocytosis
Granulocyte disorders
Quantitative disorders
Kostmann syndrome
Reticular dysgenesis
Cyclic neutropenia
Cartilage-hair dysplasia
Dyskeratosis congenita
Shwachman-Diamond syndrome
Qualitative disorders
Neutrophil actin deficiency – Abnormal chemotaxis
Leukocyte adhesion (CD11/18) deficiency – Abnormal adhesion
Chediak–Higashi syndrome – Abnormal granulation
Chronic granulomatous disease – Abnormal oxidation
Griscelli syndrome

General considerations for HCT

Timing of HCT

The timing of intervention in the phagocytic disorders with HCT depends on the nature of the disease, presence of serious infections, and the status of organ function. Even though the risk attendant to HCT may be a concern, a "wait-and-see" approach may not be in the patient's best interest. Timing of HCT should be determined by the prognosis and the anticipated risk of life-threatening infections or irreversible organ damage. The disorders with a variable clinical course need to be approached differently as opposed to disorders where delayed intervention with HCT might result in high mortality despite the provision of optimal supportive care. For example, in cyclic neutropenia, low counts can be managed with granulocyte colony-stimulating factor (G-CSF) and without intervention with HCT. On the other hand, in severe forms of reticular dysgenesis (RD), early intervention with HCT is warranted [4]. The course of chronic granulomatous disease (CGD) can be extremely variable. However, certain phenotypes of CGD can be more severe than others [5].

Choice of donor for HCT

Since the disorders discussed in this chapter affect young children, genotypically identical donors will likely be available for no more than 20–30% of patients. Therefore, 70–80% of HCT candidates are without an ideally matched related donor for HCT. Since many of these disorders occur in children of consanguineous parents, an extensive family search should be conducted to identify a suitable related donor. The siblings or any other related potential donor should be tested, if technically possible, for the presence of the disease in question before they are considered to serve as donors. Many congenital disorders of the phagocyte system are transmitted as autosomal recessive conditions. The carrier state does not result in serious clinical disease and heterozygous individuals may qualify as donors. One possible exception would be the X-linked CGD, where severely imbalanced "lyonization" may result in clinically significant disease [6]. For HCT in HLH preference should be given to the sibling donor. However, the sibling donor should be screened to rule out the presence of any occult disease. If all the available tests including low NK-cell activity are negative, the sibling may be used as a donor. The caveat that should be kept in mind is that no definitive tests exist to exclude the carrier state. In the event that the sibling is suspected to be a possible carrier, a search for a matched unrelated donor should be performed [7]. Identification of such a donor may require several months and HCT risks may rise during the search process. In general, T-cell-depleted haplo-identical grafts from parents have been successfully used as matched unrelated donors to perform HCT in these patients. However, the difficulties and risks with these HCT procedures remain a major concern.

Preparative regimens

Most of the HCT procedures for phagocytic disorders have been performed utilizing myeloablative conditioning. The two major chemotherapeutic agents in use are busulphan (BU) and cyclophosphamide (CY). Cyclophosphamide is used, under certain circumstances, in combination with total body irradiation (TBI). It remains the physician's choice to use a particular regimen, as both radiation-based and non-radiation-based regimens are relatively well tolerated by children. Since the introduction of hyperfractionated TBI, many of the previously observed long-term effects have diminished. Recent experiences suggest that lower doses of TBI may result in prolonged mixed and eventual complete chimerism, which may be effective and less toxic in HCT procedures for some disorders [8]. More data need to be generated to prove the usefulness of this approach for children with the conditions discussed in this chapter. At this time, reduced-intensity conditioning (RIC) regimens seem to be attractive and should be explored. The RIC approach can be especially useful where mixed chimerism could restore the cellular function, for example in patients with CGD.

Macrophage disorders

Hemophagocytic lymphohistiocytosis

Hemophagocytic lymphohistiocytosis (HLH) is the most frequent disorder of the macrophage system. HLH is characterized by multisystem inflammation, a reactive process resulting from the hyperactivation of antigen-presenting cells (macrophages, histiocytes) and CD8+ T cells. In this disorder, accumulation of activated macrophages along with hemophagocytosis of the hematopoietic cellular elements takes place resulting in organ enlargement with dysfunction and cytopenias. The pathologic findings in HLH arise from persistently elevated levels of proinflammatory cytokines. It is now believed that increased exposure to the cytokines generated by uncontrolled activation of histiocytes and T cells leads to progressive organ dysfunction that leads to death in affected patients. Ongoing hypercytokinemia results from failure of natural immune downregulation due to defective NK and cytotoxic T-lymphocyte function [9].

Several genetic defects have been described in HLH. The first genetic defect causing HLH was reported in 1999 involving Perforin gene mutation (PRF1) [10]. Perforin is stored in the NK, CD8+ T and NK-T cells, and is required to deliver granzyme B to target cells that initiate apoptotic programs leading to cell death [11]. The perforin gene mutation is shown to be involved in 20–40% of all affected families, and up to 50% of North American families [12]. In 2003, another gene mutation in UNC 13D on chromosome 17q25 was shown to lead to HLH [13]. The affected protein, Munc 13-4, is essential for cytolytic granule secretion preceding vesicle membrane fusion. The mutated Munc protein results in defective cytolytic granule exocytosis. A third gene defect associated with HLH has recently been identified encoding a protein Syntaxin 11. This involves the gene *STX11* on chromosome 6q24. The Syntaxin 11 has been postulated to play a role in intracellular trafficking [14]. Mutations in the UNC 13D and STX11 affect 20% and 10% of HLH patients, respectively [15]. From a diagnostic standpoint, a rapid screening assay for PRF mutations by flow cytometry has been developed. Genetic sequencing of PRF and MUNC 13-4 genes is also available on a clinical basis in the United States [16].

Hemophagocytic lymphohistiocytosis (HLH) includes two different conditions: (1) primary or familial HLH, an autosomal recessive disorder, and (2) secondary HLH which includes an infection-associated hemophagocytic syndrome and a malignancy-associated hemophagocytic syndrome. Since HCT procedures have been performed only for autosomally recessive primary HLH, we will discuss this entity here.

Primary HLH has been reported from all continents and all ethnic groups. In a retrospective study the incidence of the disease in Swedish children was estimated to be 0.12 per 100,000 children per year [17]. Since primary HLH is an autosomal recessive disorder, an increased incidence has been reported in families with consanguinity [18]. Most patients develop the disease in early life with about 70% of children presenting in the first year of life. However, the age of presentation may be up to 8 years. The age of onset is usually similar among the multiple affected siblings. However, on occasion, a difference of more than 1 to 3 years in age has been noted in affected siblings.

The diagnosis of HLH may be difficult. Diagnostic guidelines have been developed based on clinical, laboratory and histopathologic criteria by the Histiocyte Society in protocol 2004 [12,19]. In the absence of family history or genetic diagnosis, five or more of the eight diagnostic criteria need to be met for the diagnosis of HLH. The guidelines encompass clinical, laboratory and histopathologic findings in the disease (Table 75.2).

The clinical manifestations may vary widely. Although serious central nervous system (CNS) involvement may occur in the disease, the most common early findings include fever, hepatomegaly, and splenomegaly.

The laboratory findings include cytopenias, hypertriglyceridemia, elevated ferritin, and hypofibrinogenemia. Markedly reduced or absent natural killer (NK) cell and T-cell activity have been found to be associated with primary HLH [20]. The association between NK activity and HLH is of great interest due to its potential diagnostic value. It has been found that almost all children with verified familial disease have extremely low or absent NK-cell activity and this value only normalizes after successful HCT. By contrast, those patients with secondary HLH who have low NK-cell activity at presentation normalize their NK-cell activity as the primary condition resolves. The differentiation of familial HLH from secondary HLH may be difficult as about half of the patients present with infections and the family history at the time of presentation may or may not be helpful. In this situation, testing of the NK-cell activity and its normalization pattern may be helpful in distinguishing the primary and the secondary disease. This observation makes it mandatory to test for the NK-cell activity prior to the initiation of HCT.

Table 75.2 Revised diagnostic guidelines for HLH

The diagnosis of HLH can be established if either (A) or (B) below is fulfilled.

A. A molecular diagnosis consistent with HLH: pathologic mutations of PRF1, UNC13d, Munc18-2, Rab27a, STX11, SH2DIA, or BIRC4

OR

B. Diagnostic criteria for HLH fulfilled (five out of the eight criteria below)
Initial diagnostic criteria (to be evaluated in all patients with HLH)
1. Fever
2. Splenomegaly
3. Cytopenias (affecting >2 of 3 lineages in the peripheral blood:
   Hemoglobin <9 g/dL (in infants <4 weeks: hemoglobin <100 g/L)
   Platelets <100 × 10^9/L
   Neutrophils <1.0 × 10^9/L
4. Hypertriglyceridemia and or hypofibrinogenemia:
   Fasting triglycerides >3.0 mmol/L (i.e., >265 mg/dL) and/or
   Fibrinogen <150 mg/dL
5. Hemophagocytosis in bone marrow or spleen or lymph nodes
   No evidence of malignancy
6. Low or absent NK-cell activity
7. Ferritin >500 μg/L
8. Elevated soluble CD25 (i.e., alpha chain of soluble IL-2 receptor) >2400 U/mL

The histopathologic criteria of diagnosis include infiltration of the bone marrow, lymph nodes, liver and spleen with non-malignant lymphocytes and histiocytes, in addition to the evidence for hemophagocytosis in these tissues [21].

HCT procedures have been performed only for autosomally recessive primary HLH. If not treated, the primary HLH is rapidly fatal with a median survival of about 2 months [22]. Several treatment strategies with cytotoxic agents have been tried in the past without much success. The most effective of these moderately effective strategies was the combination of Vinblastine and steroids. The treatment of HLH has also included etoposide (VP-16) in combination with other agents [23]. Approximately 60% of patients have also received CNS treatment with intrathecal methotrexate and cranial irradiation [18].

Immunosuppressive drugs such as cyclosporine (CSP) and antithymocyte globulin (ATG) have been shown to be effective in HLH [24] for short-term control of disease. Historically, the probability of survival at 5 years had been found to be 10% with all patients ultimately succumbing to their disease. In 1994, the Histiocyte Society developed a treatment protocol (HLH-94) designed for treatment of primary HLH [25]. The protocol includes initial treatment with CSP, VP-16, and steroids with consideration for HCT when patients attain remission.

Fischer et al. achieved the first major breakthrough in the treatment of this fatal disease. An allogeneic HCT procedure was performed successfully, which had cured the disease in the patient [26]. Since then, several reports have been published describing the efficacy of HCT using related or unrelated donors. Most of the reports have originated from single HCT centers or from transplant registries [27–30]. The current consensus indicates that HCT should be performed after an initial 8 weeks of treatment with chemotherapy if a suitable donor is available. Patients without a suitable donor should continue to receive standard dose chemotherapy. It is preferred that the patient should be in a stable or quiescent disease state before an HCT procedure is contemplated.

In a follow-up study to the first publication of results with HLH-94, it is clear that the HCT procedure for primary HLH offers an excellent chance of cure. On the HLH-94 protocol, the estimated overall 3-year probability of survival after HCT for patients recruited during the period of 1995–2000 was 64%. The specific survival between different types of grafts used, however, varied. The 3-year survival was 71% with matched related donors, 70% with matched unrelated donors, 50% with family haplo-identical donors and 54% with mismatched unrelated donors [31] . However, the results indicate that the long-term disease-free outcome was comparable for unrelated donor transplants and matched sibling donors (approximately 70% at 3 years). In contrast, the use of T-cell-depleted grafts from haplo-identical donors has met with less success (50% survival at 3 years). A study by Chardin et al. has published the largest cohort of 48 patients with HLH undergoing HCT [32]. The major determinant for the failure and death in the transplanted patients was found to be active disease at the time of HCT. In fact the combination of active disease with T-cell-depleted haplo-identical graft resulted in the worst outcome (Figure 75.2). It may be possible that the active disease hampered donor cell engraftment because of a possible inhibitory effect of cytokines on hematopoiesis. As suggested by this and other reports, it is of great importance to perform HCT as soon as complete remission has been obtained for the disease [16,32].

HLA-matched sibling donors are the preferred choice for HCT in this disorder. However, one caveat that should be kept in mind while selecting a sibling donor is that an apparently healthy sibling donor may develop HLH later. If this occurs, then it is possible that the disease will be transferred from the donor to the recipient. As mentioned earlier, presence of extremely low or no NK-cell activity could be used as a guide to select the sibling donor for HCT in primary HLH. With the mutation analysis available as a diagnostic tool it is essential that the prospective sibling donor be checked for specific mutations. In the event that a suitable sibling donor is not available, alternative donors should be sought. Alternative donor options include: unrelated donors of marrow or human umbilical cord blood and closely matched relatives.

The preparative regimens in most of the published cases have consisted of chemotherapy, containing myeloablative doses of chemotherapeutic drugs. Most of the centers have used high-dose BU, CY, and VP-16 as the conditioning regimen [30]. In some patients, additional immunosuppression has been provided by ATG or the anti LFA-1 monoclonal antibody. These regimens have been well tolerated by children with HLH. Total body irradiation has not been favored as histiocytes may not be sensitive to this measure.

During the past decade RIC regimens have become more available and results have been very promising [33,34]. The idea behind these regimens comes from the fact that mixed chimerisms are able to sustain patients with HLH in remission. Most of these regimens have included alemtuzamab and are demonstrating better post-transplant outcomes. A single-center experience has been published using direct comparison of myeloablative to the reduced-intensity regimen demonstrating the superiority of reduced-intensity protocols [35].

Following HCT, patients usually achieve full donor chimerism. However, repeated analyses should be performed at regular intervals. In some cases, a mixed chimeric state can persist with low quantities of donor cells present in the graft. It is known that mixed chimerism >10–20% is associated with stable complete remission for up to 20 years [16,32]. Secondary graft rejections have been seen below the threshold level of 10% donor cells in the chimera. In patients with declining levels of donor cells, follow-up studies are important as a progressive decrease in donor cells may indicate early relapse and the need for further intervention.

Overall, the major causes of death following HCT for HLH are transplant-related complications including graft failure, infections, and graft-versus-host disease (GVHD). In a few cases, progression of the CNS disease has occurred within weeks after HCT. Active or

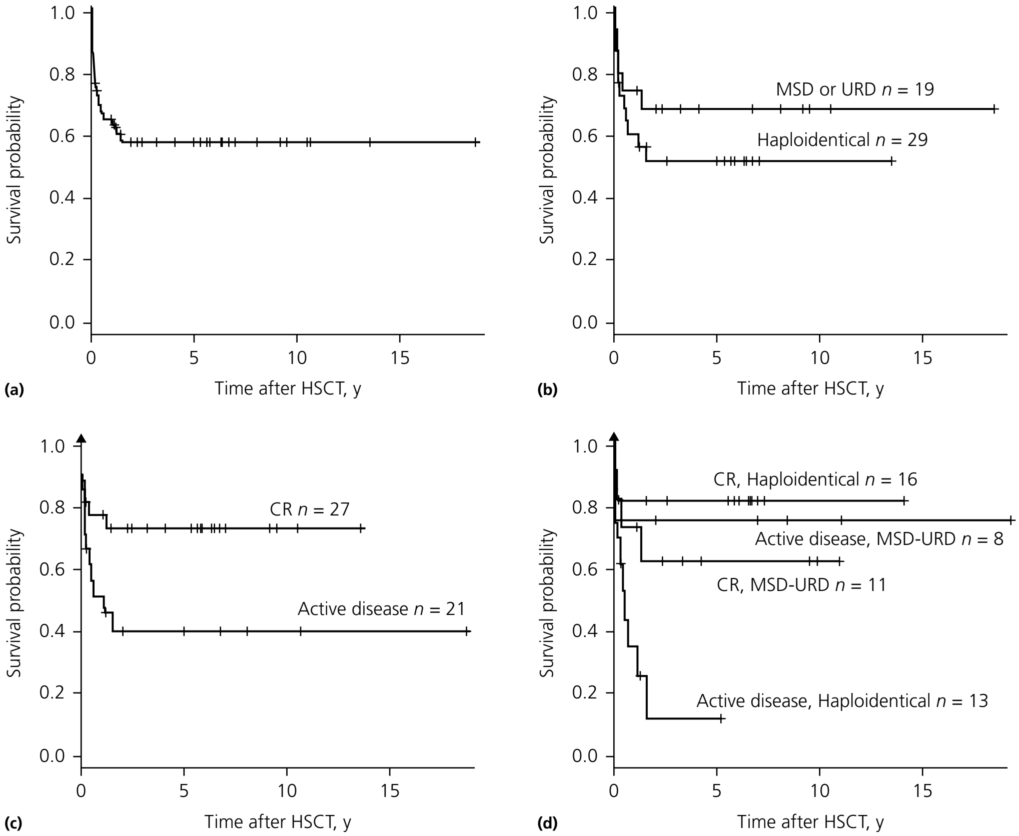

Figure 75.2 Survival of patients with HLH after HSCT. (a) Overall survival (n = 48) calculated from the time of the first attempts at HSCT. (b) Survival according to donor origin. Because of low numbers and similar data, patients who received a transplant with an MSD and a URD have been grouped together (p = 0.3 log-rank test). (c) Survival related to status of HLH at HSCT (CR vs. AD; p = 0.053 log-rank test). (d) Survival according to a combination of donor compatibility and disease activity (for patients with AD, MSD-URD vs. haplo-identical HSCT, p = 0.03 log-rank test; for patients in CR, p = 0.39 log-rank test). AD, advanced disease; HSCT, hematopoietic stem cell transplant; MSD, matched sibling donor; URD, unrelated donor. Source: Ouachee-Chardin et al. 2006 [32]. Reproduced with permission of American Academy of Pediatrics.

poorly controlled CNS disease before HCT initiation has led to deterioration after transplantation. The relapse rates for the underlying disease have been reported in the range of 0–55% [28–30]. Actuarial 5-year disease-free survival (DFS) in HLH patients who underwent HCT has been compared with those HLH patients who did not receive the HCT procedure. This DFS was reported to be 66% in HLH patients who were transplanted versus only 10% in those patients who did not receive HCT (Figure 75.3).

Most long-term survivors of HCT for HLH experience a good quality of life, and are able to discontinue all disease-related medications. Stabilization and improvement of neurologic symptoms is commonly seen. Volume loss of the cerebrum and demyelination often seen during the height of HLH disease activity is reversible within 1–3 years of successful engraftment. Long-term follow-up of survivors of BMT for HLH indicates that most children return to a normal quality of life. However, children who have severe neurologic problems in the beginning due to HLH may have persistent cognitive problems and learning disabilities. For this reason, neuropsychiatric evaluation is recommended before the transplant and on periodic basis at regular intervals post-HCT.

HLH is rare in adults and mostly presents as secondary HLH. It is difficult to diagnose secondary HLH on the basis of clinical symptoms alone as the presenting symptoms are usually of the primary disease. Therefore, it seems that secondary HLH is a heterogeneous disease. Since hemophagocytosis is the hallmark of this disease a retrospective analysis was performed in adults with features of secondary HLH who had demonstrable hemophagocytosis in the bone marrow [36]. Most patients were older than age 40 and had associated EBV infection. Genetic mutations were not known in these patients. Patients were treated as per HLH protocols. Mortality was high and patients did not greatly benefit from the HCT in this study. We feel that secondary HLH in adults seems to be a heterogeneous disease with poor understanding of the genetic basis and therefore the appropriate treatment options have not been possible so far.

In summary, there have been major strides in the understanding of HLH at the molecular level. HLH protocol 2004 includes changes reflective of increased understanding in the diagnosis and treatment of HLH .The use of HCT in this disease has provided the best results and remains the treatment of choice as chemotherapy alone is not curative. Adequate therapy to achieve remission before HCT should be attempted since the transplant outcome appears to be improved when patients are in remission.

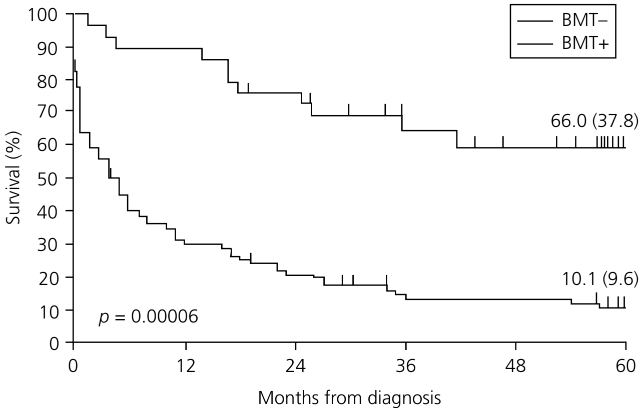

Figure 75.3 Kaplan–Meier survival estimates of 122 children with HLH treated with or without BMT. Source: Aricó et al. 1996 [18]. Reproduced with permission of Nature Publishing Group.

Granulocyte disorders

The granulocyte disorders are discussed here as the *quantitative* disorders where the number of granulocytes are low or as *qualitative* disorders where the function of granulocytes is altered. Both groups of disorders are very heterogeneous and vary widely in their clinical severity.

Quantitative granulocyte disorders

Neutropenia is defined as a condition where circulating neutrophils are less than 500/μl. Severe neutropenia means an absolute neutrophil count of less than 200/μl. Severe neutropenia is often associated with life-threatening infections, especially if it is prolonged or is associated with immune deficiency. Neutropenia is common to a heterogeneous group of disorders resulting from decreased production or release of maturing and differentiating myeloid precursor cells from the bone marrow. Neutropenia may be primary congenital or secondary acquired. This part of the chapter will cover the intrinsic maturation and differentiation defects of the early myeloid precursors.

Severe congenital neutropenia (Kostmann syndrome)

Severe congenital neutropenia (SCN), also known as Kostmann syndrome (KS), is an inherited disorder with an undefined inheritance pattern. It occurs with an estimated frequency of about 1–2 cases per million with equal gender distribution. A persistent absolute neutrophil count (ANC) of 0.2×10^9/L or less is required for making a diagnosis. Recurrent bacterial infections usually occur in the first year of life. Typically, these patients experience in early infancy recurrent pneumonia, otitis media, gingivitis, and perineal or urinary tract infections. Until recently, 50% of these patients died as a result of infections before 1 year of age, and only 30% had survived beyond 5 years of age [37].

The biology of SCN is not completely understood. At the cellular level, a maturation arrest of myelopoiesis at the promyelocyte or myelocyte stage occurs resulting in neutropenia [38]. Contrary to the earlier suggestions of defective G-CSF production or defective response of the cells to G-CSF, the current data indicate normal or even increased endogenous G-CSF biological profile and activity in cells of patients with SCN. In vitro measurements of colony-forming units – granulocyte macrophage (CFU-GM) also appear to be normal.

G-CSF receptor mutations at birth have not been noted in the SCN patients. These mutations have, however, appeared in those SCN patients who had developed leukemia or MDS later in their lives [39]. This observation suggests that these G-CSF receptor mutations are acquired and may not have a causal relationship with the genesis of this disease. The identification of mutations within the *ELA2* (Elastase-2) gene as a cause of cyclic neutropenia has led to similar studies in patients with SCN. Heterozygous *ELA2* mutations have been found in 60% of the patients with SCN [40]. Analysis of SCN families with autosomal dominant inheritance of elastase mutation and a case with mosaic expression of mutant *ELA2* has provided the strongest evidence to implicate *ELA2* mutations in the etiology of SCN. In addition to *ELA2* mutations, a number of genetic aberrations have been found in patients with disorders associated with KS. Genes that have been found to be mutated in a minor subgroup of patients are *GF1*, *CXCR4*, *SBDS*, *WASP*, Glucose-6-phosphatase translocase, Taz-1, *AP3B1*, and some others [41].

It is now well established that patients with SCN are at risk for development of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). The acquisition of G-CSF mutations in SCN patients can be used advantageously to predict the risk of subsequent development of leukemia/MDS in these patients. In a recent report from the SCN International Registry (SCINR), 375 patients with SCN were followed for the development of MDS or AML. The potential risk for the development of MDS/AML increased with the duration of SCN. The cumulative incidence of MDS/AML was 9% after 6 years of therapy. The incidence increased to 23% after 10 years and 33% after 12 years [42–44]. These ominous findings have been linked to point mutations in the G-CSF receptor gene [45,46]. Cytogenetic abnormalities such as monosomy 7 have also been noted in these patients. In light of these findings, SCN can be considered a preleukemic condition.

Based on clinical response to G-CSF treatment, SCN patients have been categorized into G-CSF responders and G-CSF non-responders. This conclusion was initially deduced from data on 304 patients enrolled on SCINR in 1994 [47]. In this study, 95% of the patients had responded to G-CSF treatment. The G-CSF responders have been treated with G-CSF therapy in doses of 3–10 μg per kilogram per day. This response was biologically explained on the basis of normal or increased expression of G-CSF receptors on the cells. Since the myeloid precursor progenitors in SCN patients retain the capacity to differentiate to normal myeloid cells in response to pharmacologic doses of G-CSF [47], a continuous treatment strategy with recombinant G-CSF has been adopted for most of the SCN patients. The continuous treatment with G-CSF results in an increase in the number of mature myeloid cells in the circulation and a concomitant decrease in the incidence of serious infections.

Those patients who do not respond to G-CSF doses of 120 μg/kg/day, and still suffer from bacterial infections, are classified as the G-CSF non-responders or patients refractory to this treatment. This population constitutes about 5% of the patients in the SCINR study group mentioned earlier. In the non-responders, the G-CSF gene defect has not been elucidated. The G-CSF non-responders and those patients who develop cytogenetic and G-CSF receptor mutations during the course of the disease are considered candidates for HCT. All SCN patients who develop MDS/AML are also considered candidates for HCT. The monitoring for cytogenetic and G-CSF receptor mutations is, therefore, emphasized for two reasons: first, for timely intervention with HCT, and secondly, for prediction of risk for developing MDS/AML in these patients with subsequent HCT procedure.

A limited number of patients with SCN, including those documented in the IBMTR registry, have undergone HCT procedures using HLA-identical sibling donors. One patient received CY as a single preparative agent, and attained only partial lymphoid engraftment for 6 months [48]. Another patient received an HLA-identical sibling donor transplant at 20 months of age. This patient is reported to be alive and disease-free 20 years after the HCT procedure [49].

In 2001, a successful fully matched unrelated donor transplant procedure was performed in a G-CSF non-responder [50]. The preparatory regimen contained TBI, VP-16, and CY. Anti-thymocyte globulin was administered to prevent graft rejection. The patient engrafted promptly and the pre-existing pulmonary abscesses resolved. In 2004, Goyal et al. reported successful engraftment following unrelated donor transplantation in an alloimmunized patient with SCN [51]. At our own institution, we have successfully transplanted a G-CSF non-responder with unrelated donor graft using a high dose conditioning regimen (unpublished data). This patient is 5 years post-HCT with full donor chimerism. The conditioning for matched unrelated donor transplants has been fully myeloablative with either busulfan (BU), cyclophosphamide (CY) and ATG, or total body irradiation, BU and ATG. With advances in supportive care, fully matched unrelated donor transplants have an excellent chance of success in patients with SCN. Reduced-intensity conditioning regimens are currently being developed for consideration in patients with high risk of toxicity [52].

To summarize, HCT remains the only curative therapy for SCN. The indications for HCT are in high-risk patients who fail to respond to G-CSF in doses more than 8 μg/kg/day, development of MDS or AML, and patients who have the Gly 185 Arg mutation in the *ELANE* gene [53]. As suggested by Choi and Levine, patients who do not meet these criteria should also be considered for transplant if an available matched sibling is identified, given their continued risk of death from sepsis and leukemia [54]. Alternative donor transplants are becoming more common in non-malignant conditions as improvements in donor selection, GVHD prevention and supportive care make HCT a safer option. Pre-transplant chemotherapy in SCN patients with MDS/AML has an important bearing on the outcome of HCT in these patients. In a report by Choi et al., a cohort of six patients has been reported who developed MDS/AML while on therapy with G-CSF. All six patients received HCT. Four of these six patients who died because of transplant-related complications had frank AML, and had received pre-transplant chemotherapy. Two patients who survived had early MDS and had not received any chemotherapy before the transplant. These findings emphasize the importance of frequent monitoring of all SCN patients for prompt recognition of the development of clonal chromosomal abnormalities and MDS for early intervention with HCT in these patients [43].

Reticular dysgenesis

Reticular dysgenesis (RD) is a rare congenital disorder characterized by severe combined immunodeficiency and agranulocytosis manifesting with life-threatening fulminant infections. The term reticular dysgenesis was introduced in 1959 when two male twins were reported with this syndrome who subsequently died of the disease [55]. An autopsy was performed on these patients and the findings showed depletion of lymphocytes and Hassall's corpuscles in the thymus, absent lymph nodes, tonsils or Peyer's patches, and lack of myeloid activity in the bone marrow [55]. At the molecular level, the primary defect in RD has not yet been identified. At the cellular level, a maturation arrest in the myeloid lineage beyond the promyelocyte stage has been observed along with global impairment of lymphoid maturation. However, erythropoiesis and megakaryopoiesis are found to be normal in RD indicating the existence of an intact pluripotent hematopoietic stem cell pool in these patients. These observations are utilized to make a diagnosis of RD using established clinical and laboratory data namely agranulocytosis, lymphocytopenia, bone marrow finding of early myeloid maturation arrest, failure of in vitro assays to produce mature granulocytes from precursors, lack of lymphocyte functions, and thymic aplasia. In contrast to other variants of severe combined immune deficiency with neutropenia, in RD no sustained responses to myeloid growth factors have been observed. So far, the only curative treatment described for RD is allogeneic HCT.

Untreated patients with RD die from infections within the first days or weeks of life. Prior to 1983, nine cases with this disorder were reported. Their survival ranged from 3 days to 17 weeks [56–60]. In 1983, a cure was reported for reticular dysgenesis following an HCT procedure from an HLA-identical sibling [60].

A preparative regimen of BU and CY was used and the patient achieved full hematopoietic and lymphoid reconstitution. Several other reports have since been published on successful HCT in RD patients using HLA-identical, T-cell-depleted, HLA-haplo-identical, and umbilical cord blood unrelated donor grafts [27,60–67]. In one report using unrelated umbilical cord grafts the patient failed to engraft initially but fully engrafted after a second unrelated cord blood transplantation procedure [66].

A challenging feature of HCT for reticular dysgenesis is not only the choice of the donor but also the intensity of the conditioning treatment to obtain both lymphoid and myeloid reconstitution. This observation has been repeatedly substantiated from data on HLA-haplo-identical transplants. A T-cell-depleted haplo-identical graft had been successfully transplanted following administration of BU, CY and ATG [58]. In one report, using haplo-identical donors, two patients could be re-transplanted successfully only when an intensive high dose conditioning regimen consisting of 700 cGy of TBI was utilized prior to the HCT procedure [64]. In another report, 10 patients receiving HLA-haplo-identical grafts were studied where half of them were given intensive BU/CY conditioning while the other half received either low-dose BU with CY or ATG with CY without BU [67]. Three of the five patients who had received intensive high dose conditioning survived with lymphoid and myeloid reconstitution. The group that did not receive the intensive preparatory regimen or was treated with regimen without BU failed to demonstrate stable engraftment of donor hematopoietic stem cells. This finding, again, indicates the paramount importance of using intensive conditioning prior to the HCT procedure in RD patients.

Cyclic neutropenia

Cyclic neutropenia is usually a familial disorder in which patients have regular cyclic fluctuations in the number of blood neutrophils. The genetic transmission may be autosomal dominant with variable expression [68]. The gene responsible for cyclic neutropenia has been identified as *ELA2* [69]. Clinical signs and symptoms include fever, malaise, periodontitis, mucosal ulcers, sore throat, and lymphadenopathy. Most patients live normal life spans with gradual amelioration of symptoms. Ten percent of patients experience frequent serious infections. The laboratory investigation reveals periods of severe neutropenia (<200 neutrophils/μL) lasting for 3–10 days alternating with periods of normal neutrophil counts. The cyclic fluctuation occurs usually every 21 days with a range of 14–36 days. For each individual patient the cycle length is constant. Bone marrow precursors also reveal concomitant cyclic oscillations. Bone marrow myeloid precursor cells are present in highest numbers at the time of nadir of peripheral neutropenia. Therapy with G-CSF improves the neutrophil counts and reduces the frequency of symptoms.

Clinical manifestations of cyclic neutropenia do not justify the use of HCT. However, HCT has contributed towards a better understanding of this disorder. In an autosomal recessive collie dog model cyclic neutropenia can be corrected by HCT [70]. To date, no human transplants have been reported for cyclic neutropenia. In one patient who was transplanted with an allogeneic donor graft with cyclic neutropenia, the disease was found to be transferred to the recipient during an HCT procedure [71].

Cartilage-hair hypoplasia

Cartilage-hair hypoplasia, an autosomal recessive disorder, is encountered primarily in the Amish and Finnish populations. The abnormal gene for cartilage-hair hypoplasia is located on the short arm of chromosome 9 [72]. Characteristic findings include short-limbed dwarfism, fine hair, moderate to severe neutropenia (ANC 100–200/μL) and defects of cell-mediated immunity [73,74]. The combination of neutropenia and cellular immunity defects lead to recurrent bacterial and viral infections especially reactivation of the varicella zoster infection in these patients. Allogeneic HCT has been used to correct the hematologic and immunologic defects of cartilage-hair hypoplasia [75,76]. Expectedly, the phenotypic hair and bony abnormalities have not been corrected by HCT. Six patients with cartilage-hair hypoplasia have been reported to have undergone HCT. One of the six patients received the preparative regimen consisting of CY, cytosine arabinoside, and thioguanine. This patient achieved complete lymphoid engraftment of donor origin and demonstrated otherwise mixed chimeric hematopoiesis. The remaining five patients received BU and CY and achieved full donor chimerism for lymphoid as well as all other hematologic parameters. The experience with allogeneic HCT is still limited; however, severely affected patients seem to benefit from the procedure.

Dyskeratosis congenita

Dyskeratosis congenita (DC), a rare congenital syndrome, was originally described in 1906. DC is genetically heterogeneous, and all three patterns of inheritance have been recognized [77]. However, the majority of the patients are males and the defective gene has been linked to chromosome Xq28. This genetic defect results in a defective nucleolar protein called dyskarin which is associated with human telomerase RNA (hTR) [77]. In dyskeratosis congenita, like Fanconi anemia, associated DNA repair defects have been noted. Clinically, DC is a multisystem disorder marked by nail dystrophy, oral leukoplakia, increased skin pigmentation, and variable degree of bone marrow aplasia. Bone marrow failure is the principal cause of death followed by predisposition to malignancy and pulmonary failure. Immunologic manifestations include defective cell-mediated immunity [78,79].

Allogeneic bone marrow transplantation has been used successfully to correct the hematologic abnormalities [80]. It is noted that patients with this syndrome require less intensive conditioning [80]. Twenty-two patients have been reported in the literature to have undergone HCT with high dose conditioning. Of the 22 patients, only four patients received grafts from unrelated donors. Ten of the patients survived longer than 3 years post-transplant. In addition to developing short-term complications like organ damage and sepsis, long-term complications carried a high mortality rate [80]. Veno-occlusive disease, pulmonary fibrosis, and renal microangiopathy developed months after HCT. In a published report of five patients with DC-associated severe aplastic anemia who had received HCT using HLA-identical related donor grafts, four patients died 2–8 years post-HCT with renal microangiopathy, veno-occlusive disease of the liver, Evans syndrome, or invasive aspergillosis [81]. One living patient at the time of publication had developed anemia, polyarthritis, pulmonary fibrosis, and gastrointestinal malabsorption syndrome 7.5 years after the HCT procedure. In view of the occurrence of the late HCT-associated complications, it is important that a complete baseline evaluation of kidneys, liver, and respiratory tract be performed prior to the initiation of the HCT procedure in these patients.

Since patients with DC have shown poor tolerance to high dose conditioning regimens, reports using RIC approaches have emerged [82,83]. In one report using RIC, the patient received 200 cGY of TBI and 90 mg/m^2 of fludarabine in a matched sibling donor setting.

GVHD prophylaxis was with mycophenolate mofetil and cyclosporine. This patient achieved 100% donor chimerism with excellent clinical outcome 2 years post-transplant [82]. In another report, two children with DC underwent unrelated donor transplant with RIC with fludarabine, CY and ATG. The prophylaxis for GVHD was provided with steroids and cyclosporine. Both of the patients did very well with full donor chimerism [83].

There have been several reports in the past 3 years that encouraging short-term survival can be achieved with HCT in patients with DC using reduced-intensity regimens [84–86]. Whether long-term toxicity is also reduced with low-intensity conditioning regimens remains to be seen in longer follow-up. However, the use of certain conditioning agents such as busulphan and irradiation should be avoided because of the increased predisposition of the DC patients to chromosomal instability, tumorigenecity, and pulmonary and vascular complications.

Umbilical cord blood has also been used as a donor choice in patients with DC with good engraftment [87].

Shwachman-Bodian-Diamond syndrome

Shwachman-Bodian-Diamond syndrome (SBDS) is an autosomal recessive disorder characterized by exocrine pancreatic insufficiency, metaphyseal dysostosis, and bone marrow hypoplasia [37]. Cyclic or persistent neutropenia is a common finding. However, in 10–25% of patients trilineage pancytopenia may also be seen. Recurrent bacterial infections occur early in childhood and can contribute to early mortality. Progression to myelodysplasia and transformation to acute leukemia are seen in patients who have pancytopenia as the predominant finding. Overall survival without any treatment varies with different subpopulations. In patients without hypoplasia and leukemic transformation, the projected 50% survival is 35 years. However, this expectation is reduced to 24 years in patients with pancytopenia and further decreased to 10 years in those with leukemic transformation [37].

Approximately 90% of patients meeting clinical criteria for the diagnosis for SBDS carry mutations in the *SBDS* gene. *SBDS* maps to the 7q11 centromeric region of chromosome 7 [88]. *SBDS* is a highly conserved gene of unknown function. In humans the SBDS protein is localized to both the cytoplasm and nucleus, and shuttles in and out of the nucleolus in a cell cycle-dependent manner.

Conceivably, HCT would be the only curative treatment option for the bone marrow dysfunction in SBDS [89]. However, mixed results have been observed with allogeneic HCT as a therapeutic approach for SBDS patients with hematologic abnormalities. Fifty-eight patients have undergone HCT for bone marrow failure and or leukemic transformation with SBDS. Most of the published data comes from case reports and small series of cases [90–92]. Patients have received grafts from matched sibling donors, matched unrelated donors, and human umbilical cord blood. The conditioning regimens were either with BU/CY/ATG or TBI/CY. Forty percent of the transplant patients died due to HCT-related complications of sepsis, regimen-related toxicity, and GVHD. Risk of graft failure did not appear to be significantly elevated. As expected, the patients with marrow aplasia had a relatively better survival than the patients with MDS or leukemic transformation. A single report exists for the use of RIC in a patient with SBDS/AML who received an unrelated donor transplant. Full engraftment is reported in this surviving patient [90]. At the moment follow-up of these patients is relatively short. Long-term studies will be needed to look at the real impact of HCT in SBDS patients.

Qualitative neutrophil disorders

The major role of neutrophils is to defend the host against a variety of infectious agents. To accomplish this task the neutrophils must first sense infection, migrate to the site of infection (chemotaxis), adhere to the membranes of the blood vessel wall and then destroy the offender (phagocytosis) through the release of granules that take part in the activation of oxidative dependent and oxygen-independent pathways of microbial killing. The presences of these different components of the microbial killing cascade are essential for the appropriate function of neutrophils. In the qualitative disorders discussed later, neutrophilia is usually present in response to infection. However, one or more of the functional components of the neutrophil attack may be faulty.

Neutrophil actin abnormality

This is a rare inherited autosomal recessive disorder in which defective granulocyte motility leads to inadequate chemotaxis resulting in serious recurrent infections. Granulocyte counts are, however, either normal or even elevated. This disorder was initially described in an infant in 1974, who had failed to form abscesses during recurrent life-threatening infections with gram positive and gram negative organisms [93]. A Rebuck skin window showed abnormal cellular migration. Although actin levels are normal in the affected neutrophils, the polymerization of actin is defective leading to poor or absent migration of neutrophils to the chemotactic stimuli. After the initial report, more children have been reported with neutrophil actin deficiency or its variants [94].

Allogeneic HCT has been attempted in a few patients with this disorder [95]. According to the published reports, one of three patients survived and was cured. The successfully treated patient received a high-dose regimen with BU, CY and ATG. The other two patients engrafted but died due to treatment-related complications, mainly infections. It is possible to correct the defect in neutrophil actin abnormality by HCT using a heterozygous histocompatible sibling donor. Since the disease course is very aggressive, supportive care alone is usually not sufficient to allow prolonged survival of these patients.

Leukocyte adhesion deficiency type I

First described in 1974, the leukocyte adhesion deficiency (LAD-1) is a rare heterogeneous autosomal recessive disorder in which neutrophil adhesion, chemotaxis and ingestion of the C3bi-opsonized microbes is impaired [96,97]. The neutrophils fail to migrate in the Rebuck skin window and also exhibit defects in neutrophil functions of adhesion and phagocytosis. It is usually detected in early childhood as a result of frequent, life-threatening bacterial and fungal infections, delayed umbilical cord separation and neutrophilia. The infections are mostly localized in the skin, subcutaneous tissues, middle ear, and oropharynx.

At the molecular level, the basic defect lies in the β_2 integrin adhesion molecule (CD11/CD18). The CD11/CD18 glycoprotein family consists of three heterodimeric proteins each composed of α and β chains. Translocation of α and β chains to the cell surface requires assembly of the $\alpha\beta$ heterodimer. The mutations in the CD11/18 deficiency have been found to be in the β_2 chain which prevent the assembly of the $\alpha\beta$ heterodimer and thereby translocation of the heterodimer to the cell surface [98]. The cells with the mutated glycoprotein are unable to adhere or migrate in response to chemotactic stimulation. Since the β_2 chain is constant for each family and is present on the surface of the leucocytes, flow cytometric analysis with pertinent monoclonal antibodies can help identify those affected by the disorder. The severe clinical phenotype with less than 0.3% of the normal amount of β_2 integrins is more commonly seen as compared to the moderate variant in which β_2 integrin levels of 2.5–11% of the normal are observed.

Since most of the children with leukocyte adhesion deficiency experience severe infections and die early in the course of their disease, early diagnosis and treatment, possibly with HCT, should be attempted. Several reports describe the course of HCT in patients with LAD-I. So far, 14 patients have been reported to have received HCT. Of these 14, five patients underwent HLA-matched sibling donor HCT, and nine patients received grafts from two antigen-mismatched or haplo-identical related donors [99,100]. In this study of 14 patients, the majority achieved either full or mixed chimerism. In most of the patients with mixed chimerism, a variable number of donor cells were noted with no increased incidence of infections except in one patient with 2–15% donor cells who developed mild gingivitis. Also, two patients with immunologic graft rejection achieved full chimerism after treatment with anti CD-2 or LFA-1 monoclonal antibody. There have been reports of successful unrelated HCT utilizing fully matched and one antigen-mismatched donors [101,102]. A recent report from Qasim et al. is the largest series of 36 children with leukocyte adhesion deficiency [103]. Myeloablative conditioning was used in 28 patients and RIC was used in eight patients. Survival rates after matched sibling donor and unrelated donor HCT were similar and mortality was highest with haplo-identical donors. Twenty-seven patients are alive with full donor engraftment in 17 patients and mixed chimerism in 10 patients. The study concluded that RIC was safe and mixed chimerisms were effective in preventing infections.

Beside CD11/18 deficiency, there are other leukocyte adhesion molecules called selectins. Defect in these molecules may result in significant disease. Two patients have been described with the absence of neutrophil receptors for E-selectin resulting in defective adhesion. This disorder is called LAD type II. To date, HCT has not been attempted in patients with selectin deficiency.

Chediak–Higashi syndrome

Chediak–Higashi syndrome (CHS) is an autosomal recessive disorder. Clinically, it is characterized by partial ocular and cutaneous albinism and an increased susceptibility to bacterial infections and compromised platelet function. Partial albinism and photophobia are usually noted in early infancy and increased susceptibility to respiratory and cutaneous infections soon become apparent. Decreased platelet function leads to bleeding tendency. Other organ systems involved include hair, adrenal glands, pituitary gland, and peripheral nerves [104]. The disease is manifested in two forms namely an initial stable phase followed by an accelerated phase. The stable phase disease may or may not be associated with serious infections while the accelerated phase disease may be complicated by fulminant infections and massive hepatosplenomegaly, lymphadenopathy, anemia, neutropenia, and thrombocytopenia [104,105]. The accelerated phase is uniformly fatal and no long-term survivors of the accelerated phase have been reported. In some patients the diagnosis of CHS is not apparent until the accelerated phase has developed.

Although phagocytosis per se occurs at a higher than normal rate in neutrophils, and post-phagocytic metabolic burst is also normal, the basic abnormality lies in the production of abnormal lysosomes and granule formation in cells of the myeloid cell series [106,107]. This abnormality leads to defective microbial killing and clinical manifestations of the CHS. The molecular defect has been reported to be an abnormal calcium uptake pump in CHS lysosomes resulting in an inefficient and incomplete post-phagocytic delivery of lysosomal enzymes into the phagosomes. This defect results in delayed intracellular destruction of bacteria by the Chediak–Higashi leukocytes [107]. In addition, cellular responses to chemotactic stimuli also appear to be defective.

In the laboratory, light and electron microscopy can be used to diagnose CHS by demonstrating abnormal granules and lysosomes. Neutrophils containing giant azurophilic granules along with abnormal lysosomes can be demonstrated. Monocytes and macrophages show abnormal lysosomes as well. Both lymphocytes and NK cells also have giant cytoplasmic granules with decreased NK function. In platelets, the content of dense granules is decreased leading to abnormal platelet function. The bone marrow examination of these patients displays ineffective myelopoiesis.

The first HCT procedure for CHS using a sibling donor was performed in a patient in accelerated phase [108]. CY alone was used as a preparative regimen. The patient, however, could not achieve sustained engraftment, probably due to inadequate preparation. The patient relapsed back into the accelerated phase and a second transplant procedure from the same donor was attempted. This time, however, a preparative regimen containing CY and TBI was used. This strategy resulted in stable engraftment. Another patient who was in the stable phase of the disease was similarly treated with CY and TBI resulting in full donor engraftment and correction of the functional defect [109]. A series of 10 other patients has been published [110]. Two patients were in stable disease and eight patients were in the accelerated phase of CHS. Seven patients received HCT from sibling donors and the other three received one antigen-mismatched or haplo-identical donor grafts. The preparative regimen included BU, CY and etoposide in eight patients. One patient received additional cytosine arabinoside. Three of the seven long-term survivors achieved full donor chimerism while four patients demonstrated mixed chimerism. In one patient with low level of donor chimerism, persistent bacterial infections were seen. As expected, in none of these patients were the cutaneous, ocular, and neurologic manifestations corrected.

A few reports describe patients receiving RIC leading to mixed chimerism with as low as 20% donor cells. These patients have continued [103] to do well without developing accelerated phase of the disease [111,112].

It is clear that HCT can be used successfully to treat patients with CHS in both stable as well as accelerated phase. HCT should be considered in the early stages as soon as the diagnosis is established. Patients with stable phase disease are expected to tolerate the procedure better than those treated in the advanced phase when serious organ damage might have taken place. If matched sibling donors are not available, unrelated donors can be considered. As evident from the earlier discussion, a good number of patients develop mixed chimerism in spite of the aggressive preparatory regimen used. Most of these mixed chimeric patients do not progress to the accelerated phase of CHS and continue to do well.

There are results from long-term follow-up of patients several years post-HCT. Eleven out of 20 patients who underwent HCT for CHS 20 years ago have shown signs of progressive neurological deterioration consistent with the progression of CHS [113] . Similar findings were also suggested by the earlier follow-up reports on patients with CHS [114].

Chronic granulomatous disease

Chronic granulomatous disease (CGD) is a rare clinical syndrome resulting from defective neutrophil function. Both autosomal recessive and X-linked patterns of inheritance have been described.

Although the carriers are usually asymptomatic, females with extreme "lyonization" may be affected. The median age at presentation of the disease is 7 years (range: 0.4 years to 15 years). Clinically, the course of CGD is extremely variable. Some patients present within their first year of life while others may present later. Most patients suffer from recurrent bacterial and fungal infections. Those presenting later in life may have fewer life-threatening infections than those who present early. The most common bacterial infections are from catalase-positive bacteria such as staphylococcus aureus and enteric bacilli. Aspergillus is the most common fungal infection. Infections are usually cutaneous, sinu-pulmonary and lymphadenitis. The occurrence of hepatic abscesses is characteristic in these patients. Serious and persistent infections may lead to formation of granulomas in vital organs.

In CGD, neutrophils are characterized by a defective respiratory burst formation during phagocytosis and an inability to generate superoxide. The impaired superoxide production usually occurs transiently in each phagosome and leads to recurrent bacterial and fungal infections. Those bacteria that have their own mechanism to produce hydrogen peroxide to generate hypochlorous acid (HOCl) via the myeloperoxidase pathway of the phagocytic killing pose little problem. However, microbes such as catalase-positive bacteria can produce very low amounts of hydrogen peroxide in the phagocytic vacuoles. This defect results in insufficient production of HOCl to contribute to significant bacterial killing culminating into recurrent infections with these bacteria [115].

The molecular basis of CGD has now been well defined. The respiratory burst defects have been found to be due to mutations in one of the four subunits of NADPH oxidase complex. Of the four subunits, two are membrane associated while the other two are located in the cytosol [115]. The gene for one of the membrane-associated subunits is located on the X chromosome, while the others are encoded by genes on chromosomes 1, 7, and 16. The disease is classified according to the underlying respiratory burst unit defect. In two large studies of 140 pediatric patients, defects in the X-linked gene for the gp91^{phox} subunit of cytochrome-b accounted for 62% of the cases. The remainder were found to be due to autosomal recessive defects in p47^{phox} (27%), p67^{phox} (5%), and p22^{phox} (6%). The nitrobluetetrazolium (NBT) test is a very important laboratory tool to detect the disease and also to detect the carrier state [116].

With the availability of excellent supportive care the overall survival of patients with CGD has improved with life expectancy extending into the 30-year range. Although prophylaxis with trimethoprim-sulphamethoxazole have improved the outcome in these patients [117–119], resistant fungal infections still pose significant life-threatening problems with resultant early mortality.

Allogeneic HCT may be appropriate in patients with frequent serious infections despite aggressive medical management. The availability of excellent supportive care with improved outcomes and the extreme variability in the disease course makes selection of appropriate patients for HCT very difficult. Pre-transplant evaluation is still of critical importance. The older patients are anticipated to have higher incidence of HCT-related complications including graft failure and/or rejection. Tissue damage due to chronic inflammation should be carefully evaluated. Hepatic abscesses, ileocecal inflammation, and gastrointestinal obstruction can influence the outcome of HCT. The patients should also be evaluated for other associated disease entities such as antibody negative collagen vascular disease, retinitis pigmentosa, Macleod red cell syndrome, and Duchenne muscular dystrophy. A significantly higher mortality in patients with the X-linked recessive form of the disease has been reported and should be considered while selecting the patients [120]. Also, the possibility of extreme "lyonization" should be kept in mind when considering related donors.

Allogenic transplantation has proven to be curative for patients with CGD and the number of reports of successful HCT in patients with CGD has increased during the past two decades [120–127]. Most of these HCT procedures have been performed using HLA-matched sibling or unrelated donors. With such donors, RIC preparative regimens containing CY or CY with anti-thymocyte serum were used initially. This resulted in expected autologous recovery with later recurrence of the disease. In one case, 1–2% cells from the donor could be identified for about 3 years after which time these cells also disappeared. This observation of autologous recovery suggested the necessity for using high dose conditioning. Subsequently, CGD patients were, therefore, treated with fludarabine and reduced doses of BU (8 mg/kg). This strategy has produced better outcomes resulting in mixed chimerism (10–23%) [128]. Horowitz et al. published their results on 10 patients with CGD who underwent matched sibling donor transplants with RIC and a T-cell-depleted graft [129]. The results of this study were mixed as six patients achieved 100% donor chimerism and two patients rejected the graft. Three patients died due to serious infections and GVHD.

To date, approximately 37 patients have been reported to the CIBMTR as having undergone transplant within the US and 55 in non-US centers. The overall survival at 1 year is 87% and at 3 years 83% for this group. Seventy-seven percent of these patients were transplanted at less than age 10 with 37% of the recipients less than 2 years of age. An additional 50 patients outside of the US and Canada are reported in various publications including single case reports and a survey of the European experience [130] with an overall survival rate of 94% and a success rate of 43 out of the 50 achieving stable donor engraftment. The longest reported follow-up to date from the Europeans is 5 years in a patient transplanted using a HLA-matched sibling and a BU/CY-based conditioning regimen. Similar to the US data, the majority of these patients are less than 10 years of age with only seven of the entire cohort being of age 18 or greater at the time of transplant.

The largest single reported group within the US was transplanted at the NIH, where a non-myeloablative regimen was used to treat 14 patients, including a number of adults. Two patients died due to GVHD, three patients had failure to engraft with one death as a result, and three had late graft rejection. Two patients have stable mixed chimerism, one of whom has only 43% lymphoid engraftment and 15% myeloid engraftment more than 6 years beyond his transplant but continues to be infection-free. We have also performed a second transplant in a young male who, despite a conditioning regimen consisting of Campath 1-H, melphalan, and fludarabine and four donor lymphocyte infusions, failed to maintain myeloid engraftment after a transplant using his HLA-matched sister as the donor. Using the same donor, the patient was treated with BU at 10 mg/kg, achieved 100% myeloid engraftment, and is now doing well at 3 years post-transplant [130].

Although the high dose regimens have led to more success in obtaining sustained hematopoietic reconstitution with long-term survival, the attendant increased risk of worsening infections and regimen-related toxicity cannot be ignored especially in older patients. Other complications of HCT in CGD patients include high risk of graft failure and increased risk of GVHD. Since the occurrence of repeated infections over a long period of time in the older patients may result in significant organ damage, it may predispose this patient population to higher risk of HCT-related complications such as GVHD. Therefore, active infections or inflammation should be attended in a timely and appropriate fashion as described later. The increased risk of GVHD in the CGD patients may possibly be due to the occurrence of repeated infections and/or inflammation resulting in sustained elevation of TNF-α levels in these patients [131,132]. Administration of TNFα-antagonists has been proposed to neutralize the circulating TNF-α. A study of 27 CGD patients has been reported evaluating complications (including GVHD) and survival according to different risk factors (infection and/or inflammation) present at the time of HCT [132]. Twenty-five patients received the graft from a compatible sibling donor and the remaining two patients received their HCT from HLA-matched unrelated donors. All patients were treated with high dose conditioning. In this study an overall survival of 23/27 had been reported with 22/23 patients cured of CGD (median follow-up of 2 years). Moreover, a slightly higher incidence of severe GVHD (grade III–IV) was found in those patients who had pre-existing overt infections and/or active inflammatory conditions prior to the HCT (4/11 patients). Of the 16 patients who did not have any overt infection/inflammation, only three patients developed mild grade II GVHD. Also, the high dose conditioning used in the study did not pose a problem in the majority of the patients who did not have pre-existing infections [133]. It is, therefore, important to identify the presence and nature of the infected foci clinically, by laboratory means and by using the appropriate radiological methods such as computed tomography (CT), positron emission tomography (PET), and combined PET/CT to institute appropriate therapy [134]. The active infections/inflammation should be treated adequately using antimicrobials with intracellular action. This aggressive treatment should be carried out before and during the HCT procedure.

In conclusion, patients with CGD can be cured with sibling donor or unrelated donor HCT. However, overall outcome is superior with the sibling donor transplants. Pre-transplant evaluation of patients with severe disease and treatment of active infections and inflammation prior to and during HCT plays an important role in the overall outcome of CGD patients. Optimal conditioning regimen includes complete myeloablation to prevent autologous recovery and immunosuppression to prevent GVHD in order to attain good results.

Since CGD results from single gene defects in proteins expressed in myeloid cells, this disorder can be considered as an excellent candidate for gene replacement therapy targeted to the hematopoietic cells. Gene replacement therapy can be theoretically very useful in patients with as few as 10–20% NBT positive cells. This concept is supported by the fact that the female carriers in the X-linked variant of CGD have as low as 10% NBT positive cells and are asymptomatic. So far, successful gene therapy had been limited to in vitro experiments where reconstitution of the respiratory burst activity has been displayed in Epstein-Barr virus-transformed lymphocytes [135]. The in vivo clinical attempts to mediate gene transfer into long-lived human hematopoietic cells have met with a lack of availability of appropriate viral vectors to carry out gene transfer.

Griscelli syndrome

Griscelli syndrome (GS) is an autosomal recessive disorder that affects both lymphoid and myeloid cells. The basic defect in Griscelli syndrome is characterized by lymphoid and NK-cell dysfunction with clinical manifestations of partial albinism with immunodeficiency [136,137]. The disorder is uniformly fatal and is also characterized by an abnormal regulation of the immune system resulting in an accelerated lymphohistiocytic phase along with macrophage hyperactivation as seen in HLH. Variable granulocyte dysfunction may coexist. Two different gene loci, *RAB27A* and *MYO5A*, have been identified in patients with GS [138]. Unlike *MYO5A*, the GTP-binding protein *RAB27A* is involved in the control of immune regulation and appears to be a key effector of cytotoxic degranulation. Also, all patients with GS who have mutations in the *RAB27A* gene have developed HLH.

A systematic diagnostic approach is very essential in suspected cases of GS. The diagnostic steps should include clinical, laboratory and histopathologic assessment as mentioned in diagnostic guidelines for HLH. The NK-cell activity should also be tested. Finally, mutation analysis of *RAB27A* gene should also be performed to confirm the diagnosis. This systematic approach is very important to identify those patients who have a worse prognosis and need timely intervention with the HCT.

Allogeneic HCT is the only potentially curative treatment of choice in GS. Although the data are limited due to the rarity of the disease, several reports of HCT performed in GS patients using sibling, matched related as well as matched unrelated donors exist [139]. In one report of three patients who received HLA-matched sibling graft two patients died due to transplant-related complications [139]. The third patient achieved full chimerism with normal immune and hematopoietic function and had done very well post-HCT procedure. In another published case of a six-month-old patient with Griscelli syndrome, mobilized peripheral blood hematopoietic cells from her phenotypically HLA-identical mother were used [140]. In this patient, the conditioning included BU, CY and etoposide. The patient received a $CD34^+$ cell dose of 15.4×10^6/kg along with the $CD3^+$ cell dose of 17.6×10^3/kg recipient body weight. Three months after the transplant procedure was performed, a lymphoproliferative syndrome occurred and was successfully treated. Twenty-six months after HCT, the patient has shown mixed chimerism (52% donor cells) and is well. A recent case report has shown successful HCT in a patient with GS using a matched unrelated donor graft [141]. In this patient, high doses of BU were used along with thiotepa and fludarabine as conditioning prior to the HCT. The number of nucleated cells infused was 7.8×10^8/kg of recipient body weight. Engraftment in this patient was prompt and the patient is well 30 months post-HCT with complete donor chimerisms and normal full blood cell counts.

In summary, in addition to a systematic diagnostic approach, an early intervention with HCT in GS patients with *RAB27A* gene mutation is recommended. If a sibling donor is not available an immediate search for an unrelated donor should be performed. If no histocompatible donors are available, strong consideration for an unrelated donor or haplo-identical HCT should be given as the condition is rapidly fatal.

Osteoclast disorders

Osteopetrosis (OP) is a rare metabolic bone disease characterized by a generalized increase in skeletal mass. The defect results in poor functioning osteoclasts leading to impaired bone resorption that prevents formation of bone marrow cavities. It is a heterogeneous group of disorders and the first description of a patient with increased bone density was by Albers-Schonberg in 1904 [142]. Since then a number of disorders with osteoclast dysfunction have been described and further knowledge has been gained by osteopetrotic mice and other animals. The disorder can be inherited in either autosomal recessive or dominant fashion and may vary in severity from a disease that may kill infants to an incidental radiological finding in adults [143].

Aberrant resorption and remodeling of the bone caused by defective osteoclast function can result in many potential clinical consequences:

- Fractures: bones are undertubulated so they break easily. Common fracture sites are long bones.
- CNS and eye problems: bony overgrowth or lack of remodeling results in compression of nerves or blood vessels that pass through bones especially the optic nerve leading to blindness.
- Pancytopenia due to encroachment of the medullary cavity results in extramedullary hematopoiesis.
- Growth: most children will have short stature.
- Hypocalcemia due to disturbance in calcium homeostasis.
- Poor dentition [143].

Overall there seems to be a considerable amount of variability in all forms of OP. Malignant infantile OP is often the disease with autosomal recessive inheritance that presents under the age of 2 years. Children presenting after age 2 generally have a slower progressive disease.

Until year 2000 only one causative gene was known but since than eight other genes have been identified. In 25% of children with autosomal recessive inheritance the exact genetic mutation is still unknown [144].

Table 75.3 shows the classification of OP by genetic inheritance, molecular basis, and possible response to HSCT [144].

The main agents used in the management are calcitrol, prednisone, and interferon. Neither of these agents is proven to be of benefit in the long run. The history of HCT dates back to a series of experiments performed by Donald Walker on mice. These experiments led to the first attempted unconditioned HCT in France in 1977, with some transient benefit but no documented donor engraftment. In 1980, Coccia and colleagues reported successful engraftment of a patient using HLA-identical marrow after cyclophosphamide therapy and modified total body radiation [145]. Unfortunately this patient had a slow rejection of the graft.

Table 75.3 Classification of osteopetrosis (OP)

Causative gene/protein product	OC rich or poor on bone biopsy	Frequency within AR OP (%)	Good response to HSCT
ATP6i (TCIRG1) (proton pump)	Rich	50	Yes
CICN7 (chloride channel)	Rich	15	Yes, but contraindicated in neuronopathic forms; late CNS pathology may occur
OSTMI	Rich	5	Contraindicated as neuronopathic disease not alleviated
TNFSF11 (RANKL)	Poor	3	Contraindicated as not caused by an intrinsic osteoclast defect
TNFRSF11A (RANK)	Poor	3	Yes, but long-term neurologic prognosis uncertain
CA2 (carbonic anhydrase II)	Rich	Not reported	Probably contraindicated; neurologic follow-up of patients reported in Ref. 15 awaited
PLEKHM1	Rich	<1	Not needed, mild form of OP
Kindlin-3	Not reported	<1	Not known
NEMO	Rich	<1	Not known
Unknown	Rich or poor	20–25	Not evaluable

RANK, receptor activator NF-κB; RANKL, receptor activator NF-kB ligand.
Source: Steward 2010 [144]. Reproduced with permission of Elsevier.

The largest reports on HCT for OP come from the European Group for Blood and Marrow Transplantation (EBMT) [146]. The reports are on 69 and 122 patients with some overlap. Five-year survival for sibling donors is considerably higher (73–79%) as compared to the unrelated donor group (13–43%). Most patients received the conditioning with BU and CY. Primary and late rejection rates seem to be higher. Other complications were neuropathic (in patients with neuropathic disease and post-transplant hypercalcemia [147]). In patients with ATP6i mutations the incidence of pulmonary hemorrhage and respiratory failure was high [148]. Many children have a decent quality of life, however, a decline in visual acuity may continue for years to come [149].

Because of the problems with non-engraftment and late rejections, especially in cases with unrelated donors, European groups have tried to increase the conditioning to include Thiotepa and ATG. These patients received the mega dose CD34$^+$ cells from the donor with some encouraging results [144].

It is recommended that there should be a very detailed pre-transplant work-up of the genetic mutations and associated defects including X-ray of extremities, magnetic resonance imaging of head and chest, and a detailed ENT and ophthalmic examination. Patients with autosomal recessive disease should be investigated immediately for TCIRG1 and CACN7 mutations. TCIRG1-mutated patients should be considered for transplantation as soon as possible [144]. The decision for transplantation should be made with the family after careful discussions about long-term neurologic issues that can arise. Disease-free HLA-identical siblings are the donors of choice and in the unrelated donor setting high doses of selected CD34$^+$ cells may be considered. Bone may take about 12 months or more to normalize even after full donor engraftment and hypercalcemia is a risk that needs management in most patients.

Conclusion

Allogeneic HCT remains the procedure of choice for most of the diseases discussed in this chapter. An HLA-matched sibling is the preferred donor. However, if such a sibling is unavailable, a search for a histocompatible matched unrelated or haplo-identical related donor should not be delayed. In specific cases such as X-linked CGD with severely imbalanced "lyonization" or in primary HLH, the risk–benefit ratio should be assessed before choosing a sibling versus an unrelated donor. Early diagnosis and HCT is essential in some of the disorders such as leukocyte adhesion deficiency and Chediak–Higashi syndrome due to the aggressive and fatal nature of these disorders. An efficient pre-transplant evaluation is, therefore, important.

The outcome of HCT in macrophage–phagocytic disorders has improved during the last decade. In addition to high-quality supportive care after HCT and improved GVHD prevention, the factors that affect a successful outcome with HCT include a careful early diagnosis, nature or course of the disease, pre-existing serious infections, other pre-existing comorbidities, and the status of vital organ functioning at transplantation.

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CHAPTER 76

Hematopoietic Cell Transplantation for Fanconi Anemia

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Introduction

Fanconi anemia (FA) is a genetically and phenotypically heterogeneous autosomal and X-linked recessive disorder characterized by congenital malformations, progressive marrow failure, and marked predisposition to malignancy. The prevalence of FA is greater than one in 100,000 births, with carrier frequencies ranging from 1 in 200 generally to greater than 1 in 100 in persons of Ashkenazi Jewish or South African Afrikaans descent, due to founder effects in these populations [1–4]. While the exact basic biochemical defects responsible for the syndrome are not completely elucidated, animal models and longitudinal studies evaluating the phenotypic consequences of specific mutations provide important clues about the function of FA genes and the care of patients with this disorder. In this chapter, the pathogenesis and pathology, diagnosis and treatment of FA will be reviewed with particular emphasis on the expected results of allogeneic hematopoietic cell transplantation (HCT). As of 2015, allogeneic HCT remains the only treatment modality with the proven potential of correcting the hematologic defect common to most patients with FA.

Early history

In l927, Fanconi described a family in which three male children between the ages of 5 and 7 years had pancytopenia and birth defects [5]. Based on his observations in this family and others, Fanconi's chief criteria for the diagnosis of FA were pancytopenia, hyperpigmentation, skeletal malformations, small stature, urogenital abnormalities, and familial occurrence. According to Fanconi, Naegeli suggested in 1931 that the term "Fanconi's anemia" be used to describe such patients [6]. Fanconi's proposed diagnostic criteria for FA were widely accepted well into the 1980s. Consideration of FA in the differential diagnosis of a patient manifesting clinical features of the syndrome depends on the clinician's concept of the FA phenotype. Most cases reported in textbooks and in the literature prior to the present decade present a clinical picture similar to that of the original cases described by Fanconi. Diagnosis of FA was usually made when aplastic anemia (AA) or leukemia developed in individuals with the characteristic physical abnormalities. Literature reviews have thus been biased, more likely excluding those without the "classic" phenotype. Glanz and Fraser [7], however, were among the first to report that the phenotype was quite variable. Compared to probands, affected siblings were more likely to have a milder phenotype, being diagnosed only after the diagnosis of FA in another affected family member and not because of their phenotypic presentation. In addition, patients with "Fanconi-like" marrow failure who completely lacked congenital malformations, previously described as having the Estren–Dameshek syndrome [8], were found in the same sibships with "classical" FA. While there is increasing awareness of the immense heterogeneity with regard to malformations and stature, the correct diagnosis is often delayed until the onset of marrow failure regardless of race or presence of congenital malformations [9,10].

Clinical features

The FA phenotype is extremely variable. FA patients can be developmentally normal or can have such severe abnormalities that there is spontaneous abortion or perinatal death [11]. The majority (60–70%) of FA patients have at least one congenital malformation with the classic being radial ray defects, ranging from a mildly hypoplastic thenar eminence and tapered thumb to complete absence of the thumb and radius. Abnormalities, however, can involve any of the major organ systems (Table 76.1). To maximize the chance of correct diagnosis, the clinician must first recognize the plethora of malformations possible in FA as well as the phenotypic overlap with other syndromes. For example, FA patients frequently present with ***v***ertebral anomalies, ***a***nal atresia, ***c***ardiac abnormalities, ***t***racheo-***e***sophageal fistula, ***r***enal anomalies, and radial ***l***imb and are therefore labeled as VACTERL syndrome. FA patients may also present with a phenotype characteristic of Holt–Oram syndrome, thrombocytopenia absent radius (TAR) syndrome, Baller–Gerold syndrome, Saethre–Chotzen syndrome (TWIST mutation), Dubowitz syndrome, velocardiofacial syndrome, Diamond–Blackfan anemia, dyskeratosis congenita, Seckel syndrome, and Nijmegen breakage syndrome. The clinician must not be misled by preexisting "diagnostic labels." Unless DNA cross-linking hypersensitivity has been ruled out (discussed later), FA is still the possible correct diagnosis. Such mislabeling is very common prior to the onset of marrow failure, resulting in suboptimal care and marrow surveillance as well as inaccurate reproductive counseling.

The International Fanconi Anemia Registry (IFAR) was established at The Rockefeller University in 1982 to collect clinical,

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genetic and hematologic information from a large number of FA patients over time in order to understand the full spectrum of clinical features and natural history of the disease. Based on the clinical information in the IFAR, congenital malformations associated with FA are even more variable than previously recognized [10]. For example, radial ray abnormalities in FA patients can be bilateral or unilateral. Even patients with bilateral abnormalities usually exhibit asymmetry, with limbs having different specific anomalies. Moreover, nearly one third of FA patients do not manifest any major congenital malformation. However, to the trained practitioner most FA patients will have short stature relative to the rest of the family, skin pigmentation abnormalities, hypoplasia of the thenar eminence bilaterally, and microophthalmia. Increased awareness of the complete spectrum of minor malformations observed in these patients should enable an earlier diagnosis even in the absence of marrow failure [11].

Table 76.1 List of congenital malformations in Fanconi anemia (FA)

Congenital malformation	Frequency (%)
Skin pigmentation (café-au-lait spots, hyper- and hypopigmentation)	71
Thumb and radius (thenar hypoplasia, clinodactyly of fifth digit, syndactyly of fingers, hyperextensible thumbs, arachnodactyly, absence or hypoplasia of radius)	59
Skeletal (dysplastic ulna, micrognathia, frontal bossing, spina bifida, Klippel–Feil syndrome, vertebral anomalies, Sprengel deformity)	31
Kidney and urinary tract (ectopic, horseshoe, rotated, hypoplastic or absent, dysplastic, hydronephrosis, hydroureter, reflux)	57
Genital (*males*: micropenis, undescended or atrophic or absent testes, hypospadius, phimosis, azospermia; *females*: bicornate uterus, aplasia or hypoplasia of vaginaand uterus, atresia of vagina, uterus and ovary)	50 (males)
Cardiac (patent ductus arteriosis, ventricular septal defect, pulmonic or aortic stenosis, coarcation of the aorta, double aortic arch, cardiomyopathy, tetralogy of Fallot)	29
Ears [deafness (usually conductive), atresia or dysplasa, canal stenosis]	Unknown
Eyes (microophthalmia, short or almond-shaped palpebral fissures, ptosis, epicanthal folds, hyper- and hypo-telorism)	47
Nose (flattened nasal bridge, nasal pit)	1
Gastrointestinal (esophageal atresia, duodenal atresia, anal atresia)	7
Central nervous system (CNS) (microcephaly, hydrocephalus, Bell's palsy, CNS arterial malformations, abnormal pituitary, absent corpus callosum, hyperreflexia)	1
Growth retardation (short stature)	67

Importantly, there is phenotypic variation even between siblings affected with FA. Among 53 sibships composed of 120 siblings, there was considerable variation in malformations. Even between two sets of monozygotic twins, there was phenotypic discordance [12]. While there is concordance in the presence or absence of congenital malformations between siblings, the variable expressivity even between twins underscores the need to test all siblings of a child (or adult) with FA since the absence of a major malformation itself does not rule out FA.

Short stature is particularly common in FA and is often secondary to hormonal deficiencies. The syndrome is usually associated with abnormal growth parameters both prenatally and postnatally (Figure 76.1) with most having a height, weight, and head circumference at the 5th centile based on reports in the IFAR database. However, growth may be improving in the past decade, perhaps as a result of more intensive monitoring and nutrition. In a recent study by Rose et al. [13], 43% of FA children were within two standard deviations of the 50th centile with 9% above the mean height for the general population. Still, most children and adults with FA had one or more endocrine abnormalities. In another study, 54 patients with FA (30 males and 24 females from 47 unrelated families) were evaluated for endocrinopathies prospectively. In this study, the most common manifestations of endocrinopathy were glucose/insulin abnormalities, growth hormone insufficiency, and hypothyroidism [14]. Expectedly, patients with endocrine dysfunction are more likely to have short stature with other endocrinopathies magnifying the degree of growth failure. Since correction of growth hormone or thyroid hormone deficiency may improve final height outcome and quality of life, a comprehensive evaluation is recommended for all FA children at an early age well before HCT or the use of androgens when possible.

Gonadal failure and infertility are also common. Approximately half of all female patients with FA are infertile. Delayed menarche and premature menopause (often in the fourth decade) are common. Still, 15% of females cited in the literature or reported to

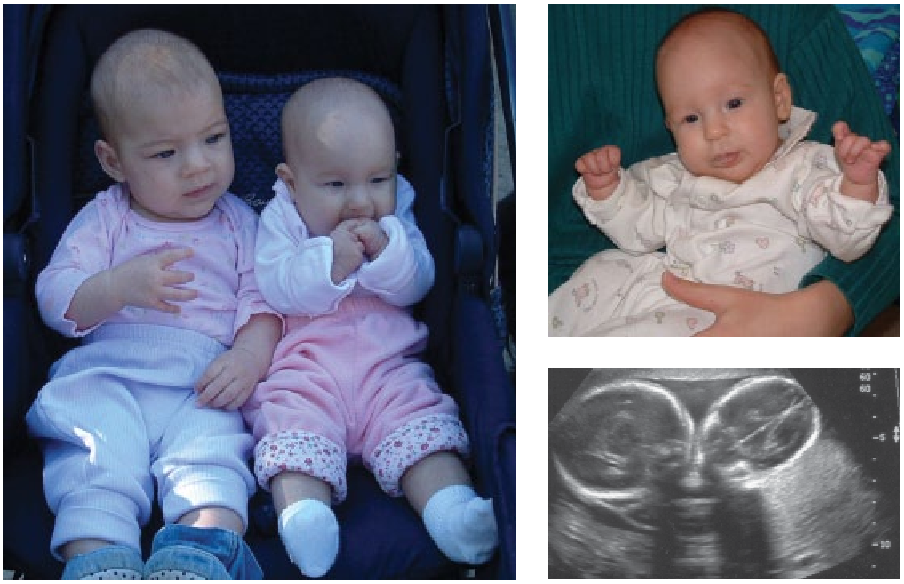

Figure 76.1 Nine-month-old twins; one healthy and one affected by Fanconi anemia (FA). Photographs demonstrate the "classical" physical features associated with FA, specifically growth retardation, bilateral hypoplastic radii, and absent thumbs, microophthalmia, and microcephaly.

the IFAR who reached 16 years of age and were not receiving androgen therapy had at least one pregnancy [15]. While pregnancy is possible in some females, it is often associated with rapid progression to severe marrow failure with transfusion dependence as well as preeclampsia and premature labour, underscoring the importance of reproductive counseling. In contrast, males are very rarely if ever fertile [16]. Genital malformations, hypoplastic gonads and low or absent sperm counts, are common findings in males with FA. Because of the high incidence of gonadal failure and its effect on growth and development as well as bone density, estrogen and testosterone levels should be routinely monitored.

Hematologic abnormalities occur in most patients with FA at a median age of 7 years (range: birth to 41 years) (Figure 76.2) [17–19]. Based on clinical data in the IFAR (*n* = 754 patients), the incidence of bone marrow failure is 90% by the age of 40 years. Although less often today due to more accurate diagnosis and enhanced monitoring, occasionally patients still present with myelodysplastic syndrome (MDS) or acute leukemia without prior diagnosis of AA. Historically, the incidence of MDS or acute leukemia in the IFAR has been 33% by the age of 40 years [18] (Figure 76.3). Similar results have been reported by Rosenberg et al. based on a survey of FA patients in North America with the median age of onset of leukemia being 11.3 years [20]. Annual marrow examinations, however, have led to earlier diagnosis of MDS and clonal cytogenetic abnormalities, in turn resulting in earlier HCT prior to disease progression. The development of clonal cytogenetic abnormalities

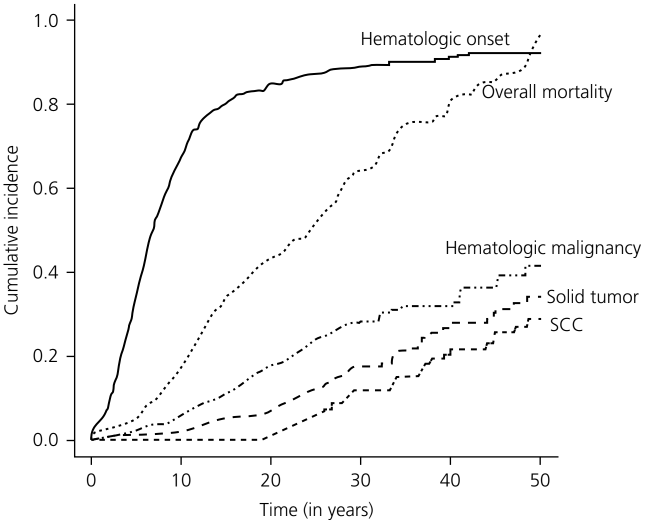

Figure 76.2 Cumulative incidence of hematologic abnormalities and malignancy over time in patients with FA. Of 754 FA patients within the International Fanconi Anemia Registry (IFAR) database, 601 (80%) experienced bone marrow failure, 120 (60%) hematologic malignancy, and 79 (40%) were non-hematologic malignancy. The risk of developing BMF and hematologic and non-hematologic malignancies increased with advancing age with a 90%, 33%, and 28% cumulative incidence, respectively, by 40 years of age. Source: Kutler et al. 2003 [18]. Reproduced with permission of the American Society of Hematology.

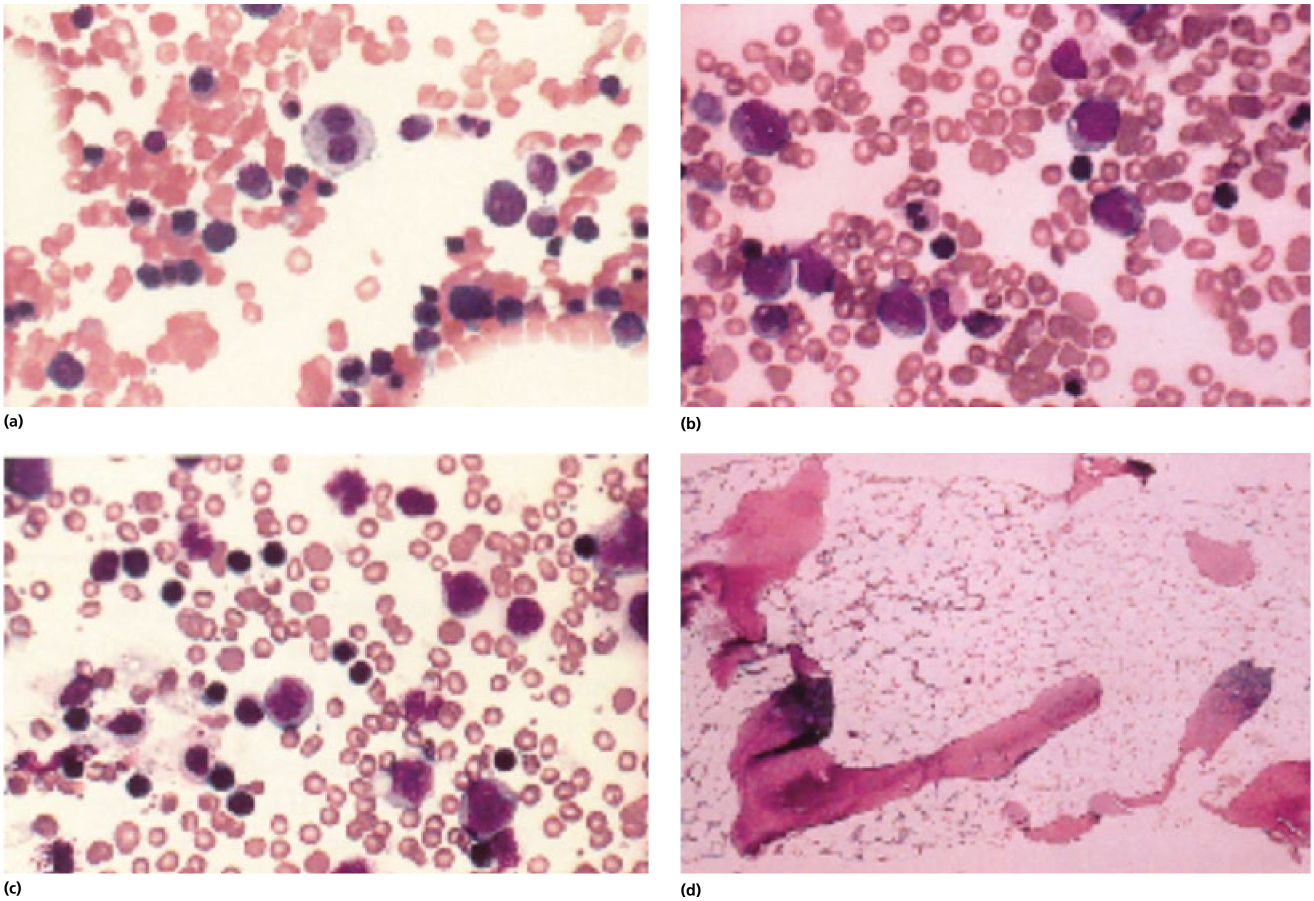

Figure 76.3 Progression of dysplasia to refractory anemia with excess blasts in a single patient with FA over a 9-month period. (a) Marrow aspirate on 02-21-96 demonstrating pancytopenia and early dysplastic changes in neutrophils; (b) marrow aspirate on 07-03-96 demonstrating progressive dysplasia and 3% blasts; (c) marrow aspirate on 09-24-96 demonstrating progressive dysplasia and 12% blasts; (d) marrow biopsy on 09-24-96 demonstrating severe aplasia. (For a color version of this figure, please see the color plate section.)

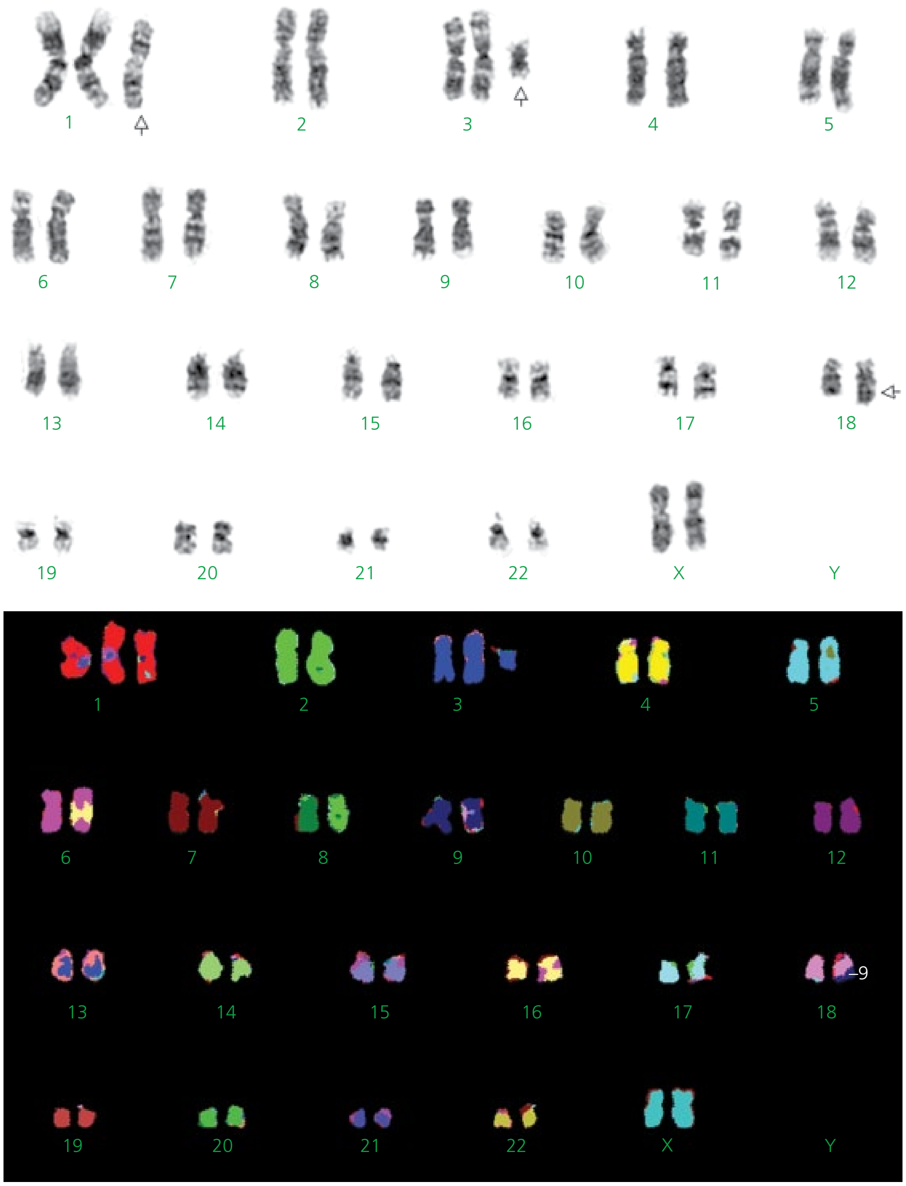

Figure 76.4 Upper panel: G-banded karyotypes from an abnormal clone in the marrow of an FA patient. This is a hyperdiploid metaphase cell with 48 chromosomes including two extra derivative chromosomes (designated by arrows); one derivative chromosome is composed of two copies of the long arm of chromosome 1 and the other contains two copies of a small portion of the long arm of chromosome 3. This cell contains a total of four copies of portions of chromosomes 1 and 3. Lower panel: A karyotype of the same abnormal clone as above, as characterized by fluorescent in situ hybridization (FISH) using a multicolor paint probe (Vysis spectral vision M-FISH). M-FISH confirmed that the two derivative chromosomes detected by G-banding represented extra copies of portions of chromosomes 1 and 3. Source: Courtesy Betsey Hirsch PhD. (For a color version of this figure, please see the color plate section.)

is increasingly more common with age. Historically, the actuarial risk of identifying clonal cytogenetic abnormalities is 67% by the age of 30 years; however, this includes patients in an era when annual marrow examinations were not the rule. Among the most frequent clonal abnormalities observed are duplications and triplications of the long arm of chromosome 1, gains of portions of the long arm of chromosome 3, and monosomy 7 or loss of material from the long arm of chromosome 7 [21]. Deletions of 5q, 11q, rearrangements of 6p and gains of chromosomes 8 and 21 have also been noted by different groups (Figure 76.4) [19,22]. In addition, acute myeloid leukemia (AML) in FA patients rarely involves the chromosomal rearrangements commonly observed in non-FA patients with AML (e.g., t[9;11], t[8;21], t[6;9], t[11;19], inv 6) and is often complex. Thus, many of the early descriptions of these karyotypes included designations of "marker" or "add," referring to structurally abnormal chromosomes that could not be completely identified by G- or R-banding. Recent advances in single and multicolor fluorescence in situ hybridization (FISH) and utilization of these and other molecular/cytogenetic techniques as adjuncts to conventional cytogenetic analysis now permits more definitive characterization of clones with the possibility of identifying new specific recurrent abnormalities (Figure 76.4). In the absence of myelodysplastic changes, leukemia or a severe cytopenia, the presence of a clonal cytogenetic abnormality in a proportion of the

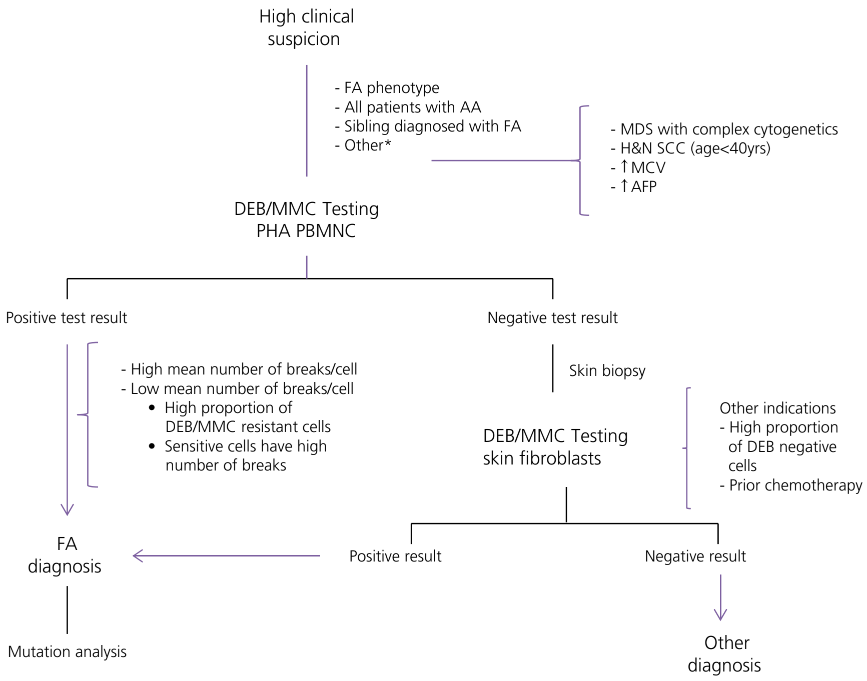
High clinical suspicion
- FA phenotype
- All patients with AA
- Sibling diagnosed with FA
- Other*
- MDS with complex cytogenetics
- H&N SCC (age<40yrs)
- ↑MCV
- ↑AFP
DEB/MMC Testing PHA PBMNC
Positive test result
Negative test result
- High mean number of breaks/cell
- Low mean number of breaks/cell
• High proportion of DEB/MMC resistant cells
• Sensitive cells have high number of breaks
Skin biopsy
DEB/MMC Testing skin fibroblasts
Other indications
- High proportion of DEB negative cells
- Prior chemotherapy
FA diagnosis
Positive result
Negative result
Mutation analysis
Other diagnosis

Figure 76.5 FA Testing Guidelines. MDS, myelodysplastic syndrome; H&N SCC, head and neck squamous cell carcinoma; MCV, mean corpuscular volume; AFP, alpha fetoprotein; AA, aplastic anemia.

cells is itself not sufficient for recommending allogeneic HCT, as abnormal cytogenetic clones have been transient in some cases. However, if repeat marrow examination reveals clonal evolution (i.e., the development of cytogenetic abnormalities in addition to the original abnormality) or clear progression in the proportion of cytogenetically abnormal cells, transplant should then be considered. In most cases, such clonal progression occurs with worsening cytopenia or progressive dysplasia in multiple lineages.

Diagnosis

Due to considerable overlap of the FA phenotype with other genetic and non-genetic diseases, the diagnosis of FA is often difficult and unreliable on the basis of clinical manifestations alone. While FA should be strongly suspected in patients with phenotypic features consistent with FA, especially if there is concomitant macrocytosis or cytopenia, many patients with FA may not have any of the classical clinical manifestations. Schroeder et al. [23] first suggested the use of spontaneous chromosomal breakage as a cellular marker for FA, but longitudinal studies of chromosome instability in FA patients showed this finding to be inconsistent. In contrast, hypersensitivity of FA cells to the clastogenic (chromosome-breaking) effect of cross-linking agents provides a reliable cellular marker for the diagnosis of this disorder (Figure 76.5). Diepoxybutane (DEB) and mitomycin C (MMC) are the two agents most widely used to make the diagnosis of FA. Extensive experience with DEB and MMC testing has demonstrated the sensitivity, specificity, and reproducibility of the results [24,25]. Cross-linker hypersensitivity can be used to identify the preanemic patient as well as the patient with AA, MDS, or leukemia who may or may not have the physical stigmata associated with FA. It is recommended that all patients exhibiting any congenital malformation known to be associated with FA or diagnosed with aplastic anemia at any age, have a peripheral blood sample tested for cross-linker hypersensitivity [10]. Children with cancer, in particular MDS or acute myeloid leukemia, found to be peculiarly hypersensitive to chemotherapy, or young adults (age <40 years) diagnosed with epitheloid tumors of the head and neck might also be considered for FA testing. Importantly, *all full siblings of an FA patient should also be screened without exception*, recognizing that there is a lack of concordance of the FA phenotype among affected siblings.

Data from DEB testing indicate that there is great variability in the degree of hypersensitivity in FA patients, although there is no overlap with the normal range [24]. In approximately 25% of FA patients, DEB testing will reveal two populations of phytohemagglutan A (PHA)-stimulated peripheral blood lymphocytes; one demonstrating an FA phenotype with chromatid breaks and exchanges and the other a normal one [12,26]. While there is no obvious correlation between the degree of cross-linker hypersensitivity and a specific FA gene mutation, severity of the phenotype in FA patients or individual patient sensitivity to chemoradiotherapy (JE Wagner and AD Auerbach, unpublished data), there may be an increased risk of graft rejection after HCT in patients with a low mean number of breaks per cell after exposure to DEB. This finding, however, is related to the proportion of DEB-resistant cells rather than a difference in the cross-linker sensitivity [12,27]. Beyond the possible correlation between DEB-resistant PHA-stimulated T lymphocytes and graft failure after HCT, patients with marked skewing toward DEB-resistant cells may be misdiagnosed as not having FA. In an analysis of DEB results at Rockefeller University, 25% patients had 25% or more of their cells without chromosomal breaks on treatment with 0.1 ug/mL DEB and 2% had >90% of cells without chromosomal breaks. Despite a low mean number of breaks per cell (which in some cases may overlap with or approach the normal range), FA patients have >1 break/cell within the aberrant cell population not seen in non-FA patients [12]. However, under such circumstances, testing of skin fibroblasts is recommended. Still, even within these patients with a substantial proportion of DEB-resistant cells, it is critical that the diagnostic laboratory report not just the mean breaks per cell but also the number of breaks per aberrant cell.

Importantly, the clinician should know that the chromosomal breakage test can also be applied to the study of fetal cells obtained by chorionic villus sampling (CVS), amniocentesis, or percutaneous umbilical blood sampling. If the specific mutation is known within the family, testing for the presence of the mutation on limited quantities of uncultured cells is possible and rapid. Alternative diagnostic methods for FA, such as by cell-cycle analysis using flow cytometric methods on lymphocytes exposed to cross-linking agents, are sometimes used as a research tool. While it is clear that duration of the G2/M phase is increased in FA cells as compared to unaffected cells, clinical diagnostic laboratories do not often use this method. In contrast, the immunoblot assay of FANCD2 protein monoubiquitination is a rapid diagnostic test for FA. However, rare patients with mutations in FA genes downstream of FANCD2 (i.e., downstream factors discussed later) or patients with significant somatic mosaicism would be missed by this approach. Finally, it has been reported that measurement of serum alpha fetoprotein (AFP) levels may be a sensitive and reliable test for FA [28]. Of 63 FA patients evaluated between 2005 and 2011 at the University of Minnesota, 84.4% had elevated AFP levels (normal range varies by age but is 0–8 ug/L for children $\geq$6 years and adults) (R Mitchell, H Zierhut, and ML MacMillan, unpublished data). Whether AFP levels can differentiate FA subpopulations is unknown; however, six of six patients with biallelic mutations in FANCD1/BRCA2 gene have had particularly high AFP levels (range, 43.5–106.6 ug/L) well above most other FA patients. Together, these data suggest that AFP may provide supportive evidence for the diagnosis of FA in general; however, AFP does not replace the need for the standard chromosome breakage test.

Once a patient has been diagnosed with FA by cross-linker hypersensitivity, molecular genetic testing is recommended. For example, the specific gene affected may be associated with differences in time to marrow failure and length of survival (Figure 76.6). Knowing the FA gene defect can be important for reproductive counseling and carrier testing (see section on "Genetic counseling").

The FA pathway and DNA repair

FA or FA-like syndromes are caused by mutations in at least one of 17 distinct genes (Table 76.2). These FA genes act cooperatively to coordinate DNA repair, particularly interstrand cross-links. While there is no consensus on the precise nature of the FA pathway, models exist that explain network interactions between FA proteins and other repair networks, including the breast cancer (BRCA) susceptibility protein [29–35]. Disruption in the pathway, however, leads to dysregulation of the signaling pathways that guide cell proliferation and apoptosis as well as inefficient DNA repair, leading to the congenital malformations, hematopoietic failure, and cancer characteristic of FA.

The FA pathway is evolutionarily conserved and FA proteins interact with numerous DNA repair circuits to maintain genome stability. Functionally, the FA-BRCA pathway can be subdivided into three compartments: (1) a large multisubunit upstream core complex with E3 ubiquitin ligase activity, (2) two monoubiquinated central effector proteins, and (3) downstream factors (Figure 76.7). As a group, FA proteins interact with additional proteins to repair interstrand crosslinks, participating in homologous recombination and translesion synthesis, as well as processes that maintain genomic stability during DNA replication.

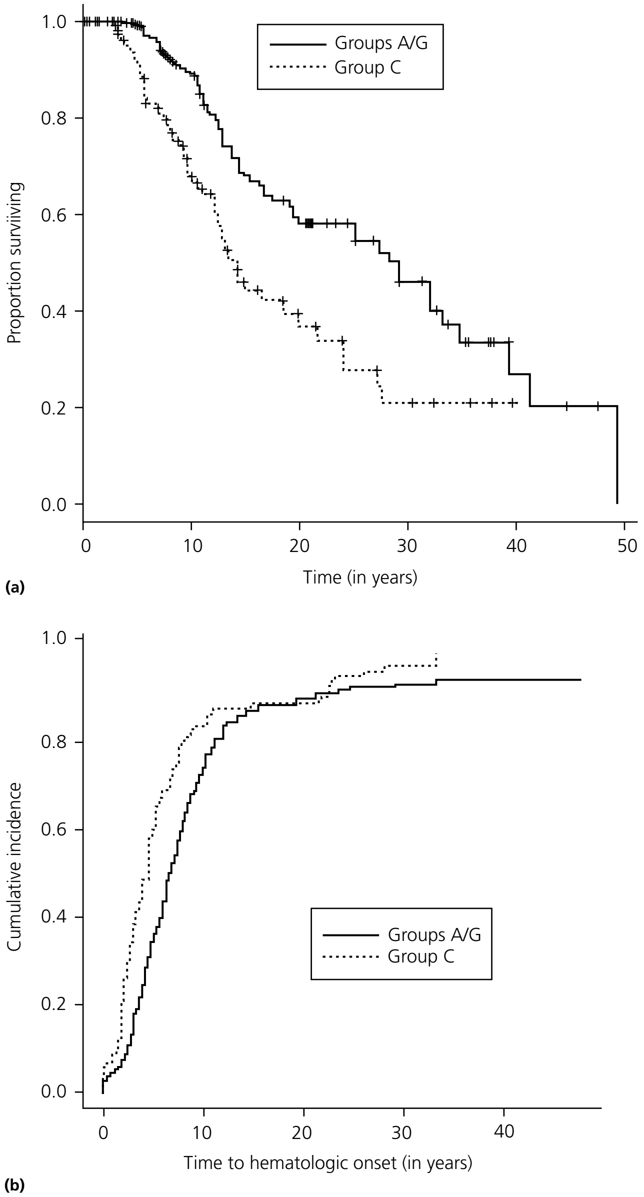

Figure 76.6 Survival and time to marrow failure was compared for FA patients in complementation groups A, G, and C. Survival and incidence of marrow failure was similar in groups A and G. Comparison of the combined groups A/G with group C showed that patients in group C had a significantly shorter survival (a) and higher incidence of bone marrow failure (b). Source: Kutler et al. 2003 [18]. Reproduced with permission of the American Society of Hematology.

Upstream core complex. The FA core complex proteins assemble in the nucleus and are necessary for monoubiquination of *FAND2* and *FANCI*, the central effectors in protection of genome integrity. Eight FA proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM) and three FA associated proteins (FAAP100, FAAP24, and FAAP20) are members of the nuclear core complex. Importantly, three of these upstream core complex genes, *FANCA*, *FANCC*, and *FANCG* are responsible for nearly all cases of FA (62%, 15%, and 9%, respectively). Additional information regarding FA mutations can be obtained from the Fanconi Anemia Mutation Database at http://www.rockefeller.edu/fanconi/mutate/ (accessed February 4, 2015).

Table 76.2 The Fanconi anemia genes: description and frequency in the IFAR database

FA Group	FA Gene	MIM #	Location	Exons	Amino Acids	Frequency	Protein Function
Core Complex Factors							
A	*FANCA*	607139	16q24.3	43	1455	60%	Core complex member
B	*FANCB (FAAP95)*	300515	Xp22.31	10	859	2%	Core complex member
C	*FANCC*	227645	9q22.3	15*	558	14.8%	Core complex member
E	*FANCE*	600901	6p21.3	10	536	1%	Core complex member; binds FANCD2 directly
F	*FANCF*	603467	11p15	1	374	2%	Core complex member
G	*FANCG (XRCC9)*	602956	9p13	14	622	9%	Core complex member
L	*FANCL (PHF9/FAAP43)*	608111	2p16.1	14	375	0.5%1	Core complex member; E3 ubiquitin ligase
M	*FANCM (Hef/FAAP250)*	609644	14q21.3	22	2048	0%	Helicase, localizes the core complex to DNA
Central Factors/ID Complex							
D2	*FANCD2*	227645	3p25.3	44	1451	3%	Binds DNA structure, exonuclease activity
I	*FANCI (KIAA1794)*	609053	15q26.1	38	1268	1%	Aids in proper monoubiquination of FANCD2
Downstream Factors							
D1	*FANCD1 (BRCA2)*	605724	13q12.3	27	3481	3%	Binds ss and ds DNA; stimulates RAD51 strand exchange (HR mediator)
J	*FANCJ (BRIP1/BACH1)*	605882	17q22	20	1249	3%	Helicase that unwinds DNA with 3′-5′ polarity; interacts with BRCA1
N	*FANCN (PALB2)*	610832	16p12	13	1186	0.5%	Helicase, translocase; stabilizer of FANCD1
O	*FANCO (RAD51C)*	613390	17q22	9	376	0%	Binds ss DNA (HR mediator)
P	*FANCP (SLX4/BTBD12)*	613951	16p13.3	15	1834	0.2%	Scaffold for structure-specific nucleases (Holliday junction resolvase)
Q	*FANCQ (ERCC4/XPF)*	615272	16p13.12	11	916	0.1%	Associates with ERCC1 to form a FANCP/SLX4-dependent interstrand crosslink unhooking nuclease; participates in nucleotide excision repair
S	*FANCS (BRCA1)*	113705	17q21.31	22	1863	0.1%	HR; inhibition of non-homologous end joining

Of 1168 FA patients in the IFAR database, the complementation group assignment has been made for 790 North American IFAR patients.
*intron 4 is now intron 5 based on the RefSeq used for mutation nomenclature (see: http://chromium.liacs.nl/LOVD2/FANC/refseq/FANCC_codingDNA.html) (accessed March 6, 2015).

In addition to the monoubiquination of the FANCD2 and FANCI, the FA core complex may play additional roles. There is emerging evidence that FA core complex suppresses the activation state of intracellular effectors of apoptosis and to facilitate survival signaling pathways, including the JAK-dependent STAT activation pathway, thus promoting cell survival. For example, FANCC interacts with the molecular chaperone heat shock protein 70 (Hsp 70) to protect HSC from cytotoxicity and apoptosis on exposure to interferon gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) [36]. Together these interactions protect cells from a variety of environmental factors, including oxidative stress and radiation, as well as suppress double-stranded RNA-dependent protein kinase activity and caspase 3 activation [37]. While there has been tremendous progress in our understanding of the FA proteins and their role in DNA repair, cell-cycle checkpoints, oxygen metabolism, and induction of apoptosis, there is much to be fully elucidated.

Central effectors. Following DNA damage (by DEB, MMC, and other DNA-damaging agents) and during S-phase of the cell cycle, FANCD2 and FANCI are monoubiquitinated by the FA core complex which contains FANCL, an E3 ubituitin ligase. FANCD2 and FANCI are also phosphorylated as a consequence of DNA damage by cell cycle checkpoint kinases, ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and RAD3-related (ATR). While phosphorylation is critical for checkpoint signaling during S phase and for monoubiquination of FANCD2/FANCI (ID complex), the precise role for this DNA damage-dependent phosphorylation of multiple FA proteins is unclear [38–44]. The FA-ID complex associates with chromatin and forms the functional fulcrum of the FA pathway SLX4 (FANCP), a downstream FA protein, which is recruited to the site of damage, and a variety of FA-associated nucleases, including XPF-ERCC1, MUS81-EME1 and SLX1, and FAN-1 cooperate with other proteins to excise the intrastrand crosslink, induce double strand breaks and prime the broken DNA strands for repair [35,45–47]. Failure to monoubiquinate FANCD2, as is observed in FA individuals with biallelic loss-of-function mutations in nuclear core complex genes, also causes hypersensitivity to cross-linking agents and poor maintenance of genome stability. Presence of the monoubiquinated long forms of FANCD2 and FANCI, however, does not exclude FA as some patients have mutations downstream of the central effectors [48].

Downstream factors. After cross-link excision, the FANCN/PALB2, 5′-3′ helicase FANCJ/BRIP1, FANCD1/BRCA2, FANCO/RAD51C, FANCS/BRCA1, RAD51, and other proteins assemble at the 3′-flap of single strand DNA. This enables invasion of the remaining broken strand and repair by homologous recombination. More recently, FANCP/SLX4 was identified as a novel FA gene. FANCP/SLX4 acts as a docking platform for multiple structure-specific nucleases. FANCP/SLX4 binds to the XPF (ERCC4) and MUS81 subunits of the XPF-ERCC1 and MUS81-EME1 nucleases, critical to DNA interstrand cross-link repair. Because cells deficient in FANCP/SLX4 have normal monoubiquination of FANCD2, FANCP/SLX4 is classified as a downstream factor. As other downstream Fanconi anemia proteins (i.e., FANCD1/BRCA2, FANCS/BRCA1, FANCN/PALB2, and FANCJ/BRIP1) are breast cancer susceptibility genes, it is possible that mutations in FANCP/SLX4 may also be associated with breast cancer; recent studies support this claim [46,47].

Why this is significant to the transplant team? The disrupted FA pathway does not respond appropriately to DNA damage, resulting in the accumulation of damage during DNA replication and loss of genomic integrity. This may lead to apoptosis and consequently high risk of developmental defects during

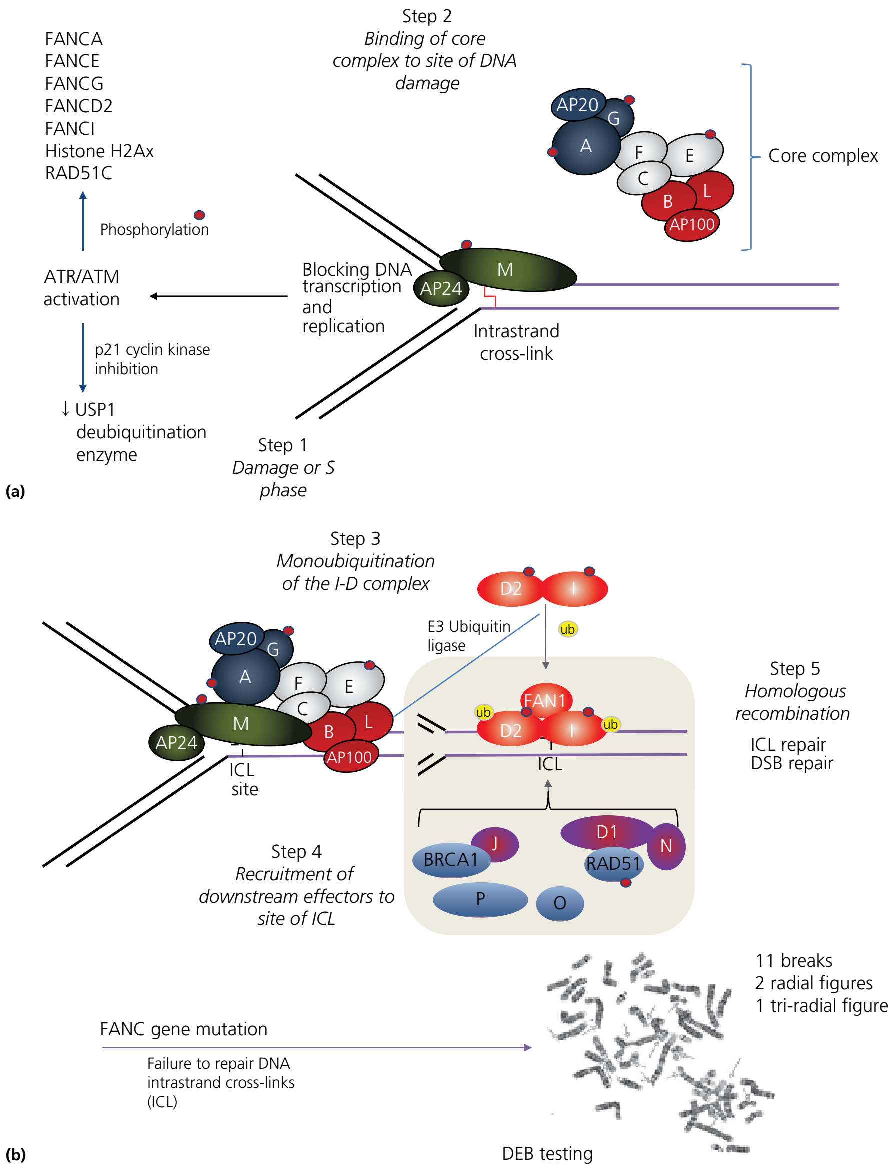
FANCA
FANCE
FANCG
FANCD2
FANCI
Histone H2Ax
RAD51C
Phosphorylation
ATR/ATM activation
p21 cyclin kinase inhibition
↓ USP1 deubiquitination enzyme
Step 2
Binding of core complex to site of DNA damage
AP20
G
A
F
E
C
B
L
AP100
Core complex
M
AP24
Blocking DNA transcription and replication
Intrastrand cross-link
Step 1
Damage or S phase
(a)
Step 3
Monoubiquitination of the I-D complex
D2
I
E3 Ubiquitin ligase
ub
FAN1
ICL site
ICL
Step 5
Homologous recombination
ICL repair
DSB repair
BRCA1
J
D1
N
RAD51
P
O
Step 4
Recruitment of downstream effectors to site of ICL
11 breaks
2 radial figures
1 tri-radial figure
FANC gene mutation
Failure to repair DNA intrastrand cross-links (ICL)
DEB testing
(b)

Figure 76.7 Model of the FA pathway in DNA repair compiling the most recent data. (a) Step 1: DNA damage and stalled replication fork signal ATR results in phosphorylation of the FANCA, E and G of the core complex, and FANC D2 and I of the I-D complex, RAD51C and histone H2AX as well as activation of p21 which downregulates USP1 deubiquitination enzyme and in turn permits ubiquitination of FANC D2 and I. Step 2: Upon recognition of the intrastrand DNA cross-link (ICL), FANCM and FAAP24 and FANCM-associated histone fold 1/2 bind near the site of the intrastrand cross-link and recruit the 8-member core complex which assembles the core nuclear ubiquitin ligase complex which monoubiquinates FANCD2 and FANCI. (b) Step 3: The modified I-D protein complex associates with chromatin and recruits FAN1 critical to excision of the ICL and formation of 3′ flaps of ssDNA for repair. Step 4: Downstream proteins FANCN, D1, J, O and P, and BRCA1 assemble and nucleate the single strand DNA with RAD51C. Step 5: This enables homologous recombination and ICL/double-strand break (DSB) repair. Mutations any of the FANC genes leads to inability to repair ICL efficiently. Loss of the FA pathway leads to chromosomal aberrations upon exposure to cross-linking agents such as diepoxybutane (DEB), leading to an infrequency of chromosomal breaks and radial figures as shown in the side panel. Source: Courtesy of Betsey Hirsch PhD. Model based on a compilation of data [29–52]. (For a color version of this figure, please see the color plate section.)

embryogenesis and bone marrow failure over the first two decades of life, as well as a series of mutations and consequent marked predisposition to leukemia and solid tumors. This knowledge of the FA pathway also explains the marked sensitivity of FA patients to (a) chemoradiotherapy and the need for marked dose reductions of cross-linking agents, such as daunomycin, carboplatin, cyclophosphamide, irradiation in the treatment of leukemia and cancer, in contrast to antimetabolites, like fludarabine, cytarabine and methotrexate, or (b) the use of psoralens with ultraviolet A (PUVA) therapy for the treatment of mucocutaneous chronic graft-versus-host disease (GVHD). Because of the possibility of accumulated DNA damage in residual HSC of FA patients, any proposed trials of gene-corrected HSC come with substantial risk, particularly in older patients with FA. The malignant transformation of gene-corrected HSC would be particularly difficult to treat since the corrected cells will be relatively chemotherapy-resistant (compared to uncorrected cells), requiring higher dose therapy in patients who cannot tolerate high-dose therapy. In summary, an understanding of the FA pathway helps the practitioner understand the rationale for recommending minimizing exposure to ionizing and UV radiation (i.e., routine dental radiographs, importance of sunscreens), hypersensitivity of FA patients to chemoradiotherapy prior to transplant and treatment of leukemia and solid tumors and the importance of a complete family medical history, need for FA gene mutation analysis and potential consultation with a genetic counselor [i.e., particularly if downstream factor genes associated with cancer risk in the carriers (e.g., parents) are mutated].

Pathophysiology of marrow failure and leukemogenesis

Children and adults with FA have an extraordinary predisposition for the development of marrow failure and leukemia. At birth, the blood counts are typically normal. Overtime, the first sign of any hematologic abnormality is often an increase in mean corpuscular volume (MCV). While anemia is classically considered the hallmark of FA, any one or all three hematopoietic lineages may be affected at the time of initial presentation. Other hematologic abnormalities include elevated fetal hemoglobin, low frequency of CD34 cell counts by flow cytometry, and low colony-forming capacity even at the time of birth based on assessments of umbilical cord blood (UCB) from affected neonates. Based on the genomic instability inherent in all the tissues of affected individuals, but in particular rapidly dividing cells like those in the hematopoietic progenitors, progressive depletion of HSC and clonal expansion of mutated cells ultimately lead to marrow failure and leukemia in almost all.

In order to better understand the pathophysiology of bone marrow failure, a number of murine models have been developed. Knockout models exist for FA core complex (fanca, fancc, fancg, fancf, and fancm), ID complex (fancd2), and downstream factors (fancd1 and fancp) [53–67]. In fanca–/–, fancc–/– and fancg–/– mice, the blood counts are normal (except for mild thrombocytopenia in fanca–/– mice); however, marrow progenitors are hypersensitive to IFN-γ and TNF-α both in vitro and in vivo, and have reduced numbers of HSC and reduced competitive repopulation potential relative to normal controls [56]. Similarly, fancd2–/– mice and mice with a hypomorphic mutation in fancd1, also have a reduced frequency of HSC and engraftment capacity albeit more severe in the fancd1 hypomorph. While none of the murine models completely recapitulate the human FA phenotype, in particular, progressive marrow failure, all models demonstrate genomic instability, cell cycle defects and hypersensitivity to DNA cross-linking agents. As such, these models may be useful in further exploring hypotheses derived from observations in FA patients (and FA cells in vitro) and identifying cellular and environmental factors that may impact the pace of HSC depletion. For example, Wang et al. [68] studied the function of constitutively expressed normal human FANCC protein in mouse hematopoietic cells. HSC from FANCC over-expressing transgenic mice were resistant to the cytolytic effect of a *fas*-triggering antibody, suggesting the *fas*-mediated apoptosis is involved in the pathogenesis of marrow failure in FA. Antisense oligonucleotides complementary to FANCC messenger RNA (mRNA) inhibited the in vitro clonal growth of normal erythroid and granulocyte–macrophage progenitor cells, even in the presence of exogenous growth factors. In contrast, peripheral blood CD34$^+$ cells isolated from a FA-C patient and infected with *FANCC* cDNA exhibited a 5- to 10-fold increase in the number of progenitor colonies in vitro. These results suggest that the *FANCC* gene plays a direct role in the survival of hematopoietic progenitor cells [69–71]. Rathburn et al. [72] proposed that marrow failure in patients with FA results from the accumulated losses of HSC caused by repeated episodes of low IFN-γ released in response to recurrent minor inflammatory stimuli. The capacity of IFN-γ to induce TNF-α gene expression is increased in the serum of children with FA [73]. Whitney et al. [74] postulated that the apoptotic phenotype in hematopoietic stem and progenitor cells creates an environment for the selection of IFN-γ resistant clones. Their findings argue that therapies designed to correct the abnormal pattern of IFN-γ sensitivity in FA cells may prevent or delay the onset of marrow failure and development of MDS and leukemia. Briot et al. [75] also showed that activation of two major stress signaling pathways, the mitogen activated protein kinases (MAPK) and the NF-κB transcription factor, induces many cytokines, including TNF-α. Together these data provide strong rationales for new clinical trials designed to correct the abnormal pattern of IFN-γ sensitivity in FA cells or reduce the levels of TNF-α which could prevent or delay the onset of marrow failure and development of MDS and leukemia. Among the hypotheses to be tested, recent focus on the role of aldehydes is particularly noteworthy. Garaycoechea and Patel et al. [76] considered the possibility that reactive aldehydes might be a cellular source of DNA cross-linking agent that may deplete FA HSCs based on the observation that FA-deficient cell lines are hypersensitive to acetylaldehyde and formaldehyde in vitro. Interestingly, bone marrow failure occurred in FA mice that were also deficient in acetylaldehyde detoxifying enzyme ALDH2 (fancd2–/– aldh2–/–) upon exposure to ethanol, the metabolic precursor to acetaldehyde. Also of interest was the spontaneous development of acute leukemia in these mice.

Why this is significant to the transplant team? Understanding the pathophysiology of FA marrow failure and leukemogenesis could help us find ways to ameliorate the disease progression as well as explain certain clinical observations. For example, in children with FA, blood counts often plummet after viral illnesses, in particular varicella zoster and respiratory viruses, which will recover over a period of 2–4 weeks but rarely back to baseline. In vitro and in vivo data suggest that FA HSC are hypersensitive to IFN-γ and TNF-α, both explaining these clinical observations and underscoring the importance of preventing viral illnesses. The models may also be useful in the development of preventative strategies (i.e., limiting alcohol exposure and aldehyde-generating foods) and optimization of forthcoming treatments, such as gene-corrected autologous HSC where exogenous factors (e.g., IFN-γ) might be needed to give the corrected HSC a competitive growth advantage over uncorrected HSC.

Somatic mosaicism

Somatic mosaicism is the state when genetically distinct populations of somatic cells can be found within an organism. Mosaicism can be caused by DNA mutations, epigenetic alterations of DNA, chromosomal abnormalities, and the spontaneous reversion of inherited mutations. As in many other inherited disorders, somatic mosaicism has been found in FA [12]. In fact it is relatively common – up to 65% of FA patients will have a population of T lymphoblasts that is resistant to DEB. The question now is whether mosaicism occurs beyond the lymphoid lineage and whether it has any clinical significance specifically in patients with FA.

To date, several reports of genetic reversion in the HSC with longitudinal data suggest that such reversion may prevent marrow failure. In the first case, Gregory et al. [77] demonstrated somatic reversion in the HSC as evidenced by loss of the maternal *FANCA* mutation in a substantial proportion of myeloid and erythroid colony-forming cells and sorted T- and B lymphocytes. The blood counts were stable for about 20 years despite an 11q23 deletion in the residual non-revertant cells which led to MDS subsequently developing. In a second report in monozygotic twins (patients 2A and 2B), Mankad A et al. [78] demonstrated increased sensitivity to DNA cross-linking agents in skin fibroblasts and not the lymphocytes or committed hematopoietic progenitors. Expectedly, molecular analysis of *FANCA* revealed two mutations in the skin [a frameshift causing deletion in exon 27 (c.2555delT) and an exon 28 missense mutation (c.2670G>A/p.R880Q)]. Surprisingly, an acquired exon 30 missense change (c.2927G>A/p.E966K) was detected only in the hematopoietic cells and in neither parent. This compensatory mutation existed in cis with the maternal exon 28 mutation, restoring function. Both sisters were free of hematologic symptoms for more than 20 years. Gross et al. [79] described the types of reversions in five mosaic FA patients who were compound heterozygotes for single base mutations in *FANCA* or *FANCC*. Intragenic crossover could be shown as the mechanism of self-correction in a *FANCC* patient. Restoration to wildtype via back mutation or gene conversion of either the paternal or maternal allele was observed in the *FANCA* patients. The environments of these reversions were indicative of high mutability. As in two patients, Gross also demonstrated in vitro phenotypic reversion as a result of the emergence of a compensating missense mutation 15 amino acids downstream of the constitutional mutation. In this series, four of five patients showed improvement of their hematologic status during a limited follow-up period.

Regardless of the mechanism for the genetic reversion, the event is relatively common in T cells of FA patients and very rare in the HSC. As genetic reversion should be a rare event, hematopoiesis should be clonal. X-inactivation studies in the monozygotic twins confirmed clonality. Notably, this has also been observed in an unreported case with clonal hematopoiesis confirmed by X-inactivation studies (JE Wagner & AD Auerbach, unpublished data). Like the prior cases, hematopoietic progenitors were insensitive to low doses of MMC, paralleling the dose–response curve of progenitors from an unaffected donor (Figure 76.8).

While it is clear that many FA patients may have mosaicism in the T-cell compartment [12], this observation does not predict

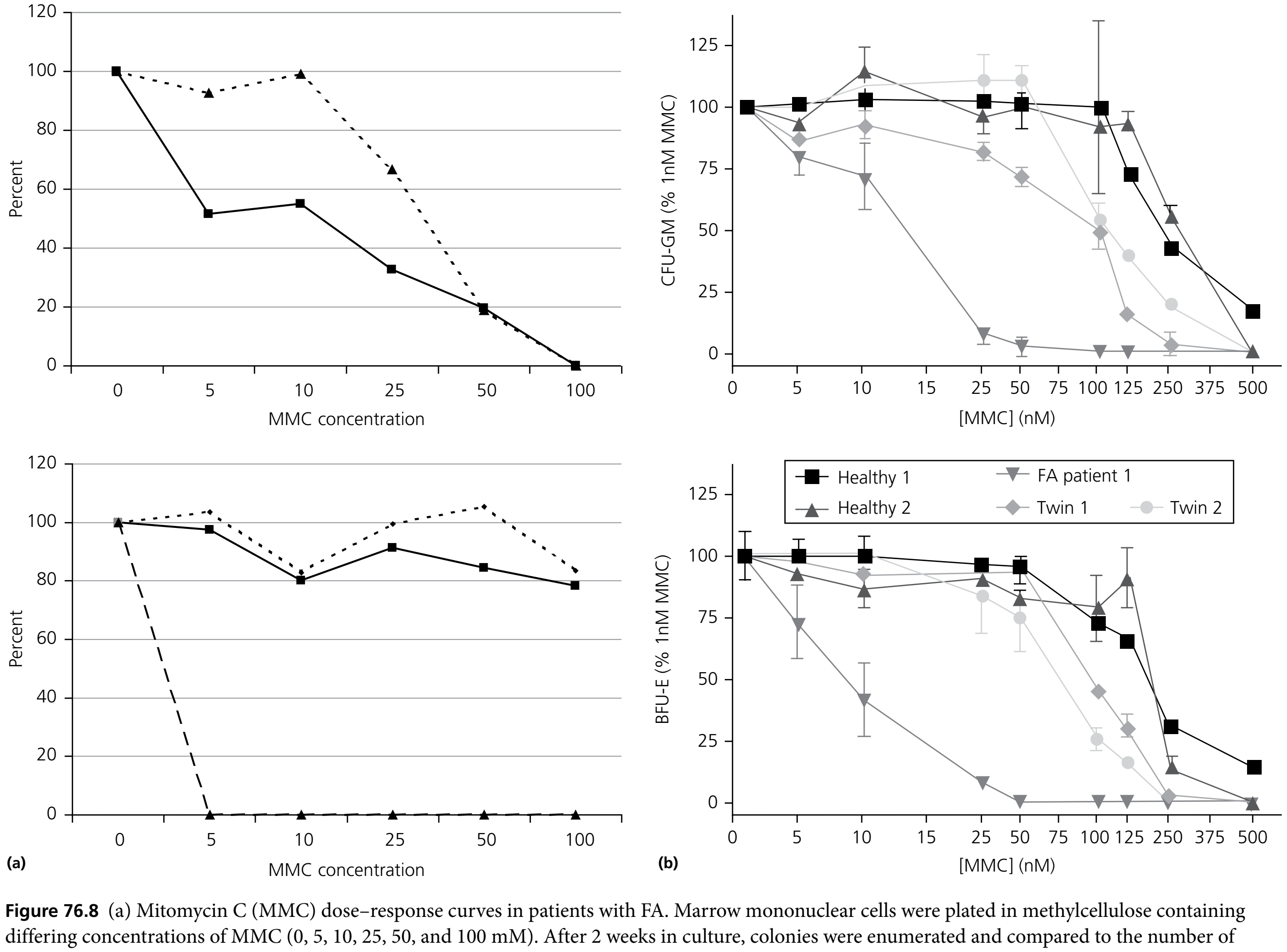

Figure 76.8 (a) Mitomycin C (MMC) dose–response curves in patients with FA. Marrow mononuclear cells were plated in methylcellulose containing differing concentrations of MMC (0, 5, 10, 25, 50, and 100 mM). After 2 weeks in culture, colonies were enumerated and compared to the number of colonies in the untreated control. Results demonstrate the colony-forming cell (CFC) MMC resistance in Patients 1 and 2. The upper panel (patient 1) demonstrates partial resistance; the lower panel (patient 2) demonstrates complete MMC resistance comparable to marrow CFCs from a normal healthy volunteer. The dashed line in the lower panel reveals marked MMC sensitivity of marrow progenitors obtained from the patient's affected sibling. (b) MMC dose–response curves of bone marrow derived clonal progenitors from one Fanconi anemia patient (FA Patient 1), two healthy volunteers (Healthy 1 and 2), and twins (Twin 1 and 2) are shown with CFU-GM in (A) and BFU-E in (B). (b) Source: Mankad et al. 2006 [78]. Reproduced with permission of the American Society of Hematology.

mosaicism in hematopoietic progenitors. Certainly, HSC mosaicism may be associated with normal blood counts at least for decades. The clinical significance of T-cell mosaicism is unclear. Some reports suggest a higher risk of graft rejection in patients with DEB resistant T cells [80]. Perhaps revertant host T cells survive the FA dose-adjusted chemoradiotherapy and are capable of rejecting the transplanted HSC. More recent reports, however, do not demonstrate such an association perhaps due to the increased use of fludarabine, a potent immunosuppressant.

Why this is significant to the transplant team? In addition to genotype, the presence of somatic mosaicism may be of important predictive value regarding the clinical course of the patient with FA. While marrow failure may be less likely in those few patients with genetic reversion in the HSC, perhaps the more important impact of somatic mosaicism is the diagnostic challenge it might impose. As outlined in Figure 76.5, if there is a high clinical suspicion of FA based on morphologic parameters or genetic history (all the cases above had affected siblings), a skin biopsy should be considered. In addition, somatic mosaicism originating in a pluripotent HSC can be viewed as "spontaneous" or "natural" gene therapy. The clinical course of the patients described earlier may be instructive with regard to the future potential of autologous HSC gene therapy for this disease. The main premise of gene therapy in FA patients is that corrected FA cells will have a proliferative advantage over non-corrected cells. In the cases described earlier, hematopoietic function was distinctly better than that in affected siblings. However, the first case raised the possibility that HSC gene correction alone may not be sufficient. This case illustrates the potential risk of myelodysplasia in residual FA HSCs with eventual suppression of normal hematopoiesis. Therefore, the risk of malignant transformation of residual FA HSCs remains a risk and suggests the need to eradicate residual non-reverted stem cells in those with somatic mosaicism (and uncorrected stem cells after gene therapy).

Genotype–phenotype correlations

As molecular data have become increasingly available, we can now explore the possibility of genotype–phenotype correlations. While it would be helpful to know which patients are at higher or lower risk of early marrow failure, leukemia, or solid tumors, this has turned out to be the exception rather than the rule. Today, there are only a few subgroups of patients for whom we can reliably predict a higher risk of cancer and early marrow failure.

In a retrospective analysis, the IFAR data [18] confirmed that specific complementation groups play a significant role in both the timing of bone marrow failure and survival (Figure 76.6). FA-C patients, for example, have a significantly earlier onset of bone marrow failure (Figure 76.6) and poorer survival compared to FA-A and FA-G patients As reported by Gillio et al. [81], patients with *FANCC* genotypes can be divided into three clinical groups: (i) patients with the c.456+4A>T (IVS4) mutation; (ii) patients with at least one exon 14 mutation (p.R548X or p.L554P); and (iii) patients with at least one exon 1 mutation (c.322delG or p.Q13X) and no known exon 14 mutation. IVS4 and exon 14 subgroups are associated with a severe phenotype manifested by multiple major congenital malformations, early onset of hematologic disease and poorer survival compared to exon 1 patients and to the non-FA-C IFAR population (Figure 76.9) [18].

However, it is the group of patients with mutations in FANCD1/BRCA2 that have the most conspicuous genotype–phenotype correlations. While there are similarities in the response to clastogenic agents, abnormal skin pigmentation, intrauterine growth retardation and short stature, biallelic mutations in *FANCD1/BRCA2* are associated with an extremely high predilection to leukemia and cancer at a very early age [82]. For all patients with biallelic mutations in *FANCD1/BRCA2*, the incidence of leukemia is 41% at age 5 years in contrast to FA in general where incidence is 1% during the first 5 years of life (Figure 76.10). In addition, biallelic *FANCD1/BRCA2* mutations are also associated with development of solid tumors, most often of the brain and kidney [83]. Of interest, medulloblastoma is particularly common in children of Ashkenazi Jewish descent who carry the BRCA2 founder mutation c.6174delT. The only other FA patients with a similar phenotype have mutations in another downstream factor, FANCN. Biallelic mutations in the BRCA2-interacting protein PALB2 (*FANCN*) is associated with embryonal cancers and early death [84]. As expected, the family history is particularly important in BRCA2 families, as many (but not all) of these kindreds have significant histories of breast and other cancers.

Neveling et al. [85] has also reported that some phenotypes are more likely found in specific complementation groups. For example, patients with the most mild phenotype are absent in complementation groups B, E, and F. Similarly, patients with the few congenital anomalies but high risk of cancer more commonly fall within the FANCA and FANCD2 groups.

Why this is significant to the transplant team? The ability to correlate genotype with outcome has considerable clinical significance. While the exception, some genotypes are associated with the pace of hematologic abnormalities, cancer risk, and survival. This knowledge aids the family and clinician in timing the various treatment options as well as prescribing a tailored surveillance plan. For example, patients with *FANCC* IVS4 or exon 14 mutations or biallelic mutations in BRCA2 should be followed more closely for early marrow failure and development of clonal cytogenetic abnormalities and, in the case of BRCA2, prophylactic allogeneic HCT might be considered.

Non-transplant treatment strategies

Androgens

Primary treatment for bone marrow failure in patients with FA has historically been androgens with or without prednisone. Androgen therapy (most commonly oxymetholone initiated at 2 mg/kg/day) was first evaluated in patients with FA by Shahidi and Diamond [86]. Hematologic improvement occurs in 50% [87]. Patients responding to androgens typically do so within several months of drug initiation [88]. In addition, prednisone (5–10 mg every other day) was frequently added in an attempt to promote vascular stability and decrease bleeding tendency in those with severe thrombocytopenia [88]. For those that respond, Alter and Young [88] observed a 3-year survival advantage in those treated with androgens versus other non-HCT treatments.

Very recently, some hematologists have argued for androgens for all newly diagnosed FA patients based on the fact that some patients may avoid transplant altogether and a recent unpublished observation that a switch in androgen formulation may further prolong the response to androgen and thus delay HCT. Certainly, the use of androgens should be considered for any patient who is considered a poor risk candidate for HCT. However, for the good HCT candidate, androgens are generally not considered because of the high survival rates expected after

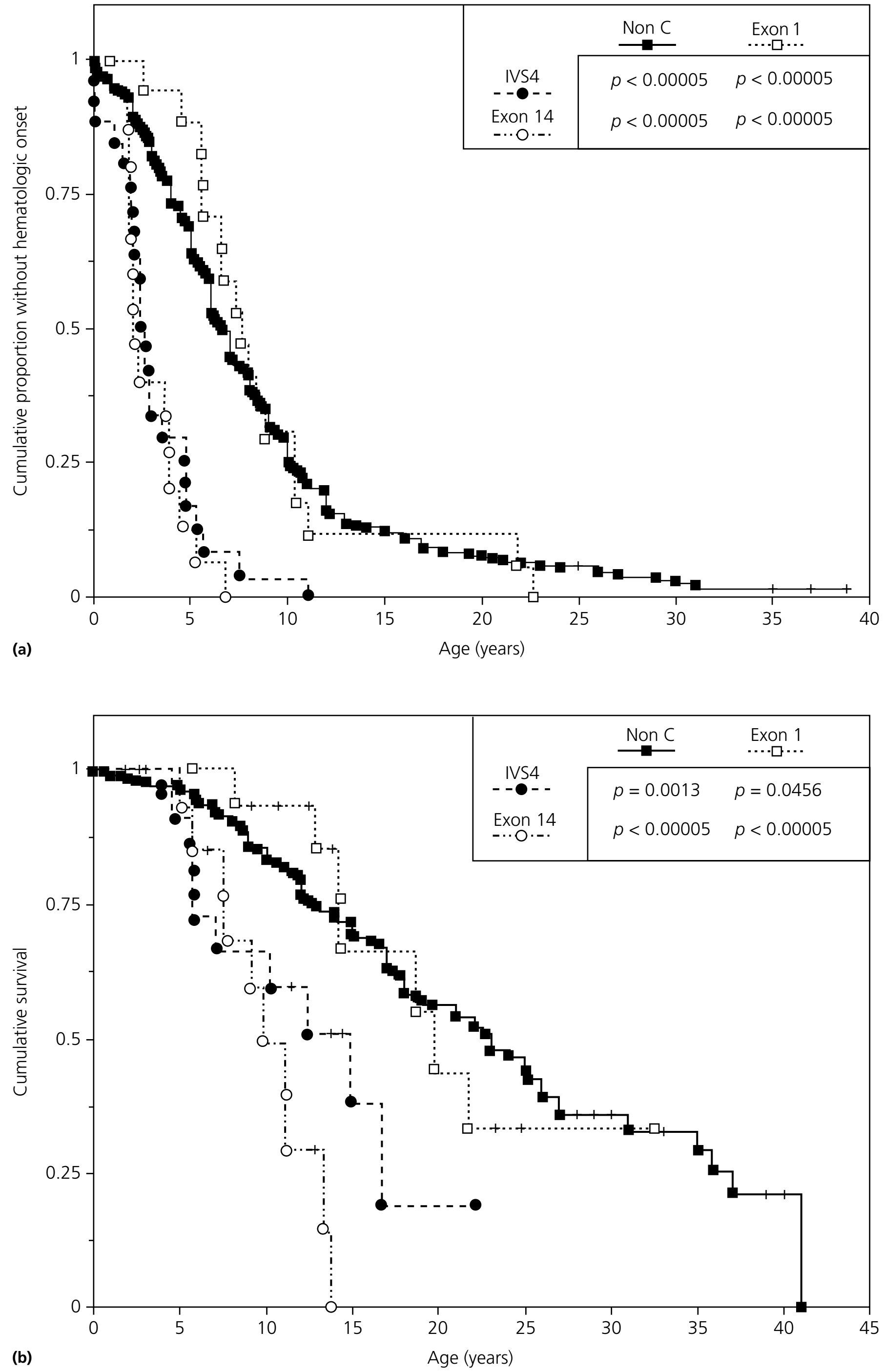

Figure 76.9 Within complementation group C patients ($n = 78$ in the IFAR database) marrow failure occurs at an earlier time point in patients with at least one intron 4 (IVS4+4A>T) or at least one exon 14 (*R548X* or *L554P*) mutation ($n = 50$) compared to patients with at least one exon 1 mutation (*322delG* and *QX13*) and no mutations in exon 14 or intron 4 ($n = 27$). (a) The median age of hematologic disease onset is a 2.7 years (range 0 to 11 years) in the IVS4 group and 2.1 years (range 0.8 to 5.2 years) in the exon 14 group. In comparison, the exon 1 subgroup displays a mild phenotype with few major congenital malformations and a median age of onset of hematologic disease is 7.6 years (range 2.6 to 21.8 years). (b) Earlier hematologic disease onset and a greater number of congenital malformations are associated with poorer survival. IVS4 and exon 14 subgroups experienced median survivals of 14.9 and 9.7 years, respectively. In contrast, exon 1 patients had a median survival of 19.7 years ($p < 0.00005$). Source: Gillio et al. 1997 [81]. Reproduced with permission of the American Society of Hematology.

transplant and potential deleterious effect of androgens on transplant outcome. The physical, mental and psychologic effects, including increased acne and body hair, voice change, testicular shrinkage, acne, mood swings, aggressiveness, mania and depression, cannot be underemphasized. In addition, patients on androgens must be monitored for peliosis hepatis, hepatic adenomata and hepatocellular carcinoma (Figure 76.11) by ultrasound, computerized tomography and/or magnetic resonance imaging

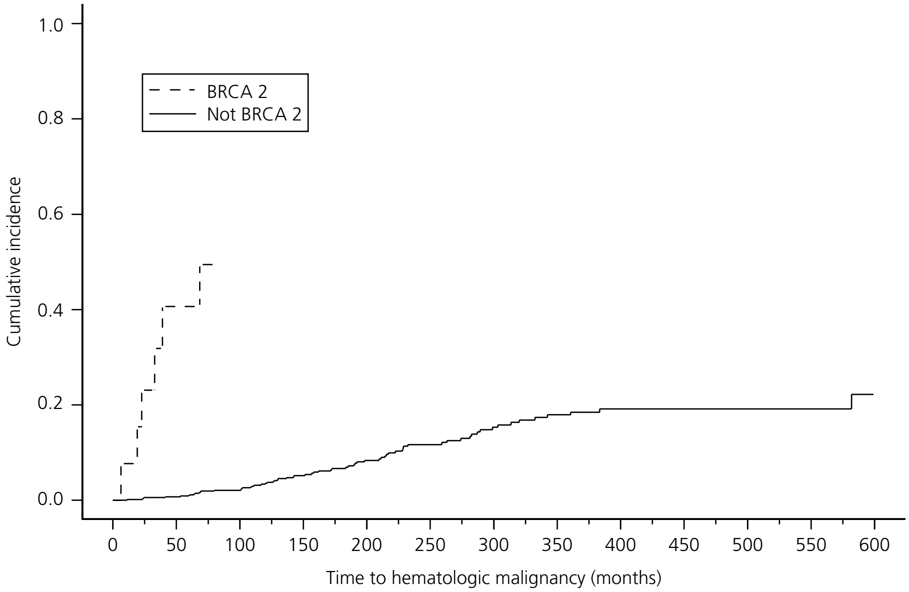

Figure 76.10 Cumulative incidence of hematologic malignancy among *BRCA2* mutation carriers (14 patients) versus all other complementation groups in the IFAR (746 patients). Among FA patients with biallelic mutations in BRCA2, the cumulative incidence of hematologic malignancy is 41% (95% CI, 20–85%) at 5 years of age. In contrast, the incidence of hematologic malignancy in non-*BRCA2* FA patients in the IFAR registry at 5 years is 1% (95% CI, 0.5–2.1%). Source: Wagner et al. 2004 [82]. Reproduced with permission of the American Society of Hematology.

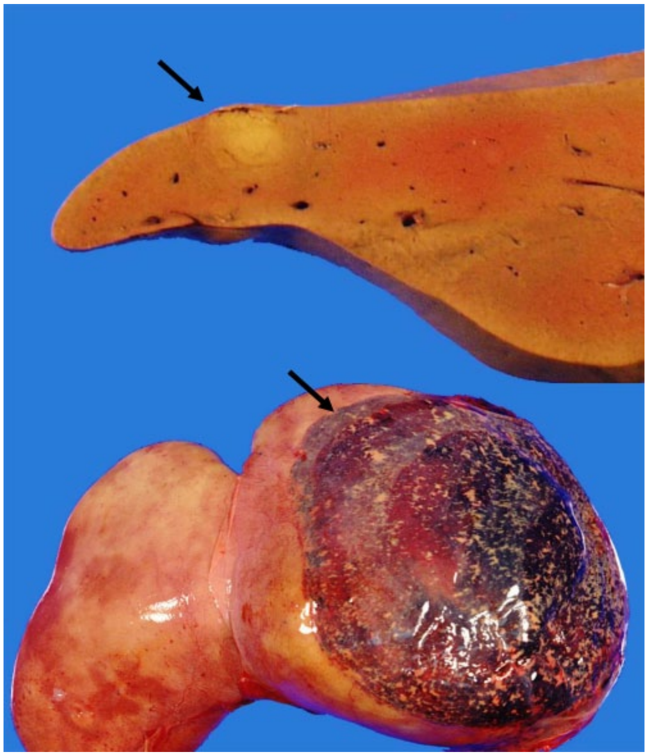

Figure 76.11 Hepatic adenoma. Pathological specimen obtained at autopsy from a patient with FA with multiple adenomata not revealed by ultrasound prior to HCT. Lower panel shows area of massive intrahepatic hemorrhage in one previously unidentified adenoma. Source: Kumar et al. 2004 [89]. Reproduced with permission of Lippincott Williams & Wilkins. (For a color version of this figure, please see the color plate section.)

and alpha fetoprotein levels. Multiple reports in the transplant literature suggest that prior exposure to androgens is an adverse risk factor, limiting the successful use of transplant. As detailed later in "Hematopoietic cell transplantation," outcomes with HCT are no longer poor, making such a recommendation for use of androgens more challenging.

In contrast to idiopathic aplastic anemia, use of antithymocyte globulin (ATG), cyclosporine, high-dose methylprednisolone and other immunosuppressants should have little impact if the mechanism of FA aplasia is loss of the HSC due to constitutive hypersensitivity to TNF-α and IFN-γ or aldehyde toxicity rather than immune attack [73–76]. While there have been some anecdotal reports of benefit after immunosuppression [88], immunosuppressive therapy is generally not expected to be an effective strategy for patients with FA, although this has never been formally evaluated.

Vitamins and antioxidants

Shahidi and others have recommended the use of antioxidants to potentially reduce free radical oxygen toxicity that may play a role in the pathogenesis of the progressive marrow failure and epitheliod malignancy observed in patients with FA [90]. Daily intake of beta-carotene, vitamin C, vitamin E, and selenium has been suggested. A clinical trial with quercetin, a plant flavonoid, has recently been opened to evaluate the safety and optimize the dose. Whether such therapies alter the natural history of marrow failure or risk of hematologic or non-hematologic malignancies (i.e., cervical carcinoma or head and neck cancers) in FA patients is unknown. In contrast, there is probably a role for folic acid and iron supplementation in those receiving therapy to increase the red cell mass. Iron levels, however, should be evaluated prior to the initiation of iron supplementation as some patients will already have excess iron stores for unclear reasons.

Hematopoietic growth factor therapy

Only two pilot studies [91,92] have been reported on the safety and efficacy of hematopoietic growth factor therapy in the treatment of neutropenia in patients with FA nearly a decade ago. While GM-CSF and G-CSF have both been used successfully to increase the neutrophil counts with little systemic toxicity, it is unknown whether such therapy will alter the risk of MDS, development of clonal cytogenetic abnormalities or risk of leukemia, if used long term. Erythropoietin has also been used to increase red cell counts and decrease the need for red cell transfusions but no formal study has been published. However, the fact that many FA patients have highly elevated endogenous erythropoietin levels may limit the usefulness of this treatment in the majority. While long-term use of hematopoietic growth factor therapy has not been studied, the most common use in FA patients is as a bridge to transplant or immediately after transplant to speed neutrophil recovery from the donor graft.

Supportive care

Eventually, FA will result in marrow failure, necessitating blood product support for treatment of thrombocytopenia and anemia. Epsilon-aminocaproic acid and hematopoietic growth factor therapy with antibiotics are short-term remedies for FA patients with thrombocytopenia and bleeding or neutropenia and fever, respectively. For the patient not pursuing allogeneic HCT as a treatment option whether because of severe comorbidities, age, or lack of a suitable donor, such combination therapy can aid in sustaining life for years. Risk of iron overload, however, should be minimized by early chelation therapy. For the patient considering allogeneic HCT, use of red cell and platelet transfusions should be avoided if possible to reduce the risk of alloimmunization. If transfusions are required, an effort should be made to transfuse only irradiated, cytomegalovirus-negative (or filtered) red cell or single donor platelet products. Whether specific, selected volunteer donors should be identified to limit exposure to human leukocyte antigens (HLAs) has not been proven. As detailed in the next section, history of transfusions and prior opportunistic infections are adverse risk factors after transplant. Therefore, moving to transplant prior to severe cytopenia would be advantageous if it is possible.

It is important that the clinician consider strategies for reduced exposure to agents potentially affecting hematopoiesis even though a decline is ultimately unavoidable in most FA patients. For example, every effort should be made to avoid marrow suppressive drugs, such as antibiotics, like Bactrim®, often used in the treatment of chronic otitis media or urinary tract infections. As summarized earlier in "Mechanisms of marrow failure," FA HSC hypersensitivity to TNF-α argues for examining ways for reducing exposure to viruses generally and TNF-α specifically. While it is not possible to eliminate all exposures, young children with FA should be immunized against Varicella-Zoster virus and influenza which has been associated with severe marrow suppression after such viral infections. Alternatively, treatment with anti-TNF-α receptor Fc fusion protein (Etanercept; Enbrel) may prevent or reduce the rate of progression of marrow failure in children and adults with FA. This study was recently completed and results are pending.

Hematopoietic cell transplantation

As of 2015, allogeneic HCT remains the only treatment with the potential of correcting the hematologic manifestations of FA. Although allogeneic HCT for FA is still associated with risk, the risk profile has substantially diminished over time. Poor outcomes in the 1980s and 1990s were primarily the result of excessive regimen-related toxicity (RRT), graft failure and severe acute graft-versus-host disease (GVHD) [93–96]. The earliest conditioning regimens were based upon those designed for patients with acquired AA and acute leukemia, administering cyclophosphamide (CY) at doses of 200 mg/kg with or without irradiation and cytotoxic agents. This dismal early clinical experience prompted in vitro laboratory studies that confirmed the hypersensitivity of FA cells to alkylating agents [97,98] and irradiation [99]. As a result, Gluckman et al. proposed the use of low-dose CY (20 mg/kg) and single fraction of thoraco-abdominal irradiation (TAI, 500 cGy) in the early 1980s, resulting in markedly reduced RRT and enhanced survival after HLA-matched sibling donor HCT [100]. Besides better donor-recipient HLA matching and supportive care applied to all patients, the specific addition of fludarabine (FLU) and incorporation of marrow T-cell depletion reduced the risks of graft rejection and severe GVHD, leading to improved survival. Current efforts are now aimed at reducing the risks of opportunistic infections and late effects, especially endocrinopathies, infertility, and malignancy [101].

HLA-identical sibling donor HCT

Historical perspective

For FA patients with HLA-identical sibling donors, HCT using the Gluckman strategy of reduced-intensity radiation was generally associated with excellent outcome if performed prior to the development of MDS or leukemia, and within the first two decades of life. Factors associated with higher survival included younger age at transplant, higher pre-transplant platelet count, lower dose conditioning regimen, and improvements in GVHD prophylaxis [94]. In an analysis of 209 FA patients with an HLA-identical sibling donor transplanted between 1994 and 1999, the 3-year survival was 81% in patients aged <10 years of age ($n = 109$) and 69 ± 10% in older patients ($n = 100$) (Figure 76.12) [102]. Since that time, survival has only continued to improve in most series.

Current results

Today fewer patients with an HLA-identical sibling donor receive radiation in an attempt to reduce the late effects commonly associated with radiation. Outcomes with and without irradiation are summarized in Tables 76.3 and 76.4 [103–110]. Bonfirm et al. [110] reported results in 85 FA patients (median age 9 years, range 3–34) treated in Brazil between 1999 and 2011. Patients either had aplastic anemia ($n = 82$) or MDS ($n = 3$), were conditioned with CY 15 mg/kg × 4 days (60 mg/kg total dose) and received methotrexate (MTX) and cyclosporine (CSA) as GVHD immunoprophylaxis. All patients received bone marrow from HLA-identical siblings ($n = 74$) or other relatives ($n = 11$). Neutrophil recovery occurred in 77/83 (93%) with primary graft failure in two and secondary graft failure in four, occurring at a median of 244 (range, 152–365) days after HCT. Of the six patients with graft failure, four are alive after second transplant using the same donor. Notably, all three patients with MDS relapsed after HCT and died from progressive disease despite second transplant. Overall, the survival is 85% at 5 years with 96% survival in those aged <10 years ($n = 48$) at the time of transplant. Of the 81 evaluable patients, 17 developed grade II–IV and 7 grade III–IV acute GVHD. Of 78 evaluable for chronic GVHD, 23 had chronic GVHD (extensive in 12). In multivariate analysis, only acute and chronic GVHD were factors associated with lower overall survival. Transplant-related mortality at day 100 and 1 year were 6% and 10%, respectively, with the major causes of death being graft failure and GVHD. To date, four patients have developed squamous cell carcinoma (SCC) of the oral cavity between 2 and 5 years after HCT with all SCC patients having had a history of extensive chronic GVHD with oral mucosa involvement with only one alive and free of disease.

In an attempt to reduce the risk of acute and chronic GVHD, Tan and MacMillan et al. ([105], updated by M MacMillan, personal communication) incorporated ex vivo and in vivo marrow T-cell depletion. Based on promising results by Aker et al. [111], patients were conditioned with CY 5 mg/kg × 4 days (20 mg/kg total dose), FLU 35 mg/m^2 × 5 days (175 mg/m^2 total dose), and antithymocyte globulin 30 mg/mg × 5 days (ATG; 150 mg/kg total dose) followed by the infusion of T-cell-depleted marrow by CD34 selection with CsA and methylprednisolone [and later mycophenolate mofetil (MMF)] immunoprophylaxis. Thus far, 23 patients (median age 8.5 years; range: 3.2–43.3) have been treated [bone marrow in 15 and umbilical cord blood (UCB) in 8] [105]. Primary neutrophil recovery occurred in 100% and no patient had grade III–V regimen-related toxicity with only one patient having grade II–IV acute GVHD and one chronic GVHD. The overall survival is 82% at 5 years with a median follow-up of 5.4 years (range 2.1–11.2 years). Transplant mortality at day 100 and 1 year was 4% and 13%, respectively. To date, no patient has developed SCC. Of the three deaths, one each died of chronic GVHD, recurrent MDS, and donor-derived AML. This regimen was also used in one other patient with an HLA-matched maternal donor who died from chronic GVHD after a second HCT for the treatment of secondary graft failure.

In 2008, on behalf of the CIBMTR, Pasquini et al. [112] compared transplant outcomes in recipients conditioned with (n = 77) and without (n = 71) irradiation-containing regimens prior to HCT with HLA-identical sibling donors. Hematopoietic recovery, acute and chronic GVHD, and mortality were similar between the two groups. In multivariate analysis, higher mortality was associated with age >10 years, prior use of androgens, and cytomegalovirus (CMV) seropositivity in either the donor or recipient. With a

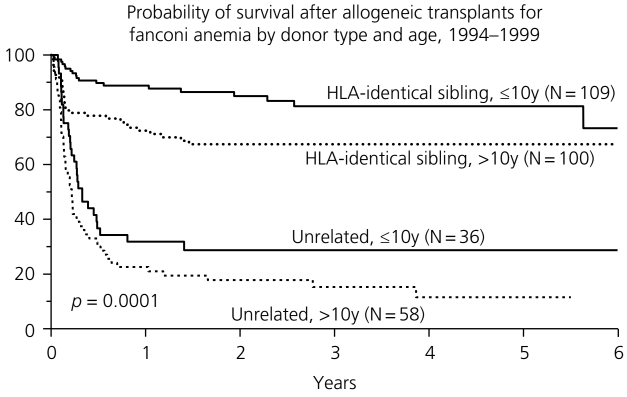

Figure 76.12 Probability of survival after human leukocyte antigen (HLA)-identical sibling donor HCT in patients with FA as reported to the International Bone Marrow Transplant Registry (IBMTR). Source: Reproduced with permission of IBMTR/Autologous Blood and Marrow Transplant Registry (ABMTR) 2002 [102].

Table 76.3 HLA-identical sibling donor HCT: clinical results with radiation-based regimens

Reference	Conditioning regimen	GVHD Prophylaxis	# cases	Median recipient age (years, range)	Sustained engraftment	Acute GVHD (grades II–IV)	Chronic GVHD	Survival
Dufour et al. 2001 [103]	CY 20 mg/kg + TAI 5–6 Gy (n = 12) CY 20–80 mg/kg, TBI 3–6 Gy (n = 10)* CY 100–200 (n = 5)	CSA (n = 18) CSA + MTX (n = 8) MTX alone (n = 1)	27	9 (2.5–19.5)	92%	36%	13%	81% alive at 3 years
Farzin et al. 2007 [104]	CY 20 mg/kg + TAI 4 Gy + ATG	CSA +MP	30	7.6 (2.7–22.9)	97%	23%	12%	89% alive at 10 years

*Includes two patients who received phenotypic identical parental donor HCT.

ATG, antithymocyte globulin; CSA, cyclosporine A; CY, cyclophosphamide; GVHD, graft-versus-host disease; MP, methylprednisolone; MTX, methotrexate; TAI, thoraco-abdominal irradiation; TBI, total body irradiation; TCD, T-cell depletion.

Table 76.4 HLA-identical related donor HCT: clinical results with chemotherapy only-based regimens

Reference	Conditioning regimen	GVHD Prophylaxis	# cases	Median recipient age (years, range)	Sustained engraftment	Acute GVHD	Chronic GVHD	Survival
Tan et al. 2006 [105]*	FLU 125 mg/m^2 + CY 20 mg/kg + ATG	CSA/MP	23	8.5 (3.2–43.3)	96%	4%	none	87% alive (median follow-up 5.4 years)
Torjemane et al. 2006 [106]	CY 40 mg/kg + BU 6 mg/kg ± ALG	CSA/MTX	17	11 (3–22)	82%	23%	13%	72% alive (median follow-up 16 mos)
Ayas et al. 2008 [107]	CY 60 mg/kg + ATG	CSA	34	8.2 (1.5–14.9)	100%	9%	6%	97% alive (median follow-up 33.7 mos)
Ertem et al. 2009 [108]	BU 6 mg/kg + CY 40 mg/kg (n = 2) FLU 150 mg/kg + CY 20 mg/kg + ATG (n = 6)	CSA +/- MTX	8	12 (6.5–14.0)	100%	none	13%	7/8 alive (median follow-up 2.5 yrs)
Ayas et al., 2012 [109]	FLU 150 mg/m^2 + CY 20 mg/kg + ATG	CSA +/- ATG	19	9.1 (1.4–12.9)	100%	5%	5%	100% alive (median follow-up 19 mos)
Bonfirm et al. 2012 [110]	CY 60 mg/kg	CSA/MTX	85	9 (3–34)	98%	21%	29%	85% alive at 5 years

*Updated.

ALG, lymphoglobulin; ATG, antithymocyte globulin; BU, busulfan; CY, cyclophosphamide; FLU, fludarabine; GVHD, graft-versus-host disease; MP, methylprednisolone; MTX, methotrexate.

median follow-up of more than 5 years for both groups, the overall survival was 78% and 81% at 5 years ($p = 0.61$), respectively. Whether a difference in late effects, for example infertility/sterility, cataracts, endocrinopathies or risk of SCC, remains to be determined. While not yet proven, use of radiation may be important in those with MDS or more advanced disease.

Alternative donor HCT

Unfortunately, the majority of FA patients do not have an HLA-identical unaffected sibling donor. Although deliberate conception and in vitro fertilization (IVF) with preimplantation genetic diagnosis (PGD) have been considered as a means to have a healthy sibling that is also HLA identical with the existing child with FA ([113,114], see "Genetic counseling"), time, maternal age, cost and personal beliefs limit its wider use. However, the need for such an option has decreased as the results with alternative (i.e., an HLA-mismatched related or HLA-matched or -mismatched unrelated) donor HCT are increasingly comparable to that observed after HCT with an HLA identical sibling donor.

Historical perspective

Before 2000, the principal obstacle to the successful use of alternative donor HCT was graft failure. Excessive conditioning led to unacceptable rates of regimen-related toxicity. Insufficient conditioning led to unacceptable rates of graft failure. Overall, approximately 25–35% of FA patients failed to engraft after alternative donor HCT [94–96]. Conditioning regimens most often consisted of CY (15–25 mg/kg), TBI (300–800 cGy) with or without ATG. In some cases, the presence of DEB-resistant T cells may have increased the risk of graft rejection; however, in others it was likely multifactorial potentially due to HLA mismatch, early infections and/or the presence of anti-HLA antibodies in those heavily transfused. After 2000, with the addition of FLU to the conditioning regimen, the risk of graft failure dramatically decreased. Based on early observations, many centers incorporated FLU into the conditioning regimen with markedly improved results [115–119]. In 2007, Wagner et al. [80] reported the outcomes of unrelated donor HCT in patients with FA ($n = 98$) on behalf of the CIBMTR. This was the first multi-institutional analysis documenting improved engraftment in recipients of FLU-containing regimen compared to those without FLU (Figure 76.13). MacMillan et al. [119] more recently reviewed the outcomes of FA patients who underwent alternative donor HCT at the University of Minnesota since 1990. While higher dose TBI (600 cGy) failed to reduce the risk of graft failure, addition of FLU significantly enhanced the probability of neutrophil recovery ($p < 0.01$). Recipients of a FLU-based regimen were nearly three times more likely to achieve neutrophil engraftment (RR 2.9; 95% CI, 1.6–5.1; $p < 0.01$). Use of androgens prior to HCT (RR 0.6; $p = 0.04$) and UCB (RR 0.2; $p < 0.01$) as a stem cell source were two factors adversely affecting engraftment.

The second major hurdle to successful alternative donor HCT was GVHD. In 2000, Guardiola et al. [95] on behalf of the European Group for Blood and Marrow Transplantation (EMBT) and the European Fanconi Anemia Registry reported the outcomes of unrelated donor HCT in 69 patients with FA and found that GVHD was the primary cause of death ($n = 22$). The incidence of grade II–IV and grade III–IV acute GVHD was 43% and 34%, respectively. In multivariate analysis, factors associated with GVHD were absence of T-cell depletion (RR, 20.0; $p = 0.004$), malformations of the urogenital tract and/or the kidneys (RR, 6.60; $p < 0.02$), elevated serum alanine/aspartate transaminases prior to conditioning (RR, 2.52; $p < 0.04$), and limb malformations (RR, 2.55; $p < 0.04$). In a more recent CIMBTR analysis, Wagner et al. [80] reported a high risk of grade II–IV acute GVHD in recipients of a non T-cell-depleted graft (RR, 4.29; 95% CI, 1.82–10.10; $p < 0.001$). In the cohort conditioned with FLU, the incidence of grade II–IV acute GVHD at day 100 was 70% and 21%, respectively in FA recipients of non T-cell-depleted and T-cell-depleted bone marrow. At the University of Minnesota, T-cell depletion (first with counterflow elutriation and subsequently with CD34 selection) was incorporated into the treatment protocol in 1994. Comparing T-cell-depleted marrow to T-replete marrow and UCB, the incidence of grade II–IV acute GVHD was significantly lower in recipients of T-cell-depleted marrow (18%) as compared to 50% and 38% in recipients of T-replete marrow and UCB, respectively ($p < 0.01$). Similarly, grade III–IV acute GVHD was also significantly lower in T-cell-depleted marrow recipients (6% vs. 38% vs. 21%, respectively; $p < 0.01$). T-cell depletion also reduced the incidence of chronic GVHD (9% vs. 25% vs. 17%; $p < 0.04$). In a report by Eurocord–Netcord and the EBMT, similar rates of acute GVHD (32%) and chronic GVHD (16%) were observed in UCB recipients [120]. Importantly, in a retrospective analysis of 37 FA patients who underwent HLA-identical bone marrow transplantation at the Hôpital Saint Louis, grades II–IV acute GVHD was a significant predictor of head and neck SCC, which was only observed in FA patients experiencing one of these two complications. The 10-year cumulative incidence of head and neck SCC in FA patients experiencing grades II–IV acute GVHD was 28% versus 0% for patients without GVHD [121].

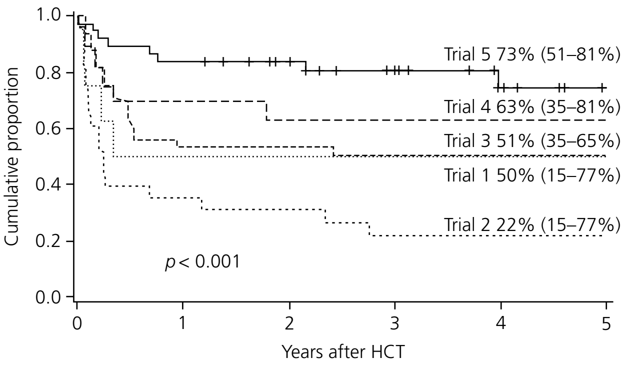

Figure 76.13 Probability of survival after alternative donor HCT for the treatment of patients with standard risk FA at the University of Minnesota between 1990 and 2012. One hundred and twenty-seven FA patients underwent AD-HCT at the University of Minnesota using one of five consecutive, prospective phase I–II total clinical trials. Trial 1: CY40 mg/kg + TBI 450 cGy + T-replete marrow; Trial 2: CY40 mg/kg + TBI 450 cGy + ATG + T-depleted marrow or UCB; Trial 3: CY40 mg/kg + FLU 140 mg/m^2 + TBI 450 cGy + ATG + T-depleted marrow or UCB; Trial 4: CY40 mg/kg + FLU 140 mg/m^2 + TBI 450 cGy (with thymic shielding) + ATG + T-depleted marrow or UCB; and Trial 5: CY40 mg/kg + FLU 140 mg/m^2 + TBI 300 cGy (with thymic shielding) + ATG + T-depleted marrow or UCB.

As shown in Figure 76.12, FA patients transplanted with marrow from alternative donors 15–20 years ago had survival rates <30% with excessive rates of regimen-related toxicity, graft rejection, GVHD, and opportunistic infections being major obstacles [94]. However, improvements in HLA typing and supportive care as well as changes in the treatment protocol led to series of incremental improvements that together completely alter the expected outcome for FA patients transplanted with marrow or UCB from an alternative donor today (Table 76.5).

Table 76.5 Alternative donor HCT: clinical results since 2000

Reference	Conditioning regimen	GVHD prophylaxis	No. of cases	Recipient age (years)	Sustained engraftment	Acute GVHD	Chronic GVHD	Survival
Guardiola et al. 2000 [95]	Varied	Varied	69	10.8 years (4.0–37.4 years)	83%*	34%	42%	33% alive at 3 years
Wagner et al. 2007 [80]	Varied	Varied	98	12 years (0.8–33 years)	89% (FLU) 69% (no FLU)	21% (TCD) 70% (no TCD)	31%	52% (FLU) at 3 years 13% (no FLU) at 3 years
Gluckman et al. 2007 [120]	Varied	Varied	93	8.6 years (1–45 years)	60%	32%	16%	74% (HLA 6/6, $n = 12$) 48% (HLA 5/6, $n = 35$) 25% (HLA 3–4/6, $n = 45$)
Chaudhury et al. 2008 [116]	CY 40 mg/kg TBI 450 cGy FLU 150 mg/m^2 rATG 10 mg/kg	TCD, tacrolimus	18	11 years (5.5–24 years)	100%	6%	6%	14/21 alive
De Medeiros 2006 [124]	Varied	Varied	47	9 years (3–31)	51%	47%	23%	38% (median 18 months)
Bonfirm et al. 2012 [118]	CY 60 mg/kg FLU 125 mg/m^2 rATG 4–6 mg/kg	CSA/MTX	33	10 years (5–18 years)	88%	39%	42%	79% at 3 years
MacMillan et al.2012 [119]	CY 40 mg/kg TBI 300 with thymic shielding FLU 140 mg/m^2 ATG 150 mg/kg	TCD+CSA	48	8.8 years (1.7–34.3 years)	100%	12%	6%	78% (median of 3 years)

ATG, antithymocyte globulin; CSA, cyclosporine A; CY, cyclophosphamide; FLU, fludarabine; rATG, rabbit antithymocyte globulin; TBI, total body irradiation; TCD, T-cell depletion.

Regardless of donor type, transplant outcome is markedly influenced by the disease status at the time of HCT. As expected, FA patients with either advanced MDS (≥5% blasts) or leukemia have a significantly lower probability of survival. The apparent successful eradication of the malignant clone in some patients with generally reduced doses of chemotherapy with or without transplant suggests that the malignant cells carry a similar hypersensitivity to alkylating agents and irradiation, characteristic of FA non-malignant hematopoiesis [122–124].

Current expectations

Today, it is expected that donors can be found for most everyone with a high likelihood of survival after HCT performed at centers experienced in FA. At the University of Minnesota, 48 patients with FA (age range 1.7 to 34.3) with lineage cytopenia and aplastic anemia in most, received FLU, CY, ATG, and low dose TBI (300 cGy) followed by T-cell-depleted 7–8/8 HLA-matched bone marrow ($n = 32$) or HLA-mismatched umbilical cord blood ($n = 16$) if an unrelated donor was unavailable. All recipients of marrow engrafted at a median of 11 days (range, 9–23). In contrast, the incidence of engraftment was 88% at a median of 19 days (range, 10–40) in recipients of UCB. The incidence of acute and chronic GVHD was low at 12% and 6%, respectively, with similar outcomes in patients transplanted with marrow and UCB. The overall survival for the entire cohort was 78% at a median of 2.9 years (range 0.6–6.3). Survival appears superior in recipients of marrow relative to those transplanted with UCB. In 2014, MacMillan et al. [123] also reported on 21 FA patients with AML ($n = 16$), acute lymphocytic leukemia (ALL; $n = 2$), or advanced MDS with >5% blasts ($n = 3$) treated with alternative donor HCT between 1988 and 2009 [123]. Five patients had biallelic FANCD1/BRCA2 mutations. Only two of seven patients who received chemotherapy before HCT achieved remission both of whom had FANCD1/BRCA-2 mutations and T-cell ALL. For the entire cohort, the median age was 15.5 (range 1.1–48.5) years. Median age for FANCD1/BRCA2 patients was substantially lower at 1.9 (range 1.4–6.6) years. Patients received CY, ATG, and either single fraction TBI ($n = 15$) or busulfan (BU) ($n = 6$), with FLU in 11, followed by alternative HCT in 18. GVHD prophylaxis consisted of CSA and MP. Probability of neutrophil engraftment was 75% without FLU and 100% with FLU-based regimens. Probability of acute GVHD was 19%. For the entire cohort, probability of survival at 1 year was 43% with a higher survival (60%) in the 11 patients who received a FLU-based regimen. In these 11 patients, the incidence of relapse was 21%. These data suggest that HCT with a FLU-based preparative regimen offers potential curative therapy for FA patients with acute leukemia or advanced MDS. Except perhaps for FANCD1/BRCA2 patients with T-cell ALL, there was no clear role for chemotherapy prior to HCT in FA patients with AML or MDS.

Recently, the results of a multi-institutional study were reported [125] exploring a non TBI-containing regimen in patients undergoing unrelated donor HCT. Between 2009 and 2012, 27 patients (median age 8.1 years) most often with lineage cytopenia or aplastic anemia (78%) received FLU, CY and ATG in combination with BU followed by transplantation of T-cell-depleted peripheral blood stem cells. All patients engrafted with one having secondary graft failure. Grade II–IV GVHD occurred in only one patient. Grade II–IV toxicities included pulmonary and hepatotoxicity ($n = 6$ for each) with one patient developing hepatic veno-occlusive disease. Although the median follow-up was short (<8 months, range 0.5–37.8 months), 19 of 23 with sufficient follow-up were alive at the time of the report. Deaths were due to acute respiratory failure, multiorgan failure, severe pulmonary hypertension, and infection.

Summary

Together, the clinical data suggest:

1. A good survival outcome can be expected for FA patients transplanted with either an HLA-identical sibling or unrelated donor HSC especially when the patient is young, has had few red cell and/or platelet transfusions, and prior to the development of MDS or leukemia.
2. HCT without radiation is increasingly the norm in FA patients with an HLA-identical sibling donor. Reduced exposure to DNA cross-linking agents, such as high-dose CY or irradiation, minimizes the risk of early and late toxicities, including ovarian failure and endocrinopathies beyond that inherent in the disease. In contrast higher dose therapy is generally required for HCT using alternative donors. While the largest experience is with low-dose CY in combination with FLU and lower dose TBI prior to the infusion of T-cell-depleted marrow or UCB,

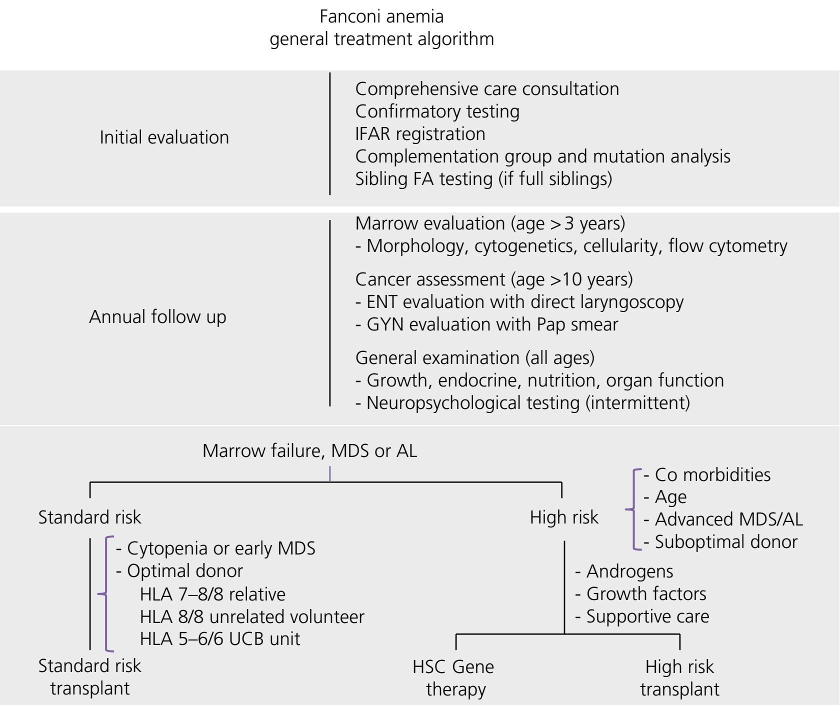
Fanconi anemia
general treatment algorithm
Initial evaluation
Comprehensive care consultation
Confirmatory testing
IFAR registration
Complementation group and mutation analysis
Sibling FA testing (if full siblings)
Annual follow up
Marrow evaluation (age > 3 years)
- Morphology, cytogenetics, cellularity, flow cytometry
Cancer assessment (age >10 years)
- ENT evaluation with direct laryngoscopy
- GYN evaluation with Pap smear
General examination (all ages)
- Growth, endocrine, nutrition, organ function
- Neuropsychological testing (intermittent)
Marrow failure, MDS or AL
Standard risk
High risk
- Co morbidities
- Age
- Advanced MDS/AL
- Suboptimal donor
- Cytopenia or early MDS
- Optimal donor
HLA 7–8/8 relative
HLA 8/8 unrelated volunteer
HLA 5–6/6 UCB unit
- Androgens
- Growth factors
- Supportive care
Standard risk transplant
HSC Gene therapy
High risk transplant

Figure 76.14 Proposed treatment algorithm for patients with FA. MDS, myelodysplastic syndrome; AL, acute leukemia; ENT, ear, nose and throat specialists; GYN, gynecology.

higher dose CY alone prior to T-replete HSC has met with similar early survival outcomes. Encouraging early results have also been reported (but not yet published) with BU, CY and FLU prior to T-cell-depleted marrow or UCB. In contrast to some non-US centers, T-cell depletion has been favored in the USA in an attempt to reduce the risks of acute and chronic GVHD, the only known risk factors for SCC in FA patients treated by HCT. As early survival has markedly improved, it should be clear in the near future what the impact of T-cell depletion is on SCC risk.

3 HCT without prior chemotherapy has curative potential in some FA patients with advanced MDS and acute leukemia. It is unclear whether pre-transplant chemotherapy to reduce the leukemia burden has any impact on long-term survival. For patients with preexisting severe comorbidities or older age (for example, age >35 years), alternate therapies, such as with androgens, transfusions with chelation, and/or hematopoietic growth factors, should be considered rather than HCT.

Over the last two decades sequential changes to the approach to alternative donor HCT has resulted in significant improvements. The two pivotal changes have been the addition of FLU to improve engraftment rates and the use of T-cell depletion to reduce GVHD. Improved outcomes are also due in part to the fact that FA patients are referred prior to the development of advanced MDS, leukemia, and opportunistic infections. Despite substantial improvement in survival, the greatest effort needs to be focused on reducing the morbidities associated with transplant, especially caused by toxicity and opportunistic infections. Additionally, emphasis is now being placed on developing approaches to HCT, which will improve quality of life by reducing late effects, particularly the risk of malignancy, sterility, and endocrinopathies. In light of the complexity of FA, it is recommended that HCT for FA be performed at select centers experienced in the care of FA patients with and without transplant, which will also provide long-term care monitoring for late effects of the disease and therapeutic interventions employed. A treatment algorithm for FA patients with bone marrow failure, MDS, or leukemia is proposed (Figure 76.14).

Cancer risk after HCT

As previously stated, FA is characterized by a marked predisposition to leukemia and solid tumors. In contrast to the general population, these cancers occur at remarkably young ages likely secondary to the underlying DNA repair defect and genomic instability. In order to better understand the relative risk of cancer after transplant, Rosenberg et al. [126] surveyed FA patients with and without transplant in the USA and in Canada. In this study 27 cancers were observed in 23 individuals of 145 respondents. Of the 27 cases, 18 had solid tumors. In contrast to the median age of leukemia diagnosis (11.3 years, range: 3–24 years), FA patients with solid tumors were older (28.9 years, range: 7–45 years). Remarkably, the observed-to-expected ratio of solid tumors of the head and neck, esophagus and vulva was 706, 2362, and 4317, respectively, relative to age-matched controls. For FA patients, the relative risk of cancer remained low (9%) until age 28 years. By ages 34 and 48 years, the relative risk was 22% and 29%, respectively. As shown in Figure 76.2, the rise in relative risk of solid tumors is non-linear and without a plateau. Reports from the IFAR are similar with a 28% incidence of solid tumors by age 40 [11]. While numerous types of solid tumors have been observed (Table 76.6), there is a marked predisposition toward SCC, especially of the upper aerodigestive and anogenital regions. Compared to the Surveillance, Epidemiology and End Results (SEER) of the general population, FA patients have a 500-fold higher risk of solid tumors [18].

In 1996, Deeg et al. [127] published the seminal paper on the risk of cancer after HCT for FA or severe aplastic anemia. In an analysis of 700 patients (79 with FA and 621with non-FA aplastic anemia) treated with allogeneic HCT in Seattle or Paris, the Kaplan–Meier estimate for developing any malignancy by 20 years after HCT was 14%. Among patients with FA, a single hazard peak for solid tumors occurred between 8 and 9 years after HCT with a probability of developing any malignancy of 42% (95% CI,

Table 76.6 Observed cancers (O), ratio of observed to expected cancers (O/E), and 95% confidence intervals (95% CI) among North American respondents with Fanconi anemia

Type of cancer	O†	O/E‡	95%CI§
Leukemia (AML)	9	785*	360–1490
Head and neck	6	706*	260–1540
Esophagus	2	2362*	265–8530
Liver	2	386*	45–1395
Vulva	3	4317*	870–12,615
Cervix	2	179*	20–645
Osteosarcoma	1	79	1–440
Soft tissue sarcoma	1	49	0.6–270.0
Brain	1	17	0.2–95.0
Total cancers	27	50*	35–80
Total solid tumors	18	48*	30–80

Source: Rosenberg et al. 2003 [20]. Reproduced with permission of the American Society of Hematology.

*$p < 0.05$ that true O/E ratio equals 1.0 (exact two-sided tests).

†Twenty-seven cancers observed in 23 patients. Two patients had two solid tumors (cervix and vulva, and vulva and esophagus) and one had three solid tumors (esophagus, liver, and cervix).

‡Expected cancer incidence rates calculated from the Connecticut Tumor Registry.

§Limits of the 95% CI rounded to the nearest 5 for values ≥10.

AML, acute myeloid leukemia.

10–74%) by 20 years after HCT with all being solid tumors. In multivariate analysis, two factors were found to be associated with risk of cancer, a diagnosis of FA (RR, 11.2; $p = 0.0001$) and treatment with azathioprine (RR, 11.7; $p = 0.0001$) used for the treatment of chronic GVHD. Within the group of patients with aplastic anemia, use of radiation was another factor associated with cancer risk. Importantly, the effect of radiation on cancer risk could not be independently evaluated in the FA cohort because use of radiation was the standard practice. Still, this study had a profound impact on the FA community.

In 2005, Rosenberg et al. [128] evaluated the risk of SCC and death in patients with FA who did and did not receive HCT. The transplant cohort ($n = 117$) underwent transplant at the Hôpital St. Louis; non-transplanted patients ($n = 145$) were those that participated in the North American Survey (NAS) [126], a retrospective natural history cohort with validated cancer diagnoses. The age-specific hazard of SCC was 4.4-fold higher and occurred at a younger age in transplanted patients (18 vs. 33 years; $p = 0.004$). However, the risk of cancer in transplanted FA patients was 33-fold higher in those with a history of acute GVHD and infinitely higher in those with chronic GVHD. Once diagnosed with SCC, survival was uniformly poor regardless of prior treatment. Similar results were observed in an independent analysis of the German Fanconi Anemia Registry ($n = 181$ FA patients). Rosenberg et al. [129] found that patients with FA were at an extraordinary risk of cancer with observed to expected ratios of 26 for all solid tumors. However, when focused on individual solid tumors, the observed-to-expected ratios were 6281 for esophageal, 2411 for vulvar, and 240 for head and neck cancers. Moreover, the risk of solid tumor was even higher after HCT (3.8-fold).

Based on these observations, there are several major conclusions: (i) the risk of solid tumor is high in FA patients regardless of transplant; (ii) in transplant and non-transplant patients with FA, solid tumors are very rare in the first decade of life, except in FA patients with biallelic mutations in FANCD1/BRCA2 and FANCN/PALB2, and the peak incidence is in the third and fourth decades; (iii) HCT is associated with an increased incidence of solid tumor and occurs at an earlier time point relative to that observed in non-transplant patients; and (iv) the increased incidence after HCT is driven by GVHD.

To address the role of HCT further, we evaluated the clinical dataset of 185 FA patients transplanted at the University of Minnesota between 1976 and 2015. As we had focused on reducing the risk of GVHD early on (1994) either by incorporating T-cell depletion in all marrow recipients or using umbilical cord blood if a closely HLA-matched donor was not available, it is possible that cancer risk was low. With a maximum follow-up of 15.4 years and 79 patients alive >3 years after HCT, six cancers were observed (three SCC of the tongue, larynx, and salivary gland; one adenocarcinoma of the lung). The interval from transplant to solid tumor diagnosis was 0.9 to 15.5 years, respectively. The age at diagnosis was 20.5, 32.3, 41.0, and 42.1 years. At 45 years of life, the incidence of solid tumor was 14% (95% CI, 2–14%), lower than expected based on prior report. Together these data suggest that higher risk of solid tumors previously observed in FA patients undergoing HCT may be driven by GVHD. Of note is the poor overall survival after treatment of FA patients with head and neck SCC, since standard treatment for SCC incorporates surgery in combination with radiotherapy and chemotherapy with DNA cross-linking drugs. In addition to severe mucositis which is difficult to manage in this population, pancytopenia is common in FA patients developing SCC without HCT and may lead to further complications, particularly bleeding and infection [130].

Why this is significant to the transplant team? All long-term survivors of FA with or without a history of allogeneic HCT are at high risk of cancer. Rigorous surveillance and cancer prevention programs are critical for the survival of FA patients particularly after the age of 20 years. However, new data suggest that SCC may not be higher in FA patients treated with allogeneic HCT as long as GVHD is prevented regardless of the conditioning (TBI vs. chemotherapy only) employed. Therefore, [in FA patients without leukemia], treatment plans that do not incorporate appropriate strategies for in vivo or ex vivo T-cell depletion are not acceptable based on the consequent high risk of cancer.

Genetic counseling

All patients and their families should receive genetic counseling from a professional knowledgeable about FA and the unique issues specific to this patient population. The consultation should include a discussion of the mode of inheritance, the phenotypic consequences of FA, genetic testing options, and reproductive counseling as well as obtaining a detailed health and family history with a particularly focus on dysmorphology, blood disorders, and types of cancers. Once diagnosed with FA, additional molecular studies are critical for the comprehensive clinical management of the patient. For some, it is essential for genetic counseling and carrier screening to be made available to extended family members; for others, previously identified genotype–phenotype correlations may help predict the clinical course. In general, the genetic counselor may choose the type of molecular test based on the history and specific needs of the family. For example, if there is a strong cancer history in the family or cancer in the patient at an early age, FAND1/BRCA2 (Myriad) or FANCN/PALP2 testing may be appropriate. However, in some cases, as in patients of Ashkenazi Jewish decent, mutation analysis may be targeted for FANCC (c.456+4A>C). For other cases, the conventional approach until recently has been complementation group analysis (i.e., correction of the DEB/MMC sensitivity by transfection of cDNA of one of the 17 FA genes) followed by gene-specific sequencing. However, large deletions or duplications can complicate molecular diagnosis. Next generation sequencing is becoming more widely available. Importantly, radical changes in methodology have recently made it possible to identify the complementation group and both mutations for FA patients in a faster and more economical way. Whole exome sequencing can now be used to detect mutations in a single study. Still, this approach is faced with pitfalls, such as failure to identify mutations that are intronic or in gene regulatory regions not covered by current exomic technologies. Furthermore, *FANCD2* mutations are complicated by the presence of two pseudogenes for *FANCD2.* Still, additional new technology such as RNA-seq, (sequencing-based RNA analysis) and aCGH (array-comparative genomic hybridization) has been shown to allow identification of both mutant alleles without prior complementation studies in all FA patients tested (Auerbach, unpublished data). The genetic counselor will help establish which tests are most appropriate for a specific patient and family and will inform patients, parents, and their extended family members of their cancer risk should mutations be identified in downstream factors, most notably FANCD1/BRCA2 and FANCN/PALB2. For the practitioner, the list of available CLIA-licensed US and non-US testing facilities can be found in the GeneTests™ Laboratory Directory (https://www.genetests.org/).

Prenatal testing

For nearly all couples at risk of having children with FA (likely because they have already had one child with the disease), reproductive counseling is mandatory. At a minimum, couples need to be aware of the risks of FA in future children so that they can make an informed decision about birth control, prenatal testing, and use of various assisted reproduction techniques, including embryo screening with PGD [131]. Chromosomal testing may be applied to the study of fetal cells obtained by chorionic villus sampling (CVS), amniocentesis, or percutaneous umbilical blood sampling. However, it should be noted that recently Fan et al. reported [132] that it is possible to non-invasively sequence the entire prenatal genome using a maternal blood sample obtained in the first trimester of gestation. Research is now ongoing to screen for mutations in single gene disorders using similar shotgun sequencing of fetal DNA in maternal plasma. In the not-too-distant future, it will likely be possible to use such techniques to prenatally diagnose FA in cases where the parental mutations are known.

Assisted reproduction

If the couple is interested in the use of IVF with PGD for the purpose of avoiding the implantation of affected embryos, identification of the mutated FA gene is required for embryo genetic screening. However, there is the additional possibility that couples can also screen for a specific HLA type, in the circumstance that an HLA-matched, unaffected child is desired to serve as an allogeneic HSC donor. In these circumstances, it is important to identify a team experienced in IVF that understands the specific goals of the couple and that can provide reliable estimates of the chance for success. Importantly, the IVF procedure does not have to be performed at the location of the PGD team or transplant center. As with IVF for infertile couples, the procedure requires daily injections to stimulate follicle development and maturation. After retrieval, each ovum is fertilized. After 2–3 days, at the 8–10 cell stage, a single cell is removed and tested for FA and HLA. Embryos that are free of disease and HLA-identical (if appropriate) are identified and implanted at that time or at a future time, depending upon the desire of the family.

Due to the risk of error inherent in testing a single cell prior to embryo transfer, CVS or amniocentesis should be strongly encouraged to confirm that the fetus is unaffected and HLA-identical if desired. In the latter setting, prior to delivery, arrangements are made for collection and shipment of the UCB from the HLA-identical healthy sibling along with maternal blood for infectious disease testing to the transplant center. Prior to HCT the newborn donor is evaluated for FA and HLA a third time, due to the critical importance of these results to the child being transplanted. To summarize, this represents the general approach toward the combined use of IVF and PGD and then UCB collection for interested couples at high risk of having a child with FA. For the practitioner, it is important to know the procedural steps at least generally and the potential ethical implications [133,134]. While the breadth of applicability remains to be identified and as the debate regarding the use of IVF and PGD to select embryos with desirable characteristics continues, it is clear from the number of referrals that there is increasing interest in the use of these technologies. Transplant physicians need to be intimately involved from the outset, not only to coordinate care but also to assess whether the patient's condition will permit sufficient time for the delivery of the healthy HLA genotypic-identical sibling donor.

Future directions

Improved understanding of the function of the FA genes has already profoundly affected our understanding of apoptosis, DNA repair, and regulation of normal hematopoiesis. Defects in the FA pathway are known to occur in somatic cells of non-FA patients, causing a variety of cancers, such as breast and ovarian cancer [30,135–138]. In addition to searching for strategies that would repair the FA defect in hematopoietic cells and eliminate the need for allogeneic HCT, others are searching for ways to induce a defect in the FA pathway that would enhance chemoradiotherapy sensitivity in otherwise highly lethal cancers. With regard to transplant physicians, the focus will continue to be close surveillance for marrow failure and cancer and development of even safer transplant strategies (e.g., use of ex vivo expansion culture of UCB HSC or reduced exposure to cytotoxic agents).

Gene therapy

The possibility of using gene transfer methods for correcting genetic diseases of HSCs has received much attention. The availability of FA genes has spurred considerable interest in the development of gene therapy for this disease for two reasons: (i) relatively few FA patients have HLA genotypic-identical HSC donors; and (ii) the risks of morbidity and mortality after unrelated donor HCT in specific high-risk subpopulations are substantial. One potential alternative to HCT could be the infusion of genetically corrected HSCs.

While there have been numerous reports [139–144] demonstrating that recombinant virus transduction of FA cDNA into FA $CD34^+$ hematopoietic progenitor cells improves clonogenic potential with phenotypic correction of CFC in vitro, as shown by resistance to MMC-induced cell death, loss of susceptibility to chromosomal breakage and restoration of cell cycle kinetics, clinical trials of gene transfer have not yet been successful. Liu et al. [140] reported the results on the first clinical trial in FA, specifically testing gene-corrected autologous HSC in patients with mutations in *FANCC.* In this study, a retroviral vector containing the *FANCC* and neomycin resistance coding sequences was used. After infusion, *FANCC* transgene was present transiently in the peripheral blood and BM. Function of the *FANCC* transgene was suggested by a marked increase in hematopoietic CFC, including colonies in the presence of MMC, after successive infusions of transduced cells. Despite a growth advantage in vitro, there was only transient evidence of gene-corrected HSCs amplification in vivo.

Since these early attempts, a great deal remains to be learned regarding gene therapy in the treatment of FA [142,145–147]. Improvements in HSC mobilization, gene transduction efficacy, long-term gene expression and safety have been made. More recent work suggests that lentiviral vectors may be a strategy for efficient gene correction of quiescent HSCs. While gene therapy is not yet proven in the context of FA, FA is a particularly attractive candidate disease for such a therapeutic approach owing to an inherent selective survival advantage of the corrected HSC. Trials with genetically modified autologous HSC for FA are in the pipeline. While of considerable interest scientifically, paucity of HSC targets, risk of accumulated chromosomal aberrations, and high success rates in recipients of allogeneic HCT regardless of donor type have slowed progress in the field of gene therapy for FA. The optimal candidate for testing the safety of HSC gene correction may be one who has a risk factor that effectively eliminates transplant as a reasonable option (e.g., poor renal function, history of solid tumor). While older age may be a high risk factor for transplant, older age is also associated with high risk of chromosomal abnormalities, MDS, and paucity of HSC. However, new strategies of HSC expansion are becoming available that could catapult the hope of gene therapy for FA in the not-too-distant future.

Stem cells and tissue repair

Embryonic stem cells are pluripotent cells derived from the inner cell mass of the blastocyst that can be propogated indefinitely in the undifferentiated state or differentiated into many cell lineages at least in vivo. More recently, inducible pluripotent stem cells (iPSC) have also been isolated from skin fibroblasts obtained from FA patients [148,149]. Irrespective of their origin, stem cells hold great promise for the treatment of many degenerative and inherited diseases. In the setting of FA, stem cells could be used as a strategy to enhance tissue repair after chemoradiotherapy, as intense local injury could itself be debilitating and life-threatening, such as that for the treatment of solid tumors. Alternatively, genetically corrected stem cells from the FA patient him/herself could be used as source of HSCs. Gene correction of patient-specific iPSC followed by differentiation into HSC is being explored as a particularly attractive strategy for treating the bone marrow failure common to most patients with FA.

Conclusion

FA is one of the truly amazing success stories in the field of allogeneic HCT. Once considered the “last resort” because of the extremely high risk of severe treatment-related toxicity, transplant is generally considered the first-line treatment for the FA patient with bone marrow failure. However, the hematologist, geneticist, oncologist, or other subspecialist making the diagnosis of FA (regardless of the patient’s age) should refer the patient to a FA Comprehensive Treatment Center as early as possible and not just at the time that marrow transplant may be needed. Such centers will provide a comprehensive evaluation focused on the unique issues of this disease from diagnostics (e.g., complementation group assignment and mutation analysis) and reproductive counseling (e.g., embryo selection, prenatal testing) to treatments and preventative care for specific organ dysfunctions (including marrow failure) and late effects (e.g., cancers, short stature, and endocrinopathies).

All patients with FA should be followed routinely by a hematologist experienced with the unique issues surrounding this disease, even prior to the onset of marrow failure when possible. Annual marrow examinations should begin early, particularly in patients with mutations in FANCD1/BRCA2. Enhanced surveillance with annual marrow examinations that include a comprehensive cytogenetic evaluation reduces the risk of an undiagnosed disease progression toward advanced MDS and leukemia. For general purposes, a surveillance plan and treatment algorithm are proposed, which will reflect those used by experts in the field (Figure 76.14). Expectedly, general approaches cannot reflect the specific issues for specific patients with FA, which may necessitate alternative considerations. However, at the time of FA diagnosis in one child, it is critical that all siblings of the proband be tested for FA regardless of age, phenotype, and HLA type. In those circumstances where severe cytopenia already exists (or is impending), a donor search should be activated urgently in order to move to transplant prior to the need for transfusions or development of opportunistic infections. While transplant is not a good option for some patients because of preexisting comorbidities (e.g., infection, cancer) or lack of a suitable donor, this is unusual. For the majority who are good transplant candidates, allogeneic HCT today, whether from an HLA-matched sibling or unrelated donor, is offered with a high expectation of success.

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SECTION 7 Complications of Hematopoietic Cell Transplantation

CHAPTER 77

Mechanisms and Treatment of Graft Failure

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Introduction

The ability of hematopoietic stem cells (HSCs) to circulate and to migrate from marrow to blood and back is an intrinsic aspect of the hematopoietic system. The biological role and physiologic significance of HSC circulation remains unclear, yet it is this trafficking that results in stable hematopoietic cell engraftment and forms the basic requirement for successful hematopoietic cell transplantation (HCT) in the treatment of hematologic and non-hematologic diseases. The failure to achieve sustained engraftment following HCT is associated with considerable morbidity and mortality, most notably infections and hemorrhagic complications due to marrow hypoplasia, and/or relapse of disease. This chapter reviews the clinical aspects associated with, and treatment of, graft failure (GF).

Overview of trafficking, homing, and engraftment of HSCs

The restoration of adequate blood cell production after HCT requires a series of balanced interactions between the infused HSC and its complex supporting marrow microenvironment.

Following HSC infusion, the cells initially adhere loosely to the vascular endothelium in the bone marrow with sufficient strength to overcome the hemodynamic shear forces of blood flow [1]. The predominant HSC cell surface molecules that mediate initial tethering are the selectin ligands, P-selectin glycoprotein ligand-1 (PSGL-1) and the hematopoietic cell L-/E-selectin ligand (HCELL), that interact with endothelial E- and P-selectin [2,3]. Thereafter, firm HSC adhesion is mediated by activation of the HSC cell surface integrin superfamily, principally VLA-4, α4β7, the α6 integrins, and LFA-1, that interact with endothelial immunoglobulin (Ig) superfamily receptors (e.g. VCAM-1), and the hyaluronate receptor CD44 [4,5]. HSCs null for cell surface α and β integrins do not adhere firmly to the endothelial surface, and are unable to home to the bone marrow (BM) niche even though they proliferate and differentiate normally in fetal liver [6,7]. Following firm adherence, transendothelial movement and homing to hematopoietic niches located within the inner endosteal surface of the bone are regulated by a gradient of extracellular matrix-bound SDF-1 (also known as CXCL12) binding to the CXCR4 receptor on HSCs [8]. Other potent HSC chemoattractants include gradients of the bioactive sphingophospholipids sphingosine-1 phosphate (S1P) and ceramide-1 phosphate (C1P) [9,10]. Following successful homing, adhesion of the HSC to its specific niche is mediated by a complex of many cell types, including mesenchymal stromal cells (MSCs) and parathyroid hormone (PTH)-receptor-bearing osteoblasts [11,12], in conjunction with a network of interacting marrow sinusoidal endothelial cells, facilitator T-cell subpopulations, adipocytes, and fibroblasts [13–15]. Successful HSC homing, adhesion, and engraftment may be compromised if the HSC, or its marrow niche, was previously damaged, possibly from prior chemotherapy and/or irradiation that the patient received. In the case of allogeneic HCT, the process is further complicated by immune-mediated host-versus-graft (HVG) and graft-versus-host (GVH) reactions. Given the intricacy of HSC trafficking from infusion to engraftment in its marrow niche, and the subsequent control of its self-renewal and differentiation, it is surprising that clinical HCT, in general, has a relatively low rate of GF.

Definitions

Graft failure is defined as the lack of hematopoietic cell engraftment following autologous and allogeneic HCT. Its criteria are predominantly operational, and the decades-old definitions of GF persist today. GF is divided into primary (early) and secondary (late) phases (Table 77.1).

Primary graft failure

Primary GF was defined as the failure to surpass a threshold absolute neutrophil count (ANC) of $0.5 \times 10^9/L$ by day 28 post-graft infusion, or $0.1 \times 10^9/L$ by day 21 [16–19]. The day of myeloid engraftment was assigned as the first of three consecutive days on which the ANC exceeded the targeted threshold. The day of platelet recovery was defined as the first day of a platelet count of at least 20, 50, or $100 \times 10^9/L$, sustained for 3 days without transfusion in the previous 7 days [16–19]. Platelet recovery was often delayed compared with myeloid recovery, or sometimes not achieved at all, especially if a higher platelet value ($100 \times 10^9/L$) was used as the threshold. The Center for International Blood and Marrow Transplant Research (CIBMTR) collects data regarding the number of days to count recovery using an ANC threshold of $\geq 0.5 \times 10^9/L$ and a platelet count $\geq 20 \times 10^9/L$. A hemoglobin level of ≥8…g/dL without transfusion support is an accepted threshold for red cell engraftment [20]. An isolated cytopenia does not necessarily invoke

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Table 77.1 Definition of graft failure and poor graft function

Transplant type	GF type	Criteria	Notes
Graft failure, autologous and full-dose allogeneic HCT	Primary	ANC <0.5 × 10^9/L by day +28, with Hb ≤8…g/dL, platelet count <20 × 10^9/L and continued transfusion support	BM biopsy confirms absent hematopoiesis Day +28 is relative to the cell infusion date
	Secondary	ANC <0.5 × 10^9/L after initial engraftment, unrelated to infection, medication, or disease progression with red cell and platelet transfusion support	BM biopsy confirms absent hematopoiesis For date of onset, use first date ANC <0.5 × 10^9/L of 3 consecutive tests
Graft failure, RIC allogeneic HCT	Primary	(i) ANC <0.5 × 10^9/L by day +28, and assay confirming ≥5% donor type cells	BM biopsy may or may not confirm absent hematopoiesis
		(ii) Failure to surpass the 5% donor type threshold, even if essentially normal blood counts	Assay to confirm the absence or presence of donor cells
	Secondary	(i) ANC <0.5 × 10^9/L after initial engraftment, continued transfusion support and assay to confirm > 5% donor type cells	BM biopsy may or may not confirm absent hematopoiesis
		(ii) Loss of donor hematopoiesis to <5% irrespective blood counts	Assay to confirm the absence or presence of donor cells
Poor graft function		Requires two or three cytopenic lines (Hb <10…g/dL, ANC <1.0 × 10^9/L, platelet count <30 × 10^9/L) for >2 consecutive weeks beyond day +28, without transfusion support	Criteria for GF not met In allogeneic HCT requires assay to confirm presence of donor cells. A $CD34^+$ selected cell boost may be considered in poor graft function following HCT

GF, as this may represent a transitory phenomenon related to a medication, viral infection, a lineage-specific immune-mediated cytopenia, or graft-versus-host disease (GVHD).

Secondary or late graft failure

Patients who satisfied the criteria for initial engraftment but subsequently developed loss of a previously functioning graft defined by at least two lines of cytopenia (i.e. ANC ≥0.5 × 10^9/L on three laboratory values with subsequent decline to < 0.5 × 10^9/L for three or more laboratory values, and a platelet count that achieved threshold with subsequent decline to <20 × 10^9/L) were considered to have late or secondary GF [17,18]. Late GF is a phenomenon more often associated with allogeneic HCT than with autologous transplantation [18]. Some possible causes of late GF include graft rejection related to residual host immunity, progressive disease for which the patient was transplanted, low donor cell yield, medication side effect, infection, or GVHD.

Graft rejection and poor graft function

The term graft rejection refers to immune-mediated rejection of the donor allograft by residual host effector cells that occur because of genetic disparity between the recipient and the donor. Therefore, graft rejection is relevant to allogeneic HCT but not autologous or syngeneic HCT. Primary graft rejection is the absence of donor hematopoiesis within the first month after transplant, and late rejection is defined as a loss of donor cells after their initial graft function [19]. The determination of graft rejection requires analysis of the blood or marrow for chimerism to confirm the absence of, or loss of, donor hematopoietic cells. Graft rejection is a cause of GF. In contrast to graft rejection, the term poor graft function describes the failure to achieve adequate blood counts following allogeneic HCT, in the presence of complete or near complete donor hematopoietic cell chimerism [21].

Mixed chimerism following allogeneic HCT

Full-dose conditioning

The initial studies of chimerism were performed in allogeneic transplant recipients with hematologic malignancies who received full-dose conditioning and unmanipulated donor grafts. These patients achieved complete (100%) donor hematopoietic cell chimerism as a result of the intensity of the transplant conditioning, and the T cells in the graft contributed to graft-versus-lympho-hematopoietic reactions. It is reasonable to forgo chimerism testing in patients who have achieved complete blood count recovery after HCT and who received unmanipulated grafts following conventional full-dose conditioning and standard GVHD prophylaxis.

Reduced-intensity conditioning

It is well established that reduced-intensity conditioning (RIC) for allogeneic HCT is associated with incomplete eradication of host hematopoietic elements [22]. As a consequence, a significant percentage of patients have multi-lineage mixed donor–recipient hematopoietic cell chimerism extending at times for months after transplantation. Primary engraftment following RIC is defined by achieving the neutrophil, platelet, and hemoglobin thresholds cited above, yet in addition, attainment of ≥5% donor T ($CD3^+$) cells is required [17]. Even in the setting of complete blood count recovery, a failure to surpass the 5% donor type threshold is considered primary GF, and secondary GF is reserved for cases that initially surpass the 5% threshold but subsequently fall below it. Full or complete chimerism generally refers to 95–100% replacement of host by donor hematopoiesis, although most assays are semiquantitative and small percentages (<5%) of host cells may be undetected. Mixed chimerism indicates the presence of both donor and recipient cells, often considered between 5 and 95% donor type [17]. The term split chimerism is used when one or more lineage is derived wholly from the donor, and one or more lineage is derived from the host. There remains a lack of consensus regarding the use of these terms, and the thresholds that define complete and mixed chimerism. Similarly, the assays to assess chimerism are not standardized, and it is uncertain whether blood or marrow and lineage selection should be part of the analysis. Importantly, the relationships between persistent mixed chimerism, the risk of GF, and disease relapse is imperfect, and needs further study. It seems reasonable that if the intensity of the transplant conditioning is altered, new GVHD regimens are being studied, or if there is graft manipulation, measurement of chimerism is important to evaluate the effects of the change in the ability to obtain complete multi-lineage donor cell engraftment.

Incidence of graft failure

The reported incidence of GF varies, in part because the consequences associated with GF (i.e. morbidity and mortality due to infection or hemorrhage) are reported, and not GF itself.

To estimate the incidence of GF following autologous HCT, it is reasonable to consider that in most centers the 100-day non-relapse mortality (NRM) is <10%, of which only a small subset can be attributed to GF. A surrogate marker for estimating the incidence of

GF following autologous HCT is the requirement for hematopoietic cell rescue using a back-up product. In a single-center study of 300 consecutive patients who underwent high-dose therapy followed by autologous hematopoietic cell rescue between 1980 and 1997, 14 (4.7%) patients required rescue with their back-up product due to primary ($n = 12$) and late ($n = 2$) GF [23]. The risk factors for GF in this study were the use of BM, a diagnosis of acute myeloid leukemia (AML), $CD34^+$ selection, and ex vivo cytokine-expanded peripheral blood mononuclear cells (PBMCs) collected by apheresis. Given that these risks factors are present in a minority of autologous transplant recipients, a more reasonable estimate of the incidence of GF for the majority of patients undergoing autologous HCT is probably between 1 and 3%.

The incidence of GF is higher in allogeneic transplant recipients, especially if the patient receives an human leukocyte antigen (HLA)-mismatched or T-cell-depleted graft, or if a single unit umbilical cord blood (UCB) transplant was performed. Depending on the specific particulars of the transplant, the risk of GF was reported to occur in 2–20% of allogeneic HCT recipients [24–26]. Some transplant centers opt to store autologous marrow or blood-derived hematopoietic progenitor cells for patients undergoing allogeneic HCT [23,24,26]. Ninety-four of 306 (31%) European centers completed questionnaires regarding their policy of storing autologous back-up cells for allogeneic HCT recipients, and just over half of the centers had such a policy [26]. The most common indications for an autologous back-up harvest were the use of a matched or mismatched unrelated donor or UCB. Some North American transplant centers also store autologous back-up cells for salvage purposes [24].

Causes of graft failure

A list of contributing risk factors for GF is given in Table 77.2.

Table 77.2 Risk factors for graft failure

Quantitative factors:
- Low $CD34^+$ cell yield
- Presence of splenomegaly
- Graft source (cord blood > bone marrow> mobilized blood)

Qualitative HSCs and marrow niche related factors:
- Extensive pre-transplantation chemotherapy and/or irradiation
- Iron overload
- Marrow fibrosis
- Disease status
  - >1–2 years from date of initial diagnosis
  - Marrow involvement with disease
  - Refractory disease
- Disease category
  - Inherited disease of erythropoiesis (SCD and thalassemia)
  - Severe acquired aplastic anemia
  - MDSs and CMNs
- Advanced recipient and/or donor age
- Storage techniques and expertise

Immunologic:
- Donor and recipient HLA disparity
- Presence of pre-transplantation DSAs
- Graft manipulation (i.e. TCD or $CD34^+$ cell selection)
- Extensive pre-transplantation transfusion history
- Post-transplantation immune suppression regimen
- Type and intensity of transplantation conditioning
- Presence of acute or chronic GVHD
- Viral infections (HHV-6, parvovirus, CMV)

CMNs, chronic myeloid neoplasms; CMV, cytomegalovirus; DSAs, donor-specific antibodies; HSCs, hematopoietic stem cells; MDSs, myelodysplastic syndromes; SCD, sickle cell disease; TCD, T-cell-depleted.

Immune-mediated graft rejection

Donor contribution

It is well established that donor T cells in the allograft promote hematopoietic engraftment as depletion of T-lymphocytes from the marrow graft before transplantation by any of a number of means was associated with a substantial increase in the occurrence of graft rejection [27–29].

The mechanism by which donor T cells inhibit graft rejection remains poorly defined. In murine transplantation models, donor $CD8^+$ T cells were reported to be much more potent facilitators of short- and long-term engraftment than $CD4^+$ T cells [30,31]. It is thought that donor T cells directly suppress, or eliminate, host effector cell populations. In a murine model of transplantation, BM engraftment across MHC class I or II barriers required donor $CD8^+$ T cells with intact perforin pathways to achieve sustained engraftment and prevent graft rejection [30]. Another possibility is that donor T cells provide cytokines that influence the ability of donor hematopoietic progenitor cells to home to the marrow niche or augment their proliferation, despite the fact that host effector cells may not have been suppressed. Secretion of granulocyte–macrophage colony-stimulating factor (GM-CSF) by donor-derived alloreactive T cells augmented HSC proliferation [32].

Efforts to characterize cell populations in the donor graft that facilitate engraftment without causing GVHD remain an area of active research. In most rodent models of bone marrow transplantation (BMT), unfractionated $CD4^+$ T cells do not possess efficient facilitating potential to support donor hematopoietic cell engraftment whereas unfractionated $CD8^+$ T cells do [30]. In a preclinical murine model of BMT, the infusion of donor-derived $CD8^+$ memory T cells at day +16 after transplantation converted MHC major and minor mismatched recipients with mixed chimerism to full donor chimerism without the development of GVHD [33]. Other $CD8^+$ subpopulations, specifically $CD8^+CD3^+$ but negative for αβTCR and γδTCR, facilitated BM cell engraftment without provoking GVHD in an MHC-disparate mouse model of transplantation [34]. Additionally, a special class of antigen-presenting cells (APCs) that carry CD8, called veto cells, deliver an apoptotic signal to T cells that recognize them [35]. Although the nature of this signaling pathway is not yet defined, it was demonstrated in murine models of BMT that the transfer of donor-derived veto cells supported HSC engraftment through the inactivation and elimination of alloreactive host T cells [36]. The infusion of donor $CD4^+CD25^+$ regulatory T (Treg) cells in a murine model of RIC for BMT in MHC-mismatched strains facilitated engraftment of donor BM cells, suggesting that donor Treg cells may regulate HVG reactions [37]. A clearer understanding of the role of various donor T-cell populations, including Treg cells, veto cell populations, and costimulation signaling pathways, has the potential to reduce HVG reactions that underlie immune-mediated allograft rejection.

Host contribution

Preclinical studies established that the increased risk of graft rejection following the infusion of a T-cell-depleted (TCD) graft was due to enhanced susceptibility of the graft to regimen-resistant host immune cells that included host natural killer (NK) cells, natural killer T (NKT) cells, γδ TCR T cells, and conventional $CD4^+$ or $CD8^+$ T cells [38–41].

Host NK cells contribute to graft rejection

Over 40 years ago and using murine models of BMT, investigators showed that parental homozygous BM allografts were rejected by their heterozygous F1 hybrids in a non-MHC-dependent manner as a result of regimen-resistant host NK cells [38,42]. Pretreatment of the conditioned host with antibody directed against NK-cell-associated antigens abrogated graft rejection [43]. Additional evidence that NK cells alone can mediate BM cell rejection was observed in severe combined immunodeficiency syndrome (SCID) mice that lacked T and B cells but had normal NK-cell function, as these mice rejected BM cell allografts, even after lethal irradiation [44]. NK-cell-mediated BM graft rejection can be regulated by host and donor Treg cells. Using the F1 hybrid resistance model in which the parental BM graft was rejected solely on the basis of NK cells, the prior removal of host $CD4^+CD25^+$ Treg cells significantly enhanced NK-cell-mediated BM rejection [45]. In contrast, when the BM graft was supplemented with donor Treg cells, NK-cell-mediated BM graft rejection was abrogated [45]. In clinical HCT, firm evidence of NK-cell-mediated resistance to engraftment is lacking, due in part to the lack of tests that discern T-cell- from NK-cell-mediated resistance. Human NK cells are highly sensitive to cyclophosphamide, and are suppressed by the use of post-transplantation immune suppression medication [46]. It is also possible that the quantity of hematopoietic cells infused in clinical transplantation may be sufficient to override resistance by host NK cells. In a murine model of BMT, NK-cell-mediated graft rejection increased as the intensity of transplant conditioning was reduced [47]. It is tempting to speculate that NK cells may play a more significant role in graft rejection following RIC regimens in humans.

Host T cells contribute to graft rejection

It is presumed that a dominant cell type mediating allogeneic BM cell rejection in non-sensitized recipients is regimen-resistant $CD8^+$ effector cytolic T cells that induce donor hematopoietic cytotoxicity primarily through Fas–Fas ligand (FasL)- and perforin-dependent pathways [48–50]. However, disruption of these two major cytotoxic pathways did not completely eliminate resistance to BM cell engraftment [48–50]. Furthermore, the elimination of any one selected cytokine in recipient mice, including tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β) and polarizing T-helper (TH)1 and TH2 cytokines, did not reduce the resistance to allogeneic BM cell engraftment [48]. Based on the aggregate literature, it is reasonable to presume that redundant host effector T-cell pathways help mediate BM cell rejection. Understanding the pathways of resistance mediated by host T-cell populations after high-dose and reduced-intensity conditioning regimens will be important for the development of effective strategies that lead to stable engraftment after allogeneic HCT.

It is reasonable to assume that BM cell rejection is faster in sensitized than non-sensitized recipients as there is no need for a T-cell effector induction phase. There is evidence that $CD44^{lo}$ naïve T cells are significantly more sensitive to graded doses of total body irradiation (TBI) than are $CD44^{hi}$ memory T cells and NKT cells [51]. In a murine model of BMT, memory T cells following immunization to major and minor mismatched alloantigens persisted after myeloablative TBI whereas naïve T cells did not, and prevented BM cell engraftment [51]. In clinical HCT, in which almost all donors are HLA matched with recipients, multiple pre-transplant recipient blood transfusions can result in an increased risk of graft rejection [19]. This is presumed to be due to white blood cells (WBCs) in the transfusions that express minor histocompatibility antigens (mHAs) and immunize recipients to cross-reactive mHAs in the donor graft. It is reasonable to consider that in humans, memory T cells directed to minor alloantigens in patients exposed to mHAs over a long period of time are also radioresistant, and can contribute to graft rejection.

Host B sells contribute to graft rejection

Antibody-mediated BM rejection either by antibody-dependent cell-mediated cytotoxicity or complement-mediated cytotoxicity has been described [52]. Preformed antibody present at the time of transplantation is not readily controlled with conventional standard conditioning regimens or with post-transplantation immune suppression medication. Strategies such as plasmapheresis, high-dose intravenous immunoglobulin, splenectomy, and immunoadsoption used in solid organ transplantation to eliminate antibody-mediated rejection are generally not part of HCT conditioning regimens and consequently their effectiveness at mitigating graft rejection has not been formally evaluated. In a murine model of BMT, it was shown that a single priming against an MHC-disparate strain resulted in antibody-mediated BM cell rejection that was rapid (<3 hours), whereas T-cell-mediated BM rejection in non-primed mice took more than 6 days [41]. In this model, BM cell rejection in sensitized hosts was overcome using the combination of a high dose of donor BM cells, with high-dose immunoglobulin and T-cell-depleting antibodies to target host T cells that survive TBI conditioning [41]. Evidence of relevance in clinical transplantation was demonstrated when pre-transplantation sera samples from patients who received HLA-mismatched grafts and developed GF were compared with an appropriate HLA-mismatched engraftment case–control cohort. Among the 37 GF patients, 24% tested positive for donor-specific antibodies (DSAs) against HLA-A, -B, and/or -DP compared with only 1% of the 78 engraftment controls [53]. Another study confirmed donor-specific anti-HLA antibodies were associated with a high rate of graft rejection in patients undergoing haploidentical HCT [54]. The presence of DSAs may also be an important contributor to GF following UCB transplantation [55]. It is reasonable to evaluate routinely for the presence of DSA in recipients of HLA-mismatched donor grafts and to consider strategies to eliminate the potential impact on GF.

The contribution of B-cell-mediated BM graft rejection in histocompatible siblings is less clear, even in the setting of prior sensitization. In human transplantation, the presence of radioresistant anti-donor cytotoxic T-lymphocyte (CTL) populations sensitized to donor mHAs in individuals who had a prior transplant, or in patients with severe aplastic anemia (SAA) who were sensitized through repeated blood transfusions, was associated with high levels of graft rejection [56,57]. In contrast, rejection did not commonly occur in recipients with cross-match antibodies, and the panel reactive antibody test in SAA recipients did not predict graft outcomes [56,57].

Understanding how donor HSCs are recognized and resisted is a complex biologic question. From preclinical models, it is evident that a variety of host cell populations contribute to resistance and that their roles are influenced by multiple factors, including genetic disparity, prior sensitization, graft content, and recipient preparation. Similarly, there are populations of cells contained within the donor allograft that effect engraftment. The continued unraveling of how transplanted HSCs are targeted and suppressed will undoubtedly contribute to developing strategies that ensure successful engraftment, and reduce the risk of GF.

Histocompatibility antigens

Comparing studies that evaluated the impact of HLA compatibility with the risk of GF following allogeneic HCT is challenging. The main reason relates to the difficulty in assessing the true degree of incompatibility in earlier studies. Also, donor selection in early studies was often based on matching criteria for HLA-A, -B, or -DR, which might not reflect matching at HLA-C, -DP, or -DQ. Consider that in 205 patients who underwent transplantation from HLA-A, -B, -C, -DRB1, and -DQB1 molecularly matched unrelated donors, a single-allele HLA-DP mismatch was identified in 90 recipient donor pairs and a two-allele mismatch was determined in 60 pairs [58]; the effect of mismatch on GF was not evaluated in this study. The disconnect between earlier and recent studies is highlighted in that virtually all earlier studies reported that an increase in the degree of HLA mismatch was associated with an increased risk of GF for sibling and unrelated donor BM grafts [59–61]. However, this is not a consistent observation in recent studies; a National Marrow Donor Program (NMDP) report evaluated HLA-A, -B, -C, -DRB1, -DQB1, -DQA1, -DPB1, and -DPA1 alleles and showed no statistically significant difference in the risk of GF between 7/8 (either allele or antigen) and 8/8 matched unrelated donor recipient pairs [62]. In another study of 709 patients with hematologic malignancies who received unrelated donor grafts following RIC, allele level HLA typing was performed at HLA-A, -B, -C, and -DRB1, and the risk of GF was not increased in recipients of HLA-mismatched grafts compared with recipients of fully matched grafts [63]. In this study, the only significant risk for increased GF was the use of BM compared with peripheral blood progenitor cells (PBPCs). Also, in this latter study, GF was not associated with a higher mortality risk, because patients with GF received effective salvage therapies, such as a donor cell boost or a second transplant [63].

ABO match

ABO incompatibility between donor and recipients occurs in approximately 25% of HLA-matched allogeneic transplants. Incompatibility was defined as major when the recipient plasma had isohemagglutinin (ISO) against donor red blood cell (RBC) antigens and minor when the donor had ISO against recipient RBC antigens. Bidirectional ABO incompatibility occurred when combined features of major and minor incompatibility were present. In the majority of studies, ABO incompatibility had no influence on the kinetics of neutrophil and platelet recovery time or the risk of GF [63,64] but has been associated with pure red cell aplasia.

Additional factors that might influence graft failure risk

Age

It remains unclear whether patient and donor age impact the incidence of GF. In autologous transplantation, higher patient age adversely impacted the ability to mobilize a sufficient number of progenitor cells to support successful engraftment in patients with multiple myeloma, Hodgkin disease, and non-Hodgkin lymphoma (NHL) [65]. However, in other reports no difference was observed in the total number of $CD34^+$ cells collected from older and younger myeloma and lymphoma patients [66]. The use of a CXCR4 inhibitor, especially when combined with granulocyte colony-stimulating factor (G-CSF), generally overcomes a low $CD34^+$ cell yield [67].

In allogeneic HCT, higher donor age was significantly associated with lower circulating $CD34^+$ cell counts and lower $CD34^+$ cell yields following G-CSF mobilization [68]. The lack of prospective studies designed to evaluate if donor age impacts the risk of GF preclude firm conclusions. At the time of writing of this chapter, the NMDP accepts healthy unrelated donors in the 18–44 years age group in recognition that physician preference is a younger donor. Donors between 45 and 60 years of age who meet health guidelines can also join, following a required US$100 tax-deductible payment.

An emerging hypothesis is that we age through accumulated damage to our stem cells including HSCs, and that the damaged stem cells have diminished cell function, which, in the case of HCT, means a reduced capacity for hematopoietic reconstitution . HSC reserve and function were evaluated in older and younger mice, and stem-cell functional capacity was impaired in older mice, which led to a loss of proliferative potential and, ultimately, functional exhaustion [69]. In another murine model, the HSC population from older mice competed poorly with the HSC from younger mice in competitive long-term repopulation assays [70]. The lower engraftment potential from older mice was due, at least in part, to a defect in the ability of HSCs to home to the marrow niche, as direct BM injections negated some of the difference in competitive repopulation assays [70]. An unanswered question remains: do old stem cells transplanted into young patients impact the likelihood of achieving sustained engraftment?

Telomeres are non-coding regions of DNA that cap the ends of eukaryotic chromosomes to protect against the enzymatic breakdown of coding DNA and prevent fusion or other chromosomal aberrations [71]. Telomeres shorten 50–150 base pairs with each cell division in cells that do not express telomerase. The maintenance of telomere length by expression of telomerase postpones senescence and its constitutive expression is a general requirement for immortalization of cells [71]. Several studies involving adult and pediatric patients showed that following allogeneic HCT, the telomere lengths in the transplanted blood and marrow cells were 0.4–2…kb shorter than those of their respective donors and age-matched controls [72,73]. This degree of accelerated telomere loss was equivalent to 15–70 years of additional ageing. Two cases of late GF following high-dose allogeneic HCT were associated with significant telomere shortening in the donor hematopoietic cells compared with the donor's native cells [74]. In these cases, GF due to medication, infection, relapse, immune-mediated rejection, or GVHD was ruled out.

Taken together, the data suggest that transplantation using a marginal graft with a limited number of HSCs, or transplantation using partially damaged HSCs from older donors, or HSCs that may have short telomeres to start with for whatever the reason, can put high demands on their proliferative potential that may eventually result in exhaustion and subsequent GF. Given the increasing number of long-term survivors of HCT and the increasing age of recipients and donors, it is important to understand age-associated regulators of HSC function. It is conceivable that this knowledge may eventually be used as selection criteria to maintain better the integrity of the repopulating HSCs and reduce the risk of GF or other adverse outcomes.

Diagnosis

The risk of GF can depend on the underlying disease, yet it is difficult to draw firm conclusions because studies often combine patients with different stages of diseases, conditioning regimens, graft source and content, and other variables that are likely important in contributing to GF.

Aplastic anemia

In general, patients with SAA have a GF rate that is higher than observed in acute leukemia [19]. This is in part because after transplantation these patients may have retained the immune-mediated recognition of stem-cell antigens that formed the targets of the pathophysiology of the disease, and they may have been sensitized before transplantation to allogeneic mHA targets that can result in graft rejection. Transplantation early in the disease course and before sensitization to histocompatibility antigens reduced the GF rate in patients with SAA, yet it is likely that only a minority of patients do not receive transfusions prior to transplantation [19].

Myeloid disorders

The GF rate in pediatric and adult patients undergoing allogeneic full-dose or RIC HCT for myelodysplastic syndromes (MDSs) and chronic myeloid neoplasms (CMNs) is increased compared with patients with acute leukemia [75–80]. Some investigators recognized that patients with MDSs and CMNs, especially myelofibrosis (MF), have a prolonged blood count recovery time, and instead considered GF as cytopenia beyond day 50 after transplantation [81]. Patients with MDSs, chronic myelogenous leukemia (CML), and other CMNs often have not received pre-transplantation intensive chemotherapy, and therefore may resist donor cell engraftment due to the presence of residual intact host effector cells. Patients with MDSs and CMNs may also have a defective marrow microenvironment that increases resistance to donor cell engraftment [78,79]. It is possible that therapy specifically targeting the underlying disease (i.e. cytadine analogs for MDSs or tyrosine kinase inhibitors for CML) given post-transplantation, or more intensive immune suppression, before or immediately after HCT, might facilitate engraftment, particularly if RIC is being considered in patients with CML, MDSs and MF.

The presence of massive splenomegaly in patients with CMNs is considered a risk factor for GF or delayed engraftment following allogeneic HCT [82]. In some patients, splenectomy, splenic irradiation, or treatment with JAK3 inhibitors is considered prior to allogeneic HCT as an intervention to improve the likelihood of successful donor cell engraftment, although this remains a debatable perspective [82,83].

Hemoglobinopathies

Thalassemia is associated with a relatively high GF rate, and risk factors include hepatomegaly (>2…cm below the costal margin), the presence of portal fibrosis in the pre-transplant liver biopsy, and the quality of iron chelation during the years before transplant (considered good if regular deferoxamine therapy was initiated within 18 months of the first transfusion and administered continuously for 8–10 hours for at least 5 days per week) [84,85]. The incidence of GF following high-dose conditioning and BMT from HLA-matched sibling donors was 10% for patients with no risk factors, compared with 25% for patients with all three risk factors [86]. The experience in patients with sickle cell disease (SCD) is similar; CIBMTR data collected in a 13-year period from 1989 to 2002 confirmed that nine of 67 (15%) recipients of HLA-matched related BM grafts developed GF following high-dose busulfan–cyclophosphamide (BU–CY) conditioning [87]. More recently, RIC containing alemtuzumab, or antithymocyte globulin (ATG) with post-transplantation CY, established persistent mixed chimerism in the majority of SCD patients who received grafts from HLA-matched sibling donors, and reported a GF rate of just under 10% [88,89]. This regimen when applied to haploidentical donors resulted in a GF rate of just over 40% [89].

Disease status

Many studies have demonstrated that in allogeneic HCT recipients with AML, CML, MDSs, and SAA, a long interval from diagnosis to transplant was associated with an increased risk of mortality, due in part to increased GF [19,59,76,80]. The increased GF rate may be the consequence of previous intensive treatments that damaged the marrow microenvironment, and/or excessive prior transfusion history resulting in HLA sensitization. The difficulty in achieving sustained engraftment in patients with advanced-stage disease may also relate to the kinetics of rapid tumor regrowth after transplantation.

Previous treatment

Experimental and clinical data showed that exposure to cytotoxic agents and radiation therapy damaged hematopoietic progenitors and impaired their repopulating ability. Animal studies confirmed melphalan, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), and BU significantly reduced the repopulating capacity of BM progenitor cells compared with CY, etoposide, and cytosine arabinoside (Ara-C) [90]. Salvage regimens containing agents that potentially affect progenitor cell function might compromise engraftment following autologous HCT. In keeping with these preclinical studies, exposure to a salvage regimen that contained BCNU or melphalan was shown to be a significant factor that adversely affected progenitor cell yield and performance of the graft, and was associated with an increased risk of GF following autologous HCT [91]. Dose intensity may also be important as patients who received higher dose cyclophosphamide, hydroxydaunomycin (doxorubicin), vincristine, and prednisone (CHOP) had a significantly prolonged time to engraftment following autologous HCT than patients who received standard-dose CHOP [92]. Lenalidomide used to treat patients with myeloma prior to autologous harvest decreased the number of $CD34^+$ cells collected, and increased the number of aphereses needed to obtain adequate cell numbers for transplantation compared with myeloma patients who received other therapies [93]. The negative impact of lenalidomide on $CD34^+$ cell collections was overcome when patients were mobilized with chemotherapy combined with G-CSF [94].

The evaluation of a bone marrow aspirate for abnormal cytogenetic clones that may have developed from prior chemotherapy therapy in patients planned for autologous harvest is important. Infusion of autologous products that harbored abnormal cytogenetic clones led to an increased risk of poor graft function with the subsequent development of post-transplantation MDS and secondary leukemia [95].

Prior exposure to radiation therapy may also impair the hematopoietic microenvironment and negatively impact graft function. In a mouse model of BMT, irradiation caused profound and lasting changes in the expression of the endothelial trafficking molecules VCAM-1 and P-selectin, which were important for hematopoietic cell recruitment to the bone marrow niche [96]. The administration of clinically relevant doses of TBI-activated marrow p38 mitogen-activated protein kinase (MAPK) induced long-term bone marrow injury that resulted in a significant reduction in hematopoietic cell clonogenic function and increased HSC senescence [97]. These findings suggest that the health of the marrow microenvironment may be a limiting factor in supporting sustained engraftment of allogeneic and autologous hematopoietic cells. The clinical relevance of these observations with respect to GF is not established, but of interest.

Preparative regimen

The transplant preparative regimen can influence the GF rate. Attempts to overcome the relatively high GF rate in patients with SAA who received multiple pretransplantation transfusions included intensifying the conditioning regimen by combining CY 200…mg/kg with TBI or total lymphoid irradiation (TLI) [19,98]. Although GF rates were reduced with the combinations, overall survival was not improved owing to the higher rates of death from GVHD and interstitial pneumonia. Animal studies showed a synergistic immunosuppressive effect between CY and ATG for overcoming marrow allograft rejection, thereby obviating the need for TBI [99]. On the basis of these models, a regimen of CY and ATG was developed for patients with SAA and a reduced GF rate and improved overall survival was reported compared with historical data [100]. However, a prospective clinical trial in which patients were randomly assigned to receive CY alone or CY in combination with ATG reported a similar GF rate in both groups [101].

Two randomized studies failed to identify differences in the time to functional platelet and neutrophil engraftment or in GF rates when conventional full-dose fractionated TBI–CY was compared with BU–CY in chronic-phase CML patients who received unmanipulated marrow grafts [102,103]. Increasing the intensity of the transplant preparative regimen in already fully myeloablative conditioning did not decrease the risk of GF as the addition of Ara-C to a standard BU–CY transplantation regimen for pediatric patients did not impact the risk of GF or the time to platelet and neutrophil engraftment in recipients of autologous or allogeneic HCT [104].

In contrast to the observations following full-dose conditioning, increasing the intensity of host conditioning following some reduced-intensity regimens reduced the incidence of GF. Non-fatal GF occurred in 20% of 44 leukemia patients who received sublethal TBI with post-transplantation mycophenolate mofetil (MMF) and cyclosporine (CSP), and autologous hematopoietic cell recovery occurred in each case [105]. In a similar patient population, the addition of fludarabine to sublethal TBI reduced the GF rate to 12% [75]. The addition of sublethal TBI to other conditioning regimens did not reduce the GF rate. In a study of a 100 patients with acquired SAA, fludarabine, CY and ATG, administered with or without 2… Gy TBI, resulted in an equivalent GF rate of 17% [106].

Transplantation following RIC is associated with multi-lineage mixed chimerism that may persist for several months [107–109]. It is difficult to assess the impact of mixed chimerism on the risk of GF because often interventions such as immunosuppressive (IS) drug withdrawal, $CD34^+$ selected donor boosts, or donor lymphocyte infusions (DLIs) were employed [24,107–110]. These interventions were to promote conversion to full donor type yet they were not without risk and resulted in the development of GVHD in some patients. A transplant regimen of CY, ATG, and thymic irradiation with postgrafting IS consisting of CSP revealed that almost 30% of the patients developed GF by day +100 if the donor T-cell chimerism level on day +30 post-transplant was <50% [108]. The GF risk following conditioning with 2…Gy TBI ± fludarabine with post-transplantation IS consisting of MMF and CSP was 50% among patients who had donor T-cell levels below 25% by day +30 post-transplantation compared with a 4% GF rate if the donor T-cell level was 51–75% by this time [111]. In other studies, the post-transplant levels of donor T- and NK-cell chimerism at days +14 and +28 were also important predictors of GF [107,112].

Controlled experimental and clinical studies are required to understand better how an alteration in a preparative regimen impacts the kinetics of conversion to donor cell type and the incidence of GF.

Post-transplantation immune suppression

The importance of suppressing HVG reactions by using post-transplantation IS medication to prevent GF has been well demonstrated in preclinical models. In a preclinical dog model of BMT using dog leukocyte antigen (DLA)-identical littermates and sublethal TBI conditioning, CSP alone was insufficient to suppress HVG reactions and all recipients had non-fatal GF [113]. By comparison, post-transplantation immune suppression with methotrexate (MTX) and CSP led to prolonged hematopoietic chimerism in all cases, yet still just over half of the animals ultimately lost their grafts. The combination of MMF and CSP or of MMF and sirolimus led to stable chimeras in all recipients for at least 4 months [113,114]. In another canine transplantation model, extending the length of post-transplantation IS medication from 35 to 100 days facilitated stable donor engraftment [115]. These preclinical studies were instrumental in designing optimal combinations of post-transplantation IS medication to help prevent GF in clinical HCT using RIC.

The use of post-transplantation CY following RIC HLA haploidentical BMT was evaluated in 210 adult and pediatric patients with leukemia, MDS, and lymphoma [116]. The median time to adequate neutrophil recovery was 15 days and the GF rate was 13%. Almost all patients with GF in this study had autologous reconstitution. Although randomized studies do not exist, it seems that post-transplantation CY appears to mitigate the negative impact of haploidentical donors on the GF rate.

Graft source

Graft source for autologous and allogeneic HCT is covered in detail in Chapter 40. Briefly, in autologous HCT, the time to neutrophil and platelet recovery is more rapid with mobilized hematopoietic progenitor cells compared with BM, and there is no apparent difference in the GF rate [117]. The same observation is true following full-dose conditioning and HLA-matched, related allogeneic HCT; G-mobilized blood mononuclear cells impart a faster time to neutrophil and platelet engraftment compared with unstimulated BM, with no difference in the GF rate [118]. In contrast, in recipients of unrelated donor transplants, G-mobilized blood was associated with a lower GF rate (3%) than unstimulated BM (9%), and GF was the cause of death in 8% of BM recipients compared with 0% in the G-mobilized group ($p = 0.002$) [119]. In recipients of unrelated donor grafts, it may be preferable to consider a G-mobilized product in patients at higher risk for GF, such as those with uncontrolled or poorly controlled disease, or those with malignant diseases who have not received cytotoxic chemotherapy prior to transplant.

The biologic features of cord blood suggest that they are highly proliferative, which may be important in overcoming the limited number of cells that are obtained for transplantation. One issue that has consistently emerged irrespective of the degree of HLA match is the delayed time to blood count recovery following UCB transplantation. A CIBMTR report of pediatric acute leukemia patients who underwent transplantation between 1995 and 2003 compared the time to platelet and neutrophil count recovery in recipients of one- or two-allele mismatched unrelated donor BM compared with one- or two-allele mismatched single-unit UCB recipients and showed a significantly delayed time to count recovery in UCB recipients [120]. A similar report from the CIBMTR, the European Group for Blood and Marrow Transplantation (EBMT), and the National Cord Blood Program of the New York Blood Center also confirmed significantly delayed neutrophil and platelet recovery times among adult UCB transplant recipients [121]. To help overcome the delay in blood count recovery, practice standards have evolved, and since about 2010 the lower acceptable nucleated cell dose limit to use a single UCB unit has been 2.5×10^7 cells/kg bodyweight.

Although the use of a double UCB transplantation assured a higher cell dose, hematopoietic recovery still remained significantly delayed compared with recipients of matched and mismatched donor BM [122]. This study emphasized that the most significant barrier to more successful UCB transplants was the delayed neutrophil engraftment time, because a delayed neutrophil recovery time led to a significantly increased NRM from infection deaths. Strategies to reduce the period of neutropenia following UCB transplantation are being explored and include ex vivo expansion culture to augment the number of HSCs and progenitors in the UCB graft, coinfusion of TCD haploidentical PBPCs to bridge the period of neutropenia, and the injection of UCB cells directly into the patient's BM to reduce non-specific losses of HSCs and improve homing and engraftment [123].

Effect of cell numbers

Most studies demonstrated that, at least to some extent, a minimum threshold number of progenitor cells was required to ensure reliable neutrophil and platelet engraftment and reduce the risk of GF following autologous and allogeneic HCT.

Historically, the nucleated cell (NC) or mononuclear cell (MNC) dose was the surrogate to assess the HSC content in BM graft. In autologous HCT, the predictive value of NC or MNC dose with respect to the rapidity of hematopoietic recovery demonstrated no statistically significant correlation between the infused numbers of NCs/kg or MNCs/kg and the time to neutrophil or platelet recovery [124]. For allogeneic HCT, it was reported that 5×10^8 NCs/kg recipient weight was a reasonable estimate of the number of BM cells required for a successful transplant, whereas a dose of $<3 \times 10^8$ NCs/kg recipient weight correlated with an increased risk of GF following full-dose conditioning [125]. Subsequent studies regarding the predictive value of BM NC dose and the risk of GF following allogeneic HCT failed to yield consistent results.

In contrast to the NC dose, an increased number of granulocyte–macrophage colony-forming units (CFU-GMs) in the autograft positively correlated with more rapid time to neutrophil and platelet recovery. A threshold dose below $0.1–3 \times 10^4$ CFU-GMs/kg resulted in significant engraftment delays [126]. The CFU-GM assay, however, has limitations: there is imprecision in CFU-GM quantification, especially when the progenitor content is low, there is a subjective nature to the data interpretation, with high interlaboratory variability, there is a requirement for expensive biologic reagents, and there is an approximately 2-week delay before results are obtained. In some transplant populations there may be value in using this assay to assess the potency of the graft. A study of the kinetics of engraftment in 435 primarily pediatric patients receiving a single-unit unrelated UCB transplant after conventional full-dose conditioning demonstrated that pre-cryopreserved higher colony-forming unit (CFU) dosing was the only graft characteristic strongly predictive of neutrophil and platelet engraftment [127]. In comparison, the $CD34^+$ cell dose and TNC/kg were only weakly predictive.

The expression of cell surface CD34 can be rapidly assessed within a few hours by flow cytometry. In autologous HCT, the infusion of $>2 \times 10^6$ $CD34^+$ cells/kg recipient weight was suggested as the minimum target dose for acceptable engraftment kinetics [128]. A more rapid neutrophil and platelet reconstitution was consistently observed by the infusion of higher $CD34^+$ cell doses [129]. Platelet recovery appeared most affected by grafts with low $CD34^+$ cell numbers. Patients considered "poor mobilizers," defined as a collection of $<1 \times 10^6$ $CD34^+$ cells/kg, safely underwent autologous HCT with no difference in overall survival, albeit 50% of the patients failed to reach a platelet count of $>10^5/\mu L$ by day +100 after transplant [130]. An absolute minimum $CD34^+$ cell dose below which autologous HCT is not recommended has not been defined. It is the opinion of the authors that BM harvesting to supplement a graft with a $CD34^+$ cell dose below 1×10^6 be considered.

There are inconsistent results regarding the impact of the $CD34^+$ cell dose and its relationship to GF following full-dose allogeneic HCT. This may reflect that a minimum threshold number of cells is required to promote engraftment above which there is no correlation. The widespread practice of infusing as many cells as possible to give the recipient the best possible chance for a rapid and complete hematologic recovery has made it difficult to analyze the effect of cell dose on the risk of GF. Furthermore, comparisons among studies is difficult because patients differ with respect to the preparative regimen, underlying disease, disease status, pre-transplantation therapy received, post-transplant IS medications, donor–recipient gender mismatch, degree of HLA compatibility, and a variety of other patient- and donor-specific factors. Overall, it appears that within the general range of cells collected in an unmanipulated graft, its composition does not appear to impact the risk of GF and the time to platelet and neutrophil recovery following full-dose conditioning with matched related and unrelated donors [131]. In mismatched and haploidentical HCT, $CD34^+$ cell doses impacted the time to engraftment [132]. When $CD34^+$ cell doses were $<8 \times 10^6$ cells/kg recipient weight, the time to blood count recovery was significantly delayed. Many centers target a $CD34^+$ cell dose of over 10×10^6 cells/kg recipient weight when considering mismatched and haploidentical HCT.

Several studies confirmed that following RIC, high doses of donor $CD34^+$ cells led to more rapid T-cell engraftment with a lower risk of GF [133]. It was also reported that a higher $CD8^+$ T-cell dose in the donor inoculum correlated with a lower risk of GF following RIC [134].

It is likely that as our understanding of $CD34^+$ and effector cell populations improves, protocols that manipulate graft content to reduce GF will be developed for autologous and allogeneic HCT.

Graft manipulation

Graft manipulation by any of a number of means may pose a potential increased risk for GF. However, the $CD34^+$ cell selection of BM and blood-derived autografts in patients with NHL and breast cancer did not adversely affect the time to neutrophil and platelet recovery or the GF rate [135,136]. In contrast, unmanipulated grafts were more effective in mediating stable engraftment following allogeneic HCT compared with grafts selectively enriched or depleted for one of a variety of cell populations, and this is true irrespective of the intensity of transplant conditioning regimen. Pan-TCD or selective $CD8^+$ and $CD6^+$ TCD of the donor inoculum reduced the risk of acute GVHD, but survival in these studies was not improved because of the increased risk of death from GF, tumor relapse, and infection [28,29].

Strategies for risk reduction of graft failure

Prior to pursuing HCT, pre- and post-transplantation strategies that address and mitigate the patient's risk factors for GF (Table 77.2) should be considered. For example, it seems reasonable to screen recipients of mismatched or unrelated donor grafts for DSAs, and if positive, treatments to decrease DSAs and reduce the GF risk should be considered [137]. Given the improved outcomes with unrelated donor HCT and the concern about advanced donor age on outcomes, including an increased risk of GF, it may be advantageous to consider a young unrelated donor, or even UCB, over an old related donor, albeit the cut-off ages for this are not defined

[138]. It may be helpful to treat patients with massive splenomegaly prior to transplant to reduce the potential negative impact that this may have on donor cell engraftment. In autologous HCT, appropriate cell numbers should be adjusted whenever possible to meet required needs, and occasionally a BM harvest to supplement a low CD34$^+$ cell yield from a patient who is a "poor mobilizer" may be required. In allogeneic HCT, a higher cell inoculum appears to enhance the probability of sustained engraftment in patients transplanted for non-malignant hematopoietic disorders such as SAA, and also in recipients of grafts from HLA-mismatched donors [139]. The higher CD34$^+$ cell dose has to be balanced against a potential increased risk of chronic GVHD [140].

Monitoring of graft performance

The evaluation of graft function may be performed at different levels, including the assessment of adequate production of blood cells, assessment of hematopoietic precursors in the marrow, and evaluation of stromal cell function. Chimerism studies of T, B, NK, and myeloid cells have become an important part of graft monitoring, particularly for patients transplanted after RIC, or recipients of mismatched grafts, or those transplanted for non-malignant diseases. Studies that evaluate the kinetic change of the proportion of donor cells are key to project the fate of the graft, determine the risk of GF, and possibly to direct interventions.

Interventions for graft failure

Strategies for the treatment for early and late GF following autologous and allogeneic HCT are highlighted in Table 77.3.

Use of autologous back-up

In circumstances where the autologous product has been manipulated, for example by CD34$^+$ cell enrichment, it is prudent to store an unmanipulated portion of the graft, as infusion of the back-up has restored hematopoiesis following GF [23]. The practice at one of the authors' institutions (R.L., Stanford University Medical Center) is to collect enough cells to ensure that all autologous products, whether manipulated or not, are split to secure a back-up cell dose of $>2 \times 10^6$ CD34$^+$ cells/kg recipient weight. If following GF autologous back-up cells are not available, a search for an allogeneic donor graft should be initiated. In this instance, an RIC regimen should be considered given the relatively close proximity to the autologous transplant. On another note, autologous rescue has been successful in restoring hematopoiesis in patients with GF after allogeneic HCT [23,24]. The relatively low risk of GF following allogeneic HCT and the inherent difficulty in collecting uncontaminated autologous cells in patients with hematologic neoplasms, or with marrow failure syndromes, make the routine decision to store autologous products in allogeneic transplant recipients problematic.

Table 77.3 Treatment strategies for graft failure

Graft failure following	Treatment
Autologous HCT	Autologous back-up should be available, especially if product is to be manipulated Hematopoietic growth factor support Early consideration for allogeneic HCT
Allogeneic HCT	Autologous back-up should be considered, especially if an alternative donor is used (cord blood or mismatched donor) Trial of hematopoietic growth factor support Alterations in immune suppression medication may help prevent graft rejection DLI or additional cell infusions to convert persistent mixed or falling chimerism to full donor type CD34$^+$ cell boost for poor graft function Regrafting using same or alternative donor

Use of growth factors after graft dysfunction

Myeloid growth factors are reported to shorten significantly the time to neutrophil recovery following the infusion of an autologous graft [141]. The impact on the time to platelet recovery is less clear. Growth factors have been used for the treatment of GF following autologous HCT, with variable improvements in blood counts leading to adequate myelopoiesis and long-term disease-free survival reported in some patients [142]. In contrast, post-transplantation growth factor support for patients with GF who received a chemically purged autologous product consistently confirmed a lack of response [143]. There are no data comparing GM-CSF with G-CSF. A trial of post-transplantation growth factors is a reasonable approach for graft dysfunction following autologous HCT while consideration for a more definitive therapeutic intervention is being evaluated.

The role of growth factors in patients with poor graft function, or GF, following allogeneic HCT remains unclear. A randomized study in patients with GF after related or unrelated allogeneic HCT demonstrated that sequential GM-CSF and G-CSF improved the 100-day survival compared with GM-CSF alone [144]. It is reasonable to consider hematopoietic growth factor support as an adjunct in the management of low blood counts following allogeneic HCT, and depending on the cause this therapy may or may not be effective.

Changes in immune suppression

Preclinical models of BM transplantation demonstrated that following RIC, suboptimal immune suppression medication immediately post-transplantation increased the risk of early GF [113], whereas adequate dosing supported engraftment by inhibiting HVG reactions [114,115]. In instances of persistent mixed or declining donor-cell chimerism, it remains unclear whether immune suppression drug withdrawal will hasten or prevent graft loss. A number of studies suggested a modest improvement in preventing GF, and subsequent disease relapse, following preemptive fast (<7 days) immune suppression medication withdrawal aimed at converting patients with declining or persistent mixed chimerism to full donor type [145,146]. Controlled studies are required to define how an alteration in post-transplantation immune suppression will affect engraftment, relapse, and GF.

Use of donor lymphocyte infusion

Unstimulated DLI was administered preemptively (preemptive donor lymphocyte infusion, pDLI) to SAA patients considered at high risk for GF because of decreasing levels of donor-cell chimerism [147]. In that study, low-dose pDLI promoted the conversion to complete donor type and no patient experienced GF.

Following RIC, persistent mixed chimerism, or declining levels of donor cells, either CD3$^+$ or leukemia lineage (i.e. B, T, or myeloid lineage) was associated with an increased risk for GF and leukemia relapse in adult and pediatric transplant recipients [146,148–152]. The administration of pDLI after day 100 to patients who, despite being withdrawn from immune suppression medication, persisted with low (<50%) or declining levels of donor CD3$^+$ cells, resulted in approximately 50% of recipients

Table 77.4 Second transplants for graft failure

Study	*n*	Graft failure	Diagnosis	Donor for 2nd transplant	Source of cells	Same donor	Median time to 2nd transplant (days)	Regimen before 2nd transplant	Response (%)	Outcome
Guardiola et al. [154]	82	28 1°GF 54 2°GF	AML CML SAA	64 MRD 18 other	72 BM 19 PB	56 Yes 26 No	62	82 Yes	73	30% 3-year OS
Min et al. [155]	20	7 1°GF 10 2°GF 3 other	ALL AML CML SAA	18 MRD 2 other	6 BM 14 PB	20 Yes	49	9 Yes 11 No	75	71% 3-year OS
Gaziev et al. [156]	32	4 1°GF 28 2°GF	Thal	32 URD	32 BM	28 Yes 4 No	675	32 Yes	58	60% 3-year OS
Jabbour et al. [157]	9	8 1°GF 1 2°GF	AML CML MDS	3 MRD 6 other	8 PB 1 UCB	8 Yes 1 No	54	9 Yes	66	20% 2-year OS
Chewning et al. [158]	16	11 1°GF 5 2°GF	AML ALL CML FA MDS	3 MRD 13 other	2 BM 13 TCD 1 UCB	6 Yes 10 No	45	16 Yes	100	35% 3-year OS
Gyurkocza et al. [159]	38	7 1°GF 6 2°GF	ALL AML CMN MDS NHL SAA RCC	11 MRD 26 URD	1 BM 36 PB 1 UCB	14 Yes 24 No	91	38 Yes Flud/TBI	87	42% 4-year OS

1°GF, primary graft failure; 2°GF, secondary graft failure; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; BM, bone marrow; CML, chronic myelogenous leukemia; CN, chronic myeloid neoplasms; FA, Fanconi's anemia; Flud, fludarabine; MDS, myelodysplastic syndrome; MRD, matched related donor; NHL, non-Hodgkin lymphoma; PB, peripheral blood; OS, overall survival; RCC, renal cell cancer; SAA, severe aplastic anemia; TCD, T-cell-depleted; Thal, thalassemia; TBI, total body irradiation; UCB, umbilical cord blood.

converting to complete donor type. The majority of the patients required multiple pDLI before converting [110,153]. Although the data are provocative, it remains uncertain how many would have become full donor chimeras without an intervention. The pDLI was associated with the development of acute GVHD in a significant proportion of recipients [108,110,153]. Selective depletion of cellular subsets within the DLI might promote conversion to full donor type, yet not precipitate GVHD. In this respect, the removal of naïve and a subset of memory T cells may be a possible candidate [33]. The timing of when to administer pDLI for GF prevention is unclear and requires careful consideration of competing risks and benefits. Is conversion from mixed chimerism to complete donor type absolutely necessary? Conversion to full donor chimerism was not required for graft versus tumor (GVT) reactions in some studies, whereas others have suggested that sustained GVT activity requires conversion to full donor chimerism [107,108,146,148–152].

Use of $CD34^+$ boost

Poor graft function after allogeneic HCT is defined by at least two lines of cytopenia beyond 28 days after transplantation in patients with complete or nearly complete donor chimerism [21]. In patients with poor graft function, a $CD34^+$ selected cell boost following G-CSF mobilization was associated with a high likelihood of hematopoietic cell recovery and a low risk of GVHD [21]. Patients did not receive conditioning prior to the $CD34^+$ cell boost. It is unclear how long one should wait before requesting a boost of cells. Once the diagnosis of poor graft function is established, we consider a few weeks of severe cytopenia to be a reasonable period, keeping in mind that it may take another several weeks actually to collect the cells. Poor graft function may result from a viral infection, a medication side effect, and GVHD, and identifying the underlying cause should be the primary focus as appropriate treatment may establish that the cytopenias are transitory.

Table 77.5 Considerations for a second allogeneic HCT for graft failure

- Should the same or a different donor be used? Likely does not matter, check for DSA
- Should BM or G-mobilized blood be used? If HLA matched may not matter, second transplants have increased risk of acute GVHD
- When to use a $CD34^+$ selected boost? Predominantly for poor graft function
- When to use an unmanipulated graft? Predominantly for graft rejection
- Should a preparative regimen be used? Yes, preference for RIC
- Is post-second transplantation IS medication necessary? Yes
- What is the role of DLI for converting mixed chimerism? Evidence supports DLI to convert mixed to full donor type, yet no evidence to support that DLI prevents falling chimerism that would otherwise lead to graft loss

Regrafting

Regrafting with a second allogeneic HCT from the same donor, or an alternative, can successfully rescue patients with GF (Table 77.4) [154–159]. The largest of the studies was a retrospective multicenter report that reviewed 82 consecutive second allogeneic transplants for primary (n = 28) and secondary (n = 54) GF in patients with acute and chronic leukemia and SAA who received full-dose conditioning for the first transplant [154]. The same donor was used for both transplants in 56 cases. All patients received RIC prior to the second transplant. The probability of neutrophil recovery was 75% and platelet recovery to $>50 \times 10^9$/L, was 30%. The estimated 3-year overall survival was 30%, which was superior to the 8% reported for GF in SAA patients without a second transplant [125]. A more recent report of second allogeneic transplants for GF in 38 consecutive patients, predominantly with hematologic malignancies, used a uniform salvage transplant regimen of fludarabine and either 3 or 4…Gy TBI [159]. Sustained engraftment was achieved in 33 patients and the estimated 4-year survival was 42%.

Some considerations regarding salvage second transplants for GF are shown in Table 77.5. There is a requirement for transplant conditioning, of which RIC is recommended. Whether the second transplant used a graft from the original or a different donor did not appear to impact the likelihood for successful engraftment; however, there appeared to be a relatively high incidence of acute GVHD, and disease recurrence after resolution of graft failure remained an important cause of treatment failure.

Conclusion

Successful HCT is achieved when the infused hematopoietic progenitor cells traffic to their BM niche and restore hematopoiesis. Graft failure refers to an inadequate recovery of blood cells following HCT and remains a relatively uncommon complication, yet its presence can be associated with significant morbidity and mortality. Graft failure is the end-stage result of many contributing factors. A detailed evaluation of the underlying cause of graft dysfunction is important because appropriate treatment may establish that the cytopenias are transitory. Although more study is needed, several treatment options are available to overcome GF and improve the long-term survival in transplant recipients.

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CHAPTER 78

Blood Group Incompatibilities and Hemolytic Complications of Hematopoietic Cell Transplantation

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Introduction

The etiologies of hemolytic disorders following hematopoietic cell transplantation (HCT) can be categorized as either immune mediated or complications related to thrombotic microangiopathy (TMA) syndromes. The majority of immune-mediated hemolytic reactions are caused by alloantibodies directed at red blood cell (RBC) antigens in blood group-incompatible donor–recipient pairs. Immune-mediated hemolysis is less common but can be seen following both allogeneic and autologous HCT. In these instances, hemolysis may be related to drugs, infections or recurrent disease (primarily in lymphoproliferative disorders) or be a manifestation of chronic graft-versus-host disease (GVHD). The factors leading to hemolysis in the context of thrombotic microangiopathy following HCT are poorly understood. The supposition has been that endothelial cells injury caused by radiation, dose-intensive chemotherapy, immunosuppressive agents such as tacrolimus (FK506), sirolimus, or cyclosporine (CSP), or cytokine release associated with GVHD are responsible for thrombus formation in small vessel beds. This chapter addresses the issues of prevention, diagnosis, and treatment of these disorders in the context of HCT.

Hemolytic complications of ABO incompatibility

The inheritance of blood group antigens (ABO, Rh, Jk, etc.) is independent of that of the human leukocyte antigen (HLA) complex present on tissues. In most series of sibling allogeneic HCT, there is discordance of ABO groups in 30–40% of donor–recipient pairs with similar or slightly higher rates in unrelated donor (URD) HCT. Although ABO incompatibility may increase the complexity of HCT, it is not usually viewed as a barrier to successful engraftment of myeloid or megakaryocytic precursors and has not been shown to affect the incidence of GVHD. Recipients of ABO-incompatible HCT products do require more RBC and platelet transfusions than those receiving compatible products with a subset of patients with major ABO mismatches developing pure red cell aplasia (PRCA), which usually resolves within a few weeks to months.

ABO incompatibility is defined as major when the recipient plasma contains isohemagglutinins (HAs) against donor RBC antigens; it is a minor mismatch if the donor has HAs directed against recipient RBC antigens and bidirectional if both features are present. There are two scenarios for the development of hemolysis in the first weeks post HCT [1–5] (Table 78.1):

1. Pre-existing HAs cause immediate and ongoing lysis of RBCs as long as the HAs persist (major or bidirectional).
2. Generation of new HAs by passenger lymphocytes in the graft as a response to foreign antigens in the recipient causes delayed hemolysis usually 2–4 weeks following HCT (minor and bidirectional).

The time of onset and duration of hemolysis are affected by the titer of HAs and the rate of clearance and also the quantity of target antigen available. In a proportion of major ABO-incompatible HCT, the HAs target not just circulating RBCs but also early marrow precursors expressing the antigen at the level of the colony-forming unit – erythroid (CFU-E), leading to PRCA [6–9]. Other factors that may have implications for the development of hemolysis are the conditioning regimen [high-dose versus reduced-intensity conditioning (RIC) versus non-myeloablative (NMA)], HCT source [marrow versus peripheral blood (PB/HCT)], and donor [sibling versus URD versus umbilical cord blood (UCB)] and immunosuppressive agents. The development of RIC regimens to accommodate a wider range of patients creates an environment in which donor and host hematopoiesis coexist for protracted periods; this has led to concerns about the possibility of higher rates of chronic hemolysis and PRCA [10–12]. Peripheral blood HCT products carry a 10-fold higher number of T-lymphocytes, which are less activated than T cells in marrow products. B-cell recovery also is reported to occur earlier post-PB/HCT with augmented immunoglobulin production early post-HCT compared with marrow, which might lead to a higher probability of delayed hemolysis in minor incompatible HCTs.

Delayed RBC production and increased transfusion requirements have been documented with ABO incompatibility in several studies in both high-dose and RIC settings. Worel et al. showed that the generation of reticulocyte counts >3% took a median of 32 days for major ABO mismatches versus 21 and 22 days for matched and minor mismatched recipients, respectively, and the number of RBC transfusions up to day 100 following ablative marrow HCT doubled (12 for major versus six for match and eight for minor) ($p = 0.045$) [13]. In 2001, Bolan et al. reported that RBC production was delayed beyond 100 days in over 60% of patients treated with RIC [14]. Early post-HCT, RBC production may appear adequate in RIC and NMA recipients due to autologous RBC recovery; however, as the chimeric balance progressively becomes donor in origin, autologous RBC production declines during the second to fourth months post-RIC HCT. The agents used in RIC (fludarabine, cyclophosphamide,

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Table 78.1 Immunohematologic problems of ABO-incompatible hematopoietic cell transplantation (HCT)

Major mismatch (20%)
Example: Recipient O, Donor A, B, AB *or* Recipient A or B, Donor AB
  Immediate hemolysis of RBC infused with graft
  Delayed hemolysis of donor RBCs by persistent recipient HAs
  Delayed erythrocyte production
  Pure red cell aplasia

Minor mismatch (22–24%)
Example: Recipient A, B, or AB, Donor O *or* Recipient AB, Donor A or B
  Immediate hemolysis of recipient RBCs by donor-derived HAs in graft
  Delayed hemolysis of recipient RBCs by newly generated HAs from donor lymphocytes ("passage lymphocyte")

Bidirectional mismatch (1–2%)
Example: Donor A, Recipient B *or* vice versa
  Both immediate and delayed hemolysis are possible with HAs of donor against recipient RBCs and recipient HAs directed against donor RBCs

melphalan, and low dose radiation) allow the persistence of recipient B cells and plasma cells for much longer periods than in ablative conditioning [15]. During the period of mixed chimerism, these cells are capable of continuing production of recipient-type HA. Although marrow cultures confirm that engraftment of erythroid precursors at the level of burst-forming units – erythroid (BFU-E) occurs in the same ratio as myeloid and megakaryocytic precursors, later erythroid precursors that express ABH antigens starting at the level of CFU-E often do not appear on marrow biopsy until immunoglobulin G (IgG) and IgM titers of recipient HA fall to 1:1 [16]. The reported incidence of PRCA following RIC has varied widely from 6 to 50%, compared with rates of 5–16% post-high-dose conditioning [14]. Griffith et al. followed chimerism studies including plasma cells and B cells post-fludarabine/cyclophosphamide conditioning in 12 ABO-mismatched recipients with renal cell carcinoma or other malignancy. Six developed PRCA that persisted for 3–4 months after T-cell and myeloid hematopoiesis had shifted to 100% donor; in all six patients with PRCA, recipient plasma cells were detectable by chimerism studies (5–42%) and persisted longer than B cells, implying that the plasma cells were the source of the HA production [17]. In a recent report from the M.D. Anderson Cancer Center at Houston, TX, covering 162 patients who received major ABO-incompatible stem cells following RIC with a busulfan-based regimen, the incidence of PRCA was 7.5%. The median time to appearance of donor RBCs was 30 days for the majority of patients without PRCA versus 218 days for the PRCA recipients who remained transfusion dependent beyond 500 days [18]. Wang et al. summarized the Seattle experience for 503 patients who received NMA conditioning regimens of a single fraction of total body irradiation (TBI) 2…Gy alone or combined with fludarabine. Transfusion requirements for both PRBCs and platelets were less for NMA recipients with 25% of patients not requiring PRBCs for NMA versus 4% for ablative regimens. As expected, rates of PRBC requirements were higher in ABO-incompatible or bidirectional pairs than in ABO-matched or minor mismatched pairs whereas there was no difference in platelet transfusion requirements. The median time to disappearance of HA was 48 days for IgG and 47 days for IgM among 102 major or bidirectionally mismatched pairs; nine recipients still had detectable titers at the time of last follow-up. Time to clearance was related to pre-transplant titers. There was no significant difference in graft failure, GVHD, or survival based on blood group mismatch. The report did not comment on the incidence of PRCA [19].

There have also been reports that GVHD regimens of CSP alone or combined with prednisone create a mileau conducive to persistence of HA production since CSP (and by inference FK506) permits B cells to remain longer than combinations that include methotrexate or mycophenolate mofetil (MMF) along with CSP [20]. The impact of sirolimus combined with FK506 on the clearance of HA is uncertain. The development of acute GVHD appears to accelerate the clearance of HA, possibly due to a graft-versus-plasma cell effect [21]. In the M.D. Anderson series which used FK506 and methotrexate, the incidence of GVHD in the non-PRCA patients was 27% but none of the PRCA patients had GVHD prior to recovery of RBC engraftment.

Delayed hemolytic reactions

In minor ABO-mismatched HCT, hemolysis, which may be severe and occasionally fatal, can occur either immediately due to existing IgM or IgG HA in the product or emerge abruptly 5–17 days post-transplant as a result of the development of new HA generated by "passenger" B-lymphocytes transferred from the donor at the time of transplantation [5,22,23]. Significant hemolysis occurs in 10–15% of minor mismatched adult HCT grafts compared with 10% for renal allografts and 70% for heart–lung recipients. The incidence of "passenger lymphocyte syndrome" increases with the B-cell content of the graft; it is therefore reasonable to expect a higher incidence of hemolysis with peripheral blood progenitor cell (PBPC) products that contain a 10-fold increase in $CD10^+$ and $CD20^+$ cells compared with marrow grafts [23,24]. The use of granulocyte colony-stimulating factor (G-CSF) to mobilize donor PBPCs can also modulate T-lymphocyte cytokine production in such a way that promotes antibody production. Although ABO antigens are the most common targets, hemolysis has also been reported against Rh, Jka, Kidd, and Lewis blood group antigens [25–28]. Several factors influence the severity of the hemolysis, including rapidity of rise of donor antibody in the recipient post-transplant, secretor status, and rapidity of engraftment (i.e. type of graft) [23,24,29]. Massive hemolysis in which most, if not all, recipient RBCs are lysed over 2–3 days seems to be complement mediated, analogous to post-transfusion purpura. The incidence of hemolysis following minor mismatched HCT has varied from 5–10% for marrow up to 33% for PB/HCT. One study evaluated the rate of HA production following 24 PBPC and 14 UCB minor mismatched products. Fifteen of 24 PBPC recipients were found to have donor-derived HAs between 6 and 88 days post-HCT; 50% of those with detectable HAs showed some evidence of hemolysis. None of the UCB recipients developed HAs, which correlates with the data that the naïve B cells present in the UCB graft lack the exposure to ABH antigens necessary to generate HAs [29].

Management of ABO and Rh incompatibility

The strategies for managing blood product support is outlined in Table 78.2. As a general rule, Group O RBCs are the preferred products in the immediate pre- and post-HCT period, thereby diluting the percentage of RBC targets for donor-generated HAs in minor or bidirectional mismatched HCT [30–33]. Group O RBCs are the RBC product of choice upon selection of an ABO-mismatched donor for an imminent HCT procedure. Prior to HCT conditioning, both recipient and donor HA titers should be ascertained. If the recipient has high titers against donor RBC antigen, the RBC content of the infused product will be minimized. Plasma exchange should be considered using 5% albumin or plasma lacking that antibody shortly before HCT in patients with titers ≥1:256.

Table 78.2 Management of blood support for ABO-incompatible transplant recipients

	RBCs	**Plasma**
Major mismatch		
Example: Recipient O Donor A	Remove RBCs from HCT product to <25…mL or <1…mL/kg Transfuse O cells pre- and post-transplant	Give donor-type platelets and plasma and volume-reduced platelet product if not available. If recipient anti-A titer is >1:256, consider: Plasma exchange pre-transplant
Minor mismatch		
Example: Recipient B Donor O	Transfuse group O cells. Red cell exchange with group O cells pre-HCT, or hyper-transfuse with group O cells	1. Remove plasma from HCT graft to prevent immediate hemolysis if donor titer ≥1:256 or antibody is hemolytic 2. Give platelet and plasma lacking anti-B isohemagglutinin or volume-reduced platelets 3. Monitor direct Coombs test beginning day +1 then every 2–3 days ending day +21 to monitor for hemolysis caused by "passenger" lymphocyte syndrome
Bidirectional mismatch		
Example: Recipient B Donor A	Transfuse O cells Remove RBCs from HCT product Red cell exchange with group O cells, or hyper-transfuse with group O cells	1. Remove plasma from HCT(M) to prevent immediate hemolysis if donor titer >1:256 or antibody is hemolytic 2. Use group AB plasma and platelet products before and after transplant, or volume-reduced platelets 3. Consider plasma exchange for isohemagglutinin titer >1:256 4. Monitor direct Coombs test beginning day +1 then every 2–3 days ending day +21 to monitor for hemolysis caused by "passenger" lymphocyte syndrome
Rh incompatible Major Minor	Transfuse with group O negative cells pre- and post-transplant. Possible RBC-depleting HPC(M) from an Rh-positive product if recipient has a high titer anti-Rh antibody	Consider Rhogam

A typical one plasma volume exchange will remove approximately 60–70% of circulating antibodies, and since drug levels will be affected by the exchange, redosing certain regimen drugs might need to be considered. RBC exchange prior to HCT has been used to minimize the late hemolytic reactions seen 5–16 days post-infusion in minor ABO-mismatched recipients with high titers against recipient RBCs; these procedures are usually performed between 2 weeks and up to day −3 before HCT infusion after immunosuppression has been started to prevent rebound phenomena. A one RBC volume exchange with group O RBCs will provide the patient with approximately 60% of group O RBCs at the end of the procedure. The pre-transplant titers are not always predictive of the severity of delayed hemolysis in the "passenger lymphocyte" syndrome, hence exchange transfusions are being used less frequently. Some suggest that RBC and platelet products should be volume reduced post-HCT to minimize infusion of HAs in the plasma [33], whereas others propose infusing donor-type fresh frozen plasma (FFP) to provide a non-cellular source of A or B antigen to absorb recipient HAs [34].

Some authors have also reported using small aliquots of donor RBC 12–24 hours before HCT. This intervention requires forced alkaline diuresis and intensive monitoring for hemolytic reaction [35]. Hyper-transfusion with group O RBCs prior to day 0 has also been utilized by some centers. One example of hyper-transfusion is to give one unit of group O RBC to an adult patient every day for 3–4 days while the patient is receiving the conditioning regimen. An important aspect in managing immediate and delayed hemolysis for ABO-mismatched transplantation is product preparation. Peripheral blood hematopoietic stem-cell product (HPC-A) usually contains less than 20…mL of RBCs, but RBC depletion is often performed on a major ABO-incompatible marrow product by using either dextran sedimentation or centrifugation on an automated apheresis machine. Reduction of HAs by reducing the plasma volume of a minor ABO-incompatible marrow or apheresis stem-cell product is also commonly performed when the donor HA titer is greater than 1:256 or if the antibody is hemolytic. Although these measures are fairly effective in preventing immediate hemolysis in major mismatches;, 10% of patients receiving myeloablative and up 35% of patients receiving RIC HCT will develop delayed hemolysis.

Following HCT, patients with ABO incompatibility should be monitored clinically, as well as by the blood bank. Typically, an anti-A and/or anti-B titer will be performed 2 weeks to 1 month post-transplant on a major or a major/minor ABO-incompatible transplants. A direct Coombs test will be performed on day +1 and then every 2 days, ending on day +21 for minor and major/minor ABO-incompatible transplants. Clinical monitoring for evidence of hemolysis includes lactic dehydrogenase (LDH), reticulocyte counts, and bilirubin two or three times per week in the first 21 days post-HCT when the risk for acute hemolysis is highest. Patients with major mismatches and high HA titers pre-HCT are particularly at risk for delay of RBC engraftment and PRCA. HA titers in recipients with major mismatches should be monitored at least twice monthly when clinically indicated. Patients with rising titers and increasing transfusion requirements merit marrow evaluation to exclude PRCA. Clearance of HAs appears to be affected by both donor source and GVHD. Investigators in Seattle examined the rate of fall of HA titers in 383 major or bidirectional ABO-mismatched HCT recipients following ablative conditioning [36]. The time to undetectable titers was significantly shorter (46 versus 61 days, p = 0.016) in unrelated compared with sibling recipients. Among related donor–recipient pairs, those who developed GVHD had a twofold more rapid decline in antibody titers. In this study, ABO incompatibility did not affect the incidence of GVHD or survival. Remberger et al. reported that major ABO mismatch was a significant risk factor for graft failure among 224 URD HCT recipients who received fully ablative conditioning and T-cell depletion as a component of GVHD prophylaxis. The rate of primary and secondary graft failure was 7.5% for ABO major mismatch and 0.6% for matched donor–recipient pairs [37]. Graft failure has not been reported with high frequency in non-T-depleted studies. In two other studies, ABO incompatibility did affect survival at day 100 in subgroups of patients (chronic myelogenous leukemia patients in one and acute myeloid leukemia and myelodysplasia in the other) [14,38]. Neither report specified the factors influencing the poorer outcomes for ABO-incompatible HCT. One may speculate that more protracted transfusional support requiring indwelling venous access devices, which placed patients at higher risk of infection, might be a factor. Iron overload is a potential deleterious condition for those patients who developed PRCA.

Two large studies of the impact of ABO incompatibility of HCT outcome were compiled by the Center for International Blood and Marrow Transplant Research (CIBMTR) and the National Marrow Donor Program (NMDP). The CIBMTR study evaluated 3103 sibling ablative HCTs performed between 1990 and 1998 with CSP/methotrexate for GVHD prophylaxis for early-stage leukemia. Two-thirds of the recipients were ABO matched and 14% were major, 14% minor and 4% bidirectional mismatches. There were no differences in survival, relapse, transplant-related mortality, or acute and chronic GVHD. The only differences were a higher incidence of grade II–IV GVHD in bidirectional mismatched pairs and a longer RBC transfusion dependence for major mismatches [39]. In the NMDP report on almost 7000 donor–recipient pairs of which fewer than 40% were ABO matched, there was no difference in mortality or survival based on ABO groupings [40].The registry data from Japan showed no difference in acute GVHD but did show a higher rate of non-relapse mortality with both major and minor mismatches and a lower overall survival for major incompatible transplants [41]. The French registry data for over 1000 reduced-intensity transplants from both sibling and URD pairs showed increased non-relapse mortality and decreased overall survival for minor mismatch recipients[42]. The reasons for these differences are unclear.

Despite immunosuppression, patients can also develop alloimmunization to non-ABO RBC antigens post-HCT, most commonly involving Rh, Kell, or Kidd (Jk) antigens. Development of non-ABO antibodies has been reported in high frequency in patients receiving ABO incompatible HCT: 9.6% versus 1.6% for those receiving ABO matched products [25]. De la Rubia et al. [26] reported a 3.7% incidence of detection of new alloimmunization to Jka, K, M, Le, and Rh antigens, usually within the first 30 days post-HCT. In the majority of cases in which the specificity of the antibody was identified by elutriation from RBCs, the antibody was directed at antigens that were not detected in either the donor or recipient at the time of HCT. The HAs could have been produced by either residual host- or donor-derived lymphocytes in response to new RBC antigens presented post-HCT. Clinically, the agglutinins that develop in response to antigens that are different from those expressed on either donor or recipient RBCs have little hemolysis associated with them compared with fairly severe hemolytic reactions that have been seen with Rh– donor lymphocytes infused into Rh+ recipients. However, there have been two reports of severe hemolysis associated with the Jka system. In both instances, the donor had previously been sensitized to Jka but the method used for the pre-transplantation antibody screen did not detect the Jka antibody. It is not uncommon for an individual with known anti-Jka to have no serologically identifiable serum antibody a few months after the antibody was originally identified, making the risk of a clinically significant hemolytic event higher in Jka+ recipients [26,28], but overall, hemolysis due to non-ABO RBC antibodies post-ABO-incompatible transplants is not common.

Treatment of pure red cell aplasia

PRCA represents the most extreme form of hemolytic reaction post-HCT with recipient HAs targeting medullary precursors at the CFU-E stage, which is the earliest point of ABH antigen presentation. Recent reports suggest that there is a difference in both incidence and timing of this complication dependent upon the conditioning regimen. In high-dose conditioning, the incidence is lower and appears as an early and prolonged transfusion dependence whereas RIC recipients often have early autologous RBC production when the donor graft is just emerging with a later development of RBC dependence. RBC production usually returns within 3–4 weeks following the disappearance of circulating HA; however, some patients have remained RBC dependent for over 5 years [43]. In the majority of cases, tapering of immunosuppression permits a "graft-versus-plasma or B-cell" effect that shuts off HA production by either memory B cells or plasma cells. Other strategies have employed pharmacologic doses of erythropoietin alone or combined with steroids [44–46]. In more refractory cases, plasma exchange or the use of rituximab has been successful; rarely, donor lymphocyte infusion has been used to promote full donor chimerism and to ablate HA production, albeit at the risk of inducing acute GVHD [47–50]. Poon and Koh recently reported their experience using 4-weekly doses of bortezomib to target the mature plasma cells that were sustaining HA production in a patient who had failed all of the above therapies. Within 1 month of starting therapy the HAs were undetectable, although it was a further 3 months before the patient became transfusion independent [51].

In addition to PRCA associated with ABO incompatibility, aplasia has also been reported in conjunction with parvovirus B19 and human herpes virus 6 (HHV6) infections in both HCT and solid organ transplantation [52,53]. Most of these patients responded to Ig administration and treatment of the infection. Two children with hepatic allografts were reported to develop PRCA associated with FK506, which resolved when the drug was withdrawn; four renal allograft recipients developed PRCA associated with MMF [54,55]. In situations where PRCA develops outside the setting of major ABO incompatibility, a change in calcineurin inhibitor from FK506 to CSP or discontinuation of MMF may produce red cell recovery.

Non-ABO-related Coombs-positive autoimmune hemolytic anemia (AIHA) can also develop late post-HCT as an autoimmune manifestation of chronic GVHD [56]. Drugs such as antibiotics and fludarabine or infections with mycoplasma may also produce hemolysis. Impaired B-cell regulation may occur as a consequence of functional or quantitative T-cell depletion of the HCT product, with an AIHA incidence of 3% in one large series.

Patients who develop AIHA after HCT usually receive the same battery of immunosuppressive agents as non-transplant patients: steroids, immunoglobulin, rituximab, or splenectomy and also less conventional approaches such as plasma immunoabsorption, vincristine, or reinfusion of aliquots of donor T cells to exert immunoregulation on the autoreactive T cells.

Hemolysis and thrombotic microangiopathy (TMA) syndrome

Thrombotic microangiopathy describes syndromes of Coombs-negative hemolysis associated with RBC fragmentation, often combined with clinical features of renal or neurologic dysfunction and thrombocytopenia suggestive of thrombotic thrombocytopenic purpura (TTP) or hemolytic uremic syndrome (HUS). Injury to the endothelial substructure early in the post-transplant setting can be attributed to high-dose chemotherapy, radiation, or cytokine production in acute GVHD. Calcineurin inhibitors used for GVHD prophylaxis (cyclosporine or FK506) are directly toxic to the endothelium and can cause thrombosis in the microvasculature [57–62]. Sirolimus is a lactone antibiotic with a different mechanism of immunosuppression. Although this agent is not directly toxic to endothelial cells when used in combination with calcineurin inhibitors, it seems to potentiate vascular toxicity. Inflammatory cytokines [tumor necrosis factor (TNF) and interleukin-8 (IL-8)] released by tissue injury associated with GVHD have been linked to increases in prothrombotic events such as increases in tissue plasminogen activator and ultralarge von Willebrand factor assembly; IL-6 inhibits ultralarge von Willebrand factor cleavage [58,59]. The role of complement activation by the classical pathway was demonstrated by C4d deposition in renal biopsies of children with post-HCT TMA by Laskin et al. [63]. The involvement of the alternative pathway has been implicated by the detection of a high frequency of deletions of complement factor H (CFH) related genes 1 and 3 (83%) in children with post HCT TMA in both the autologous and allogeneic setting compared with the normal population (24%)and the presence of autoantibodies generated against complement factor H (CFH) in allogeneic recipients [64]. The incidence of TMA has varied widely from low levels of less than 1% for autologous HCT to as high as 70% in some small series of allogeneic HCT [65–69]. In contrast to idiopathic sporadic TTP/HUS syndrome, there is no defining laboratory marker such as ADAMTS13 deficiency, decrease in large von Willebrand multimers, or markers of complement activation that defines this entity or separates it from coexisting post-transplantation complications [65,70–72]. In a 2004 literature review of 447 cases of post-transplantation TMA culled from more than 5400 transplant procedures in 35 articles, George et al. reported an overall incidence of 8.2% with the majority of cases occurring within the first 3 months after HCT [73]. However, the criteria used to define the syndrome differed in 28 of these series and included a range of clinical settings from non-transfusion-dependent anemia with schistocytes and mild renal impairment to severe anemia, thrombocytopenia with dialysis-dependent renal failure, and/or neurologic symptoms that usually were manifested as confusion or seizures. In 2005, the Bone Marrow Transplant Clinical Trials Network (BMT CTN) established criteria for classifying and grading TMA; in 2007, the European BMT International Working Group (EBMT IWG) proposed slightly different criteria [74,75] (Table 78.3). Although there is a substantial degree of overlap, the CTN criteria that emphasize organ dysfunction capture a cadre with clinically worse features whereas the IWG, which focuses more on the thrombocytopenia and hemolysis, will include a broader range of endothelial cell injury. Thus reported outcomes will vary depending on the criteria used to define TMA in a series [76]. In 2008, shortly after both of the above criteria were published, Cho et al. reported on the impact of TMA in patients with acute GVHD (greater than grade II) [77]. Over a 4-year period (2001–2005), 674 patients received allogeneic HCT primarily with ablative conditioning; 158 (28%) developed grade II or greater acute GVHD. TMA occurred in 43 (39.5%) of these patients, which was graded as definite if the patients had either renal or neurologic dysfunction (16 patients) or probable if they had increased LDH and schistocytes and a negative Coombs test (27 patients). The median onset of recognition of TMA was 53 days (range 10–108 days) and GVHD was already present in 77% of the patients at the time. Twenty seven of the TMA patients died; TMA was considered a contributing factor in 11/13 of definite cases and in only 4/14 probable cases of TMA [77].

Table 78.3 BMT CTN Toxicity Committee consensus criteria [74] and EBMT IWG criteria [75] for TMA

BMT CTN criteria	IWG criteria
1. RBC fragmentation and more than two schistocytes per high-power field on peripheral smear	1. Increased percentage (>4%) of schistocytes in peripheral blood
2. Concurrent increased serum LDH above institutional baseline	2. De novo prolonged or progressive thrombocytopenia (PLT count thrombocytopenia <50×10^9/L or a 50% decrease from previous counts)
3. Concurrent renal[a] and/or neurologic dysfunction without other explanations	3. Decrease in hemoglobin concentration or increase in blood transfusion requirement
4. Negative direct and indirect antiglobulin test results	4. Decrease in serum haptoglobin concentration

[a] Doubling of serum creatinine from baseline (baseline = creatinine before hydration and conditioning) of 50% decrease in creatinine clearance from baseline.

The median time of onset of TMA in other adult allogeneic HCT series is 47 days, although an earlier onset at a median of 25 days was reported by Cutler et al. in patients receiving a combination of sirolimus and tacrolimus [78–80]. A series from St. Jude's Hospital in Memphis, TN, showed a 9.6% incidence of TMA with a much later onset of 171 days for pediatric patients; however, in this population T-cell depletion was used as a component of GVHD prophylaxis for all unrelated or mismatched related donor GVHD prophylaxis. In this report, T-cell depletion was identified as a risk factor for developing TMA rather than either acute GVHD or URD status, which are the major risk factors for TMA in adults [81]. In addition to grade II–IV GVHD, female gender, receipt of an unrelated donor product, and diagnosis of lymphoid malignancy were the major predisposing factors for TMA in a review of over 1200 patients treated at the M.D. Anderson Cancer Center using tacrolimus-based GVHD prophylaxis [82]. The incidence of TMA in this group was 5.9% compared with a 10.8% incidence using tacrolimus and sirolimus in the series reported from Dana Farber Cancer Institute in Boston, MA. The TMA rate for the CSP-based control group in the Dana Farber series was 4.2% [78]. The use of RIC has not led to a lower incidence of TMA with an incidence of 15–23% in three small series; in several of these cases, however, these patients had had a prior myeloablative HCT often within the previous 6 months [80,83,84]. In the M.D. Anderson report, which included more than 500 RIC recipients, the risk of TMA was equivalent to that for high-dose conditioning recipients. In 2010, investigators at the City of Hope Cancer Center (COH) reported on their experience with tacrolimus/sirolimus in 85 patients; over half of the patients (46) received RIC, 28 received fractionated total body irradiation (FTBI)/etoposide and 11 received busulfan plus cyclophosphamide. TMA developed in 7% of the RIC patients, 25% of the FTBI patients, and 55% of the busulfan recipients. The median tacrolimus levels were higher in the busulfan recipients than the other groups. Acute GVHD (greater than grade II) developed in 43% overall and was present in 75% of patients who subsequently developed TMA. Treatment for TMA was primarily dose reduction or discontinuation of one or both drugs. Only one patient received plasma exchange. TMA was reversible in all cases and only one patient required dialysis [85]. In a more recent COH follow-up of 177 patients who received tacrolimus and sirolimus for GVHD prophylaxis, the group had a 17% incidence of definite TMA with an additional 5% of patients with probable TMA with a median onset of TMA at 4.6 weeks. Acute GVHD was a pre-existing condition in 81% of these patients. Risk factors for TMA were myeloablative conditioning, grade II or greater acute GVHD and a sirolimus level of 9.9…ng/mL at day 14. In this series, TMA was a negative predictor for non-relapse mortality with a 33% non-relapse mortality at 2 years for patients with TMA versus 12.25% for those without TMA [86].

Therapy for post-HCT TMA

Patients who exhibit grade I TMA by NCI common toxicity criteria for TMA actually do not meet the current CTN definition of TMA (Table 78.4) However, these patients may represent a population in which attention to factors that may increase endothelial damage and RBC fragility may lessen the risk of evolution to overt TMA. Strategies include optimizing anti-hypertensive therapy, adjusting CSP, FK506, and sirolimus levels to lower therapeutic levels in patients without active GVHD, and providing appropriate magnesium replacement. In patients with renal dysfunction or neurologic abnormalities, discontinuing CSP or FK506 for a few days may help distinguish TMA from drug-related toxicity. However, a significant number of TMA patients also have active GVHD of grade II or greater that requires ongoing therapy. In the M.D. Anderson series, FK506 was continued at reduced doses in the majority of patients and discontinued only for severe renal or neurologic impairment. In patients requiring discontinuation of FK506, either steroids were started or the dose was increased to provide additional coverage for GVHD.

The majority of patients (95%) in the M.D. Anderson trial also received plasma exchange on a daily basis with tapering of exchanges based on clinical response [82]. Resolution of TMA-associated clinical and laboratory findings occurred in 60% of patients at a median of 29 days in this report, in contrast to much lower responses to plasma exchange (18–30%) in earlier series. Mortality, however, remained high with only 50% of the responders and none of the non-responders alive at 6 months. Control of GVHD and infection appeared to be crucial for long-term survival post-TMA. Ultimately, only 11/66 plasma exchange recipients were long-term survivors at 3 years post-HCT, comparable to the 70–95% mortality in other post-HCT TMA series, and in contrast to survival of >60% in idiopathic non-HCT TMA treated with plasma exchange [87–97]. Based on these experiences plasma exchange is less commonly employed for long-term management of TMA; its role is now more often only in the setting of significant organ dysfunction as a temporizing strategy as some patients may show improvement in neurologic or renal function early following plasma exchange. Mechanistically, plasma exchange either alone or in combination with rituximab might be helpful in (1) rapidly removing toxic levels of the inciting drugs or (2) depleting autoantibodies in the minority of cases which either led to defective cleaving of large von Willebrand multimers or indirectly led to complement activation. If there is not meaningful improvement in organ function within the first 5–7 days, plasma exchange should be stopped. New treatment approaches need to be developed for this entity. One of the novel treatments being explored is the use of eculizumab, the monoclonal antibody against the C5 component of complement, which was original approved for the treatment of paroxysmal nocturnal hemoglobinuria (PNH) and has more recently been given expanded utilization in the treatment of atypical hemolytic uremic syndrome (aHUS). There has been a case report in an adult and one small series of six children with severe post-HCT TMA treated with eculizumab [98,99]. In the pediatric series, four of six patients responded with improvement of hematologic parameters within 15–45 days; they were able to discontinue the drug after 4–13 doses. Drug levels and efficacy of complement blockade (as measured by a CH50 of ≤4) were tested twice weekly. While the routine treatment schedule was weekly, those without therapeutic levels of eculizumab received additional drug at the time the low target dose became available. Two children who had grade IV GVHD and multiorgan failure at the start of therapy never achieved evidence of adequate complement blockade and died of GVHD/TMA. CH50 levels <4 correlated well with therapeutic drug levels and are a much more easily available way to monitor suppression of complement activation outside the research setting. All patients received antibiotic prophylaxis for meningococcal infection throughout therapy.

Table 78.4 NCI common toxicity criteria for BMT-associated TMA

Thrombotic microangiopathy	Version 3.0
Grade I	Evidence of RBC destruction (schistocytosis) without clinical consequences[a]
Grade II	–
Grade III	Laboratory findings present with clinical consequences (e.g. renal insufficiency, petechiae)
Grade IV	Laboratory findings and life-threatening or disabling consequences (e.g. CNS hemorrhage/bleeding or thrombosis/embolism or renal failure)

[a] Must have microangiopathic changes on blood smear (e.g. schistocytes, helmet cells, red cell fragments).

Conclusion

As the scope of HCT evolves to encompass wider age ranges, differing sources of hematopoietic cells, newer conditioning regimens, and alternative immunosuppressive agents, we need to be conscious that changes in the kinetics of chimerism or the introduction of new drugs to either conditioning or GVHD prophylaxis and treatment can have a significant impact on the incidence and timing of hemolysis post-allogeneic HCT. A transplant program should develop a process and standard to define policies in transfusion support for ABO-incompatible transplant recipients, in post-ABO-incompatible product collection manipulations, and in pre- and post-transplant patient monitoring tests. It is important to maintain a high level of suspicion and to initiate appropriate testing for hemolysis of both immune and thrombotic etiologies in patients with unexplained anemia and increasing transfusion requirements. Better definition of TMA syndromes may allow earlier intervention, which should improve outcomes.

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CHAPTER 79

Principles of Transfusion Support Before and After Hematopoietic Cell Transplantation

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Introduction

Patients who are candidates for or are undergoing hematopoietic cell transplantation (HCT) have unique transfusion requirements. These are due to the need to minimize the likelihood of alloimmunization, the severe immunosuppression that these patients undergo, their temporary inability to produce blood cells, and the fact that their blood type may change and they may become a temporary or permanent chimera. Hence an effective blood bank and transfusion medicine program are essential for a successful HCT program.

Red blood cell components

Red cells

Red blood cells (RBCs) are the cells that remain after most of the plasma has been removed from whole blood. This blood component is often called "packed red cells" or "packed cells." The unit of red cells has a volume of about 350 mL, and will contain about 200 mL of red cells with a hematocrit of about 60%. The fluid portion of the unit is primarily an additive solution preservative used to enable red cells to be stored for 42 days. About 20 mL of plasma remains from the original unit of whole blood. One unit of red cells will increase the hemoglobin and hematocrit in an average-sized (70-kg) adult by about 1 g/dL and 3%, respectively. Red cells are the component of choice for any patient with severe anemia. Most patients who require red cell replacement do not also need intravascular volume replacement because anemia has developed slowly. Therefore, almost all transfusions given for red cell replacement are packed red cells, and whole blood is rarely used.

Red cell transfusions are usually given to HCT patients because of anemia. Although the decision to transfuse is usually based on the hemoglobin level, other clinical factors should also be considered. Most patients with hemoglobins of 10 g/dL or above do not require transfusion, and most patients with a hemoglobin of less than 7 g/dL will benefit from transfusion [1–5], and this is generally used as the indication for red cell transfusion. Because of the heterogeneity of patients, the clinical condition of the patient is of primary importance, and patients should not be transfused based only on their hemoglobin level. Patients with cardiovascular disease may benefit from transfusion at hemoglobin of 9 g/dL [4,5].

When there is acute blood loss, the major threat to the patient is the loss of intravascular volume and resultant cardiovascular collapse. In most "normal" patients, the loss of approximately 1000 mL of blood can be replaced by colloid or crystalloid solutions alone. An otherwise healthy individual can tolerate the loss of up to half of the red cell mass without the need for replacement. Because many patients have some degree of cardiovascular compromise, they will require red cell replacement after smaller volumes of blood loss.

Leukocyte-reduced red cells

Red cell and platelet blood components contain leukocytes that can alloimmunize transfusion recipients, leading to multiple problems (Table 79.1). In order to avoid these problems, leukocytes are removed from the red cells. This is done at the time of blood processing using filters that remove about 99.9% of the leukocytes leaving fewer than 5×10^6 per unit. In the past, filtration was performed at the bedside, but currently this is done in the laboratory in order to obtain more consistent leukocyte removal and prevent the accumulation of cytokines produced by the contaminating leukocytes.

The indications for leukocyte-reduced red cells are as follows:

- prevention of alloimmunization to leukocytes and platelets;
- prevention of transmission of cytomegalovirus (CMV) (see later);
- treatment of patients with multiple febrile transfusion reactions;
- prevention of immunomodulatory effects of transfusion (see later).

The kinds of patient who should receive leukocyte-reduced red cells include any individuals with a disease that can potentially be treated by HCT and those who will receive multiple transfusions during their life, such as patients with hemoglobinopathies (Table 79.2). In addition to alloimmunization to leukocytes, transfusion has other immunologic effects, generally referred to as transfusion-related immune modulation [6–8]. Because the clinical situations in which leukocyte-reduced blood components are valuable continues to increase, many blood centers and some countries now leukocyte-reduce all of their blood components [9,10].

Washed red cells

Washed red cells are red cells suspended in an electrolyte solution. Most plasma, platelets, and leukocytes have been removed, although leukocyte removal is not as complete as with frozen deglycerolized red cells. After washing, the red cells can be stored for only 24 hours

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Table 79.1 Adverse effects of leukocytes in blood components

- Immunologic effects
  - Alloimmunization
    - Febrile non-hemolytic transfusion reactions
    - Refractoriness to platelet transfusion
    - Rejection of transplanted organs
  - Graft-versus-host disease
  - Transfusion-related acute lung injury
  - Immunomodulation
    - Increased bacterial infections
    - Increased recurrence of malignancy
- Infectious disease
  - Cytomegalovirus
  - Human T-lymphotrophic virus-I
  - Epstein–Barr virus

Table 79.2 Clinical situations in which leukodepleted red cells are recommended

- Hematopoietic stem cell recipients
- Acute leukemia
- Chronic leukemia
- Congenital platelet function abnormalities
- Congenital immune deficiency syndrome
- Hematologic malignancies potentially treated with stem cell transplantation
- Solid tumors potentially treated with stem cell transplantation
- Intrauterine transfusions
- Exchange transfusion for hemolytic disease of the newborn
- Hemoglobinopathy or thalassemia

because of the possibility of bacterial contamination. Since the major advantage is the removal of the plasma by washing, washed red cells are indicated for patients who have severe reactions caused by plasma.

Coagulation factor components

Fresh frozen plasma

Fresh frozen plasma (FFP) is plasma separated from whole blood and placed at −18°C or lower within 8 hours of collection. The unit of FFP has a volume of about 200–250 mL and contains all of the coagulation factors that are present in fresh blood. FFP is not considered to contain red cells and is usually administered without regard to Rhesus (Rh) type. However, occasional rare reports have suggested that units of FFP contain a small amount of red cell stroma that can cause immunization to red cells. Because the plasma contains ABO antibodies, the plasma must be compatible with the recipient's red cells.

Although FFP has been widely used for years and its use is increasing, there have been few good clinical trials to establish its efficacy [11]. The generally accepted indications for FFP [12] are documented coagulation factor deficiencies for which factor concentrates are not available, especially factor V, multiple factor deficiencies such as patients with severe liver disease or anticoagulated with warfarin, and massive transfusion. FFP is used as the replacement solution for plasma exchange in patients with thrombotic thrombocytopenic purpura, but it is not indicated for use as a volume expander or a nutritional source.

Bleeding due to dilution of multiple coagulation factor deficiency can occur in massive transfusions. This dilution occurs because red cell components contain little plasma. It is important to replace coagulation factors in platelets before coagulopathy develops [13,14]. Most hospitals have established massive transfusion protocols in which a standard combination of packed red cells, thawed plasma, and platelets is provided by the blood bank very quickly [15]. There is debate about the best ratio of the three components, but 1: 1: 1 is most often used. Patients who are undergoing massive transfusion may also develop disseminated intravascular coagulation (DIC) [16] because of the severity of the underlying problem. Thus, a bleeding diathesis may develop that is a combination of the dilutional coagulopathy of massive transfusion and the "consumption coagulopathy" of DIC.

In mild forms of DIC, transfusion is usually not necessary. However, in the more extreme forms, there is usually a deficiency of factors V and VIII, fibrinogen, and platelets. Replacement of coagulation factors in the management of DIC should be based on laboratory abnormalities and not on arbitrary formulas. When replacement is necessary, FFP is usually used to replace all factors. Coagulopathy may also develop in patients undergoing HCT. The use of FFP as prophylaxis for patients with coagulopathy who are to undergo invasive procedures is discussed later in this chapter.

Plasma (FP24)

Plasma separated from whole blood between 8 and 24 hours after collection can be frozen, stored for up to 1 year, and used as a source of coagulation factor replacement, but FP24 contains only 50–75% of factors V and VIII. This plasma product can be used in most situations requiring replacement of multiple coagulation factors and is gaining wider use.

Cryoprecipitate and fibrinogen

Cryoprecipitate is the cold-insoluble portion of FFP that has been thawed between 1 and 6°C. It is stored at −18°C or below and can be kept for up to 1 year. Cryoprecipitate contains coagulation factor VIII, fibrinogen, and von Willebrand factor. Each bag of cryoprecipitate contains about 250 mg of fibrinogen, and this is now its major use. Cryoprecipitate is not a suitable source of coagulation factors II, V, VII, IX, X, XI, or XII. Virally inactivated fibrinogen concentrate prepared from human plasma is now available. It is recommended when the fibrinogen level is <100 mg/dL, although in acute blood loss, transfusion at a level of 150 g/dL is being used. The US Food and Drug Administration (FDA)-approved indication is limited to congenital fibrinogen deficiency, but it is being used in other situations [17], especially in Europe.

Blood group compatibility of components used to replace coagulation factors

FFP need not be ABO-identical but should be compatible with the recipient's red cells and can be given without regard to Rh type. Red cell compatibility testing is not necessary. Cryoprecipitate should also be administered as ABO compatible with the recipient. Although the volume of each unit is small, most therapy involves many units, hence the total volume of plasma may be large. Cryoprecipitate can be administered without regard to Rh type. Compatibility testing is not necessary. In HCT, patients' cryoprecipitate is usually used to replace fibrinogen, but the recipient's fibrinogen may become elevated by the fibrinogen contained in cryoprecipitate if many units are given to patients who are not hypofibrinogenemic.

Platelet components

Platelet concentrates prepared from whole blood

Platelet concentrates, often referred to as "random-donor" platelet concentrates, are prepared by centrifugation of whole blood. The centrifugation technique used in Europe and Canada differs from that used in the United States [18]. At least 75% of the units of random-donor platelets must contain at least 5.5×10^{10} platelets.

Most units contain 6–8 × 10^{10} platelets, and each blood bank should be able to provide an estimate of the platelet content from their quality control data. To achieve a therapeutic dose, platelets from 4–5 units of whole blood are pooled.

Platelet concentrates prepared by apheresis

Several different instruments that separate the platelets using centrifugation are available for the collection of platelets by apheresis. The instrument operation is controlled by a microprocessor that regulates the blood flow rate, the anticoagulant added to the whole blood entering the system, the centrifuge conditions, the component separation, and recombination of the remaining components that are then returned to the donor.

The official name of this component is "platelets, pheresis," but it is usually called single-donor platelet or plateletpheresis concentrate. For quality control purposes, a unit or bag of plateletpheresis concentrate must contain at least 3 × 10^{11} platelets in a minimum of 75% of the units tested, but most plateletpheresis concentrates contain approximately 3.5–4 × 10^{10} platelets. Plateletpheresis usually requires about 1.5 hours and involves processing 4000–5000 mL of the donor's blood through the blood cell separator. Platelets obtained by plateletpheresis are processed, tested, and labeled the same as whole blood. This includes ABO and Rh typing, and required testing for all transfusion-transmitted diseases. The number of platelets contained in each concentrate is determined, although this information may not necessarily be recorded on the label. Each platelet concentrate has a volume of approximately 200 mL and contains very few (0.5 mL) red cells, so that red cell cross-matching is not necessary. The white blood cell (WBC) content varies depending upon the instrument and technique used for collection, but platelet concentrates produced by apheresis are usually considered to be leukocyte reduced and contain fewer than 5 × 10^6 leukocytes.

Platelet storage conditions and duration

Platelets are stored at 20–24°C to maintain functional effectiveness for 5 days [19]. The variables that affect the quality of platelets during storage include the temperature, anticoagulant preservative solution, storage container, type of agitation, and volume of plasma [19,20]. Citrate phosphate dextrose and citrate phosphate dextrose–adenine solutions are satisfactory platelet preservatives; undisturbed storage is inferior to gentle agitation, and horizontal agitation is preferable to end-over-end agitation. The composition, surface area, and size of the storage container influence the ability for carbon dioxide to diffuse out and oxygen to enter the platelet concentrate, and storage containers specifically designed to optimize platelet quality are now used routinely.

Maintenance of the pH above 6.0 is crucial for satisfactory platelet preservation. This combination of storage container, agitation, preservative solution, temperature, and the use of about 50 mL of plasma provides satisfactory preservation of platelets for 5 days. An electrolyte platelet additive solution used in Europe is now licensed in the United States. This replaces most of the plasma and is associated with fewer allergic transfusion reactions.

Transfusion of platelets

The decision whether to transfuse platelets depends on the clinical condition of the patient, the cause of the thrombocytopenia, the platelet count, and the functional ability of the patient's own platelets. Most platelets are transfused to patients with transient thrombocytopenia due to chemotherapy for malignancy or those undergoing HCT [21]. Most platelet transfusions are used for the prevention of bleeding (as prophylaxis) rather than the treatment of active bleeding [21].

Very few controlled studies of prophylactic platelet transfusion were carried out when platelet therapy first became available. Some studies supported the use of prophylactic transfusions, but other studies were not able to show a benefit of prophylactic platelet transfusion [22]. Two recent studies compared prophylactic transfusion with transfusion only after bleeding occurred. Wandt et al. [23] concluded that prophylactic transfusions were not necessary, whereas Stanworth et al. recommended prophylactic transfusion, especially for patients with acute myeloid leukemia (AML) [24]. Despite the lack of substantial convincing clinical trial data, it became common practice for physicians to use platelet transfusions to prevent serious bleeding when the platelet count was less than 20,000/μL, because there is little risk of serious spontaneous hemorrhage when the platelet count is more than 20,000/μL, but the risk increases with lower counts [25,26]. In stable thrombocytopenic patients, there is no increase in stool blood loss at platelet counts of 10,000/μL, and blood loss increases only when the platelet count reached 5000/μL [27].

Experience in the management of thrombocytopenic patients has made it possible to maintain patients at lower platelet counts than were previously believed to be safe. Serious bleeding usually occurs only when the platelet count is below 10,000/μL, and fatal bleeding is unlikely to occur at platelet counts above 5000/μL. Gmur et al. [28] found that the threshold for prophylactic platelet transfusion could be 5000/μL in uncomplicated patients, 10,000/μL in individuals with fever or bleeding, and 20,000/μL in patients with coagulopathy or bleeding sites. Heckman et al. [29] found no difference in red cell use, days of fever, hospitalization, thrombocytopenia, remission, death, or major bleeding complications when prophylactic transfusion thresholds of 20,000 and 10,000/μL were compared.

Rebulla et al. [30] reported that, in patients with acute leukemia undergoing first induction of remission, there was no difference in deaths, red cell transfusions, or severe hemorrhage, but there was a 21.5% reduction in platelet use using a transfusion threshold of 10,000/μL compared with 20,000/μL. Wandt et al. [31], summarizing experience in 17 centers in Germany, found no difference in bleeding complications when 10,000 or 20,000/μL thresholds were used, and hemorrhagic deaths actually occurred in two patients with platelet counts of 36,000 and 50,000/μL. In stable marrow transplant recipients, lowering the transfusion threshold from 20,000 to 10,000/μL did not increase severe bleeding or deaths due to bleeding [32], but reduced platelet use by about 25%. Aderka et al. [33] proposed, based on an analysis of 196 patients with acute leukemia, that prophylactic platelet transfusion was not necessary when the platelet count was above 10,000/μL. More recently, Zumberg et al. [34] reported no differences in the incidence or severity of bleeding in 159 HCT patients prospectively randomized to receive platelet transfusions at a threshold count of 20,000 or 10,000/μL. A National Institutes of Health Consensus Conference recommended that the 20,000/μL value traditionally used for prophylactic platelet transfusion could be safely lowered for many patients [35]. Beutler [36] recommended lowering the indications for prophylactic platelet transfusion, and now many physician and hospital guidelines use platelet counts of 10,000 or 5000/μL as the indication for prophylactic transfusion to patients with uncomplicated conditions [37].

Establishing the indications for platelet transfusion remains complex because many studies are not powered to show modest differences, most do not use bleeding as the end-point, and the patient population is heterogeneous. The guideline of a platelet count of 10,000/μL for prophylactic transfusion applies to the stable, uncomplicated patient. However, many HCT patients are febrile, have severe mucosal bleeding, or have other complications that necessitate transfusion at higher platelet counts [21]. Clinical decisions to increase the transfusion threshold are variable and do not coincide with a rigidly controlled bleeding assessment [38].

Treatment of active bleeding

Platelet transfusions are not necessary for bleeding patients with a platelet count above 100,000/μL because they have a normal bleeding time. With platelet counts below 100,000/μL, the bleeding time is increasingly prolonged [39]. The optimum platelet count to achieve in a bleeding patient is not known. In one study [40] of patients undergoing surgery, prophylactic platelet transfusions were given to those whose platelet count was less than 50,000/μL. Bleeding was similar in patients with levels less than 50,000/μL who received platelet transfusion compared with those whose platelet count was above 50,000/μL and who did not receive platelet transfusion. The bleeding did not relate to the platelet count but instead to the severity of the surgical procedure. Thus, in actively bleeding patients, platelet transfusion should be considered in those with a platelet count of less than approximately 50,000/μL. An attempt to achieve a level of above 50,000/μL is recommended. If the patient's platelets are dysfunctional due to causes such as drugs or uremia, the bleeding time may be much longer than would be expected based on the degree of thrombocytopenia. In those situations, the decision to give a platelet transfusion is made on clinical grounds alone.

Dose of platelets and outcome of platelet transfusion

There is a dose–response effect from platelet transfusion. Within 1 hour after transfusion, the platelet count increases by approximately 10,000/μL when 1×10^{11} platelets are transfused into a 70-kg patient [41]. Since a transfusion usually contains approximately $3.5–4.0 \times 10^{11}$ platelets, the platelet count should increase by 35,000–40,000/μL in an average-sized adult. In order to achieve these increases in platelet count, four to six whole-blood-derived platelet concentrates are pooled or one apheresis product is used. For some individuals, the volume of plasma in the final pooled component is too large, and plasma must be removed before transfusion. The loss of platelets during this concentration step is reported to be from 15% to as much as 55% [42]. Although concentration or volume reduction of pooled platelets can be carried out successfully, and is often done for small patients or those who are receiving large volumes of blood components or intravenous fluids, some platelets are lost in the process.

The 1-hour post-transfusion platelet count is one predictor of an effective platelet transfusion [41]. If a very accurate determination of the response to platelet transfusion is needed, the 1-hour corrected count increment (CCI) or the percentage recovery can be determined. This takes into account the number of platelets transfused and the size of the patient. The CCI is calculated as follows:

$$\text{CCI} = \frac{(\text{post-transfusion} - \text{pre-transfusion platelet count}) \times (\text{body surface area})}{\text{No. of platelets transfused}}$$

The expected CCI is about 15,000/μL × 10^{11} platelets transfused per square meter of body surface area. If the CCI is less than 5000–7500, the patient is considered to be refractory. If platelet recovery is used as an indicator, the expected result is about 65% because some of the platelets normally are sequestered in the spleen.

It has been suggested that frequent transfusions of a lower dose of platelets will reduce the number of platelets needed [43]. Conversely, when larger numbers are transfused, the time between transfusions is extended [44,45], suggesting that overall fewer platelets or transfusions might be needed. A multi-center trial involving 1272 patients established that transfusion of half or twice the current standard dose of platelets was equally effective as the standard dose [26]. The likelihood of bleeding was similar for platelet counts ranging from 5000 to 80,000/μL, but there was a difference in number of transfusions. Children in this clinical trial had a higher rate of bleeding than adults, but the platelet dose did not affect bleeding [46]. A small study of platelet dose in adults was halted prematurely because of non-statistically significant but clinically important bleeding in the low-dose group [47]. Despite the large, well-performed study [26], transfusion practice has not generally changed to the use of lower doses of platelets.

ABO (H) and Rh in platelet transfusion

ABO antigens are intrinsic to the platelet, and some are also adsorbed on its surface. When ^{51}Cr-labeled group A platelets are transfused to normal group O volunteers, the recovery is reduced [48], and the higher the ABO isoagglutinin titers, the greater is the reduction in recovery of transfused platelets. This ABO effect has been substantiated clinically in several studies. There is a reduced recovery when platelets from human leukocyte antigen (HLA)-matched donors are transfused to alloimmunized patients who are ABO incompatible with the donor platelets [49], and blood group O patients may be refractory to platelets transfused from group A donors but not from platelets transfused from group O donors [49]. Two other studies substantiated the concept that if platelets that contain ABH antigens are transfused into patients with circulating antibody directed against those antigens, the intravascular recovery of the transfused platelets is substantially decreased [50,51]. Decreased recovery of transfused platelets also occurs when the incompatibility involves transfusion of ABO antibody directed against the recipient's ABO antigens (e.g. group O platelets transfused to a group A patient) [51]. This may be due to the formation of circulating immune complexes by soluble ABH substance and ABO antibodies, and can occur with the antigen–antibody complexes from either donor or recipient antibody and donor or recipient ABH substance. Although ABO incompatibility is associated with reduced post-transfusion platelet recovery, this is not of a magnitude that would contraindicate transfusion of ABO-mismatched but HLA-matched platelets.

A separate consideration involving the ABO system and platelet transfusion is the potential administration of large volumes of ABO-incompatible plasma (e.g. group O platelets transfused to group A patients) to cause red cell hemolysis. Most institutions have a policy to avoid or limit the amount of ABO-incompatible plasma transfused in platelet products [52]. Limiting the volume of ABO-incompatible plasma that a patient can receive can be in terms of a percentage of the patient's estimated blood volume or an absolute volume limit or by using only a low ABO titer (<1: 200) for incompatible transfusion. As mentioned earlier, the platelet product can be concentrated further by removing some of the plasma.

Rh antigens are not present on the platelet surface. However, the few red cells contained in the platelet concentrate can lead to immunization, hence Rh must be considered in platelet transfusion. In Rh-negative cancer patients, reported rates of immunization to the D antigen range from 0% to 18% [53–56]. Because oncology patients and HCT recipients receive a large number of platelet transfusions, it is usually not possible to provide all Rh-negative platelets for Rh-negative patients. Rh immune globulin can be administered to prevent alloimmunization, but this is not usually done and is not recommended. Even when circulating anti-D develops, this antibody does not interfere with the circulation of Rh (D)-positive platelets [57]. If Rh immune globulin is to be administered to prevent alloimmunization, the dose of Rh immune globulin can be determined from the number of units of platelets that the patient receives. For instance, since most therapeutic doses of platelets (e.g. one single-donor unit or four to five pooled random-donor units) contain less than 1 mL of red cells, one standard dose of 300μg of Rh immune globulin is sufficient [54].

Lack of response (refractoriness) to platelet transfusion

Many patients do not attain the expected post-transfusion increment in platelet count and are said to be "refractory" to platelet transfusion. In patients who receive large numbers of platelet transfusions, the CCI may decrease for reasons that are not well understood [58]. Refractoriness to platelet transfusion can be caused by factors related to the patient and/or factors related to the platelet concentrate.

Factors related to the patient

In patients who have HLA or platelet antibodies, such as those with autoimmune thrombocytopenic purpura or patients who are immunized to antigens of the HLA system, survival of circulating platelets is extremely brief, sometimes only a few minutes [59]. Splenomegaly also causes sequestration of platelets and a reduced post-transfusion increment [59]. Factors associated with a poor response to platelet transfusion include DIC, amphotericin administration, palpable spleen, presence of HLA antibody, presence of platelet antibody, and fever [60]. It is not clear whether active bleeding is associated with a reduced response to platelet transfusion.

Hence patients can be refractory due to alloimmunization (HLA and/or platelet antibodies) or non-immune clinical factors. In some patients, refractoriness is clearly associated with the presence of HLA antibodies; however, many of these patients also have one or more clinical factors present, which could account at least partially for the refractoriness, and non-immune factors may be the major cause of refractoriness [38,61,62]. Therefore, in practice, it is very difficult to determine whether a patient is refractory due to immune or non-immune factors, and this affects the strategy used to manage these patients. In the past, the incidence of refractoriness was rather high, but during the 1990s it was only about 15% after the first 8 weeks of therapy [63]. This decrease may be due to changing transfusion practices, described in the section on prevention of alloimmunization, or to more intensive chemotherapy.

Factors related to the platelet concentrate

ABO incompatibility can reduce the intravascular recovery and survival of transfused platelets. Transfusion of platelets near the end of their storage period can also reduce recovery [64]. Transfusion of similar numbers of apheresis- or whole-blood-derived platelets should give similar increments in platelet count. There is no reason to believe that transfusion of whole-blood-derived platelets could give the misleading impression of refractoriness due to poor increments based only on the source of the platelets.

Strategies for managing patients who are refractory to platelet transfusion

HLA matching for platelet transfusion

If the use of ABO-matched platelets stored for the shortest time feasible fails to produce a satisfactory increment, transfusion of either HLA-matched or cross-matched platelets should be attempted. Platelets from HLA-identical siblings, HLA-identical unrelated donors, and even partially matched donors can provide a good post-transfusion response [65]. However, most HLA-matched platelets obtained from unrelated donors have some antigens mismatched with the patient. Although the average response to these partially matched transfusions is similar to that from fully matched HLA-identical transfusions, about 30% of HLA-matched transfusions do not provide a satisfactory response [64]. Also, since these patients require frequent platelet transfusions, many donors who are matched with the patient must be available to sustain the patient for days or a few weeks, so that in practice it is often difficult to maintain a patient on HLA-matched platelets. A different approach with some success is to identify the HLA antibody specificity and select donors who lack the corresponding HLA antigens [65,66]. It is not clear whether this results in more successful transfusions.

Platelet cross-matching for platelet transfusion

Immune platelet refractoriness is usually due to HLA antibodies, but platelet-specific antibodies may also be involved. Therefore, one approach that might be effective in overcoming the 30% failure of HLA-matched transfusions is cross-matching the patient's serum and potential donor's cells. Several different platelet antibody methods have been used, and there was great variability in results, with the positive predictive value ranging from 73% to 100% and the negative predictive value from 52% to 92% in 10 studies summarized by Heal et al. [51].

The method currently in clinical use predicts a successful transfusion outcome in 97% of patients with no clinical factors to cause non-immune platelet destruction [67]. Successful prediction of transfusion response was confirmed by O'Connell et al. [68] and Friedberg et al. [69], who, in a separate study of 962 single-donor platelet transfusions to 71 refractory patients, showed that the solid-phase red cell adherence assay was superior to HLA matching. The relatively effectiveness of HLA matching versus cross-matching has not been established. In one study [70], HLA matching and platelet cross-matching provided similar degrees of successful 1-hour CCIs (40–60%). If only transfusions were used in which four HLA-A,B locus antigens were identical between donor and recipient (A match) or in which only three HLA-A,B locus antigens were identified in the donor, HLA matching was superior to platelet cross-matching. However, Moroff et al. [70] concluded that a similar number of successful transfusions could be obtained by either HLA matching or platelet cross-matching, and this is the general belief currently. A recent smaller study [62] also found no difference in the number of successful transfusions selected by HLA matching or cross-matching, although only about 25% of transfusions using either method were successful. It was suggested that in the more than 20 years since the previous studies, patients have become more complex and there are more clinical factors that overwhelm the immunologic matching/selection of platelets [62].

Other approaches to the refractory patient

Other strategies that have been attempted but are not usually successful and are not recommended include plasma exchange, treatment of the patient's plasma with a staphylococcal protein A column, cyclosporine, and intravenous immunoglobulin.

A practical approach to the patient who is refractory to platelet transfusion

The lack of a response to platelet transfusion is often associated with bleeding. These patients are usually quite ill, with problems such as fever, sepsis, DIC, or viral infections. Therefore, clinical factors such as infection that might cause refractoriness should be sought and, if they are present, appropriate treatment should be initiated. Depending on the patient's condition and the degree of concern about the platelet count, additional simple steps that can be attempted to improve the response to platelet transfusion include the transfusion of ABO-identical platelets and platelets stored for the shortest time feasible. The techniques for platelet transfusion should be reviewed to be sure the platelets are not being damaged or lost due to improper handling after leaving the blood bank, use of incorrect filters, or improper storage conditions. An additional step is to be certain that the platelet concentrates contain an adequate number of platelets, which can be done by checking the blood bank quality control testing results and the platelet count and content of the specific concentrates being used for the patient.

If these measures fail, platelets that are matched to the recipient should be used. This can be accomplished by HLA matching of donor and recipient, or by using platelets that are compatible in a cross-match.

Although either HLA matching or platelet cross-matching can be used to obtain about the same effect, one practical difference is that usually cross-matched platelets can be obtained more rapidly than HLA-matched platelet. Cross-matching is done on platelets already collected and available in inventory, whereas HLA-matched donors must be located and scheduled for donation. A suggested practical strategy for dealing with patients who are refractory to platelet transfusion is as follows:

- Treat any correctable clinical factors present that may cause platelet refractoriness. Until these factors have been eliminated, recognize that any of the steps listed below may not be effective.
- Ensure that the patient is receiving the correct dose of platelets.
- Give at least one test transfusion of platelets that are not more than 48 hours old.
- Ensure that platelets being transfused are ABO identical.
- If these steps have failed and transfusion is urgently needed, give transfusions of either cross-match-compatible or HLA-matched platelets, whichever is available soonest. Give at least one and preferably two or three such transfusions.
- If satisfactory platelet count increase is not obtained, the refractoriness is probably due to non-immune clinical factors and there is no value in continuing HLA-matched or cross-matched platelets.
- Determine whether the patient has HLA or platelet-specific antibodies, or both. This precaution may be of value in making future decisions about platelet transfusion if the patient does not respond to HLA-matched or cross-matched platelets.
- Determine whether the patient is receiving medications such as vancomycin that might cause drug-dependent antiplatelet antibodies. If so, carry out a drug–platelet antibody test.

Some patients do not respond to either HLA-matched or cross-matched platelets. Often these are patients with many complications. One approach is to increase the dose of platelets to two or even three single-donor, or 10–20 random-donor, units per day. Although this may enable physicians to feel that they are doing something helpful, usually the patients fail to achieve a substantial increase in platelet count. It is not known whether the transfusions are helpful despite the lack of increase in circulating platelets.

Prevention of alloimmunization and platelet refractoriness

Because of the difficulty in managing patients who are refractory to platelet transfusion, there is considerable interest in preventing alloimmunization. Immunization may be caused by pregnancy but is also due to the leukocytes contained in the platelet and red cell transfusions [71]. Although the exact mechanism of alloimmunization has not been defined, it appears that intact viable leukocytes present HLA class I and II antigens for processing [72]. Therefore, strategies to modify the transfusion products have been adopted. In general, these strategies have involved limiting the number of donor exposures, removing the leukocytes, or treating the components to render the leukocytes non-immunogenic.

There is a very substantial body of data that establishes that leukocyte reduction reduces the incidence of alloimmunization and delays and/or reduces the onset of refractoriness [73–75]. In a study of more than 500 newly diagnosed patients with acute leukemia [63], the use of leukocyte-reduced red cells and platelet concentrates reduced the incidence of alloimmunization, and no difference was found between pooled random-donor and single-donor platelet concentrates. This confirms that the important factor is the reduction of the leukocyte exposure rather than the number of different donors. Filtration of the components at the time they are produced in the laboratory is more effective than bedside filtration for prevention of alloimmunization because the leukoreduction can be standardized and controlled better [76].

Ultraviolet (UV) radiation inhibits the ability of lymphocytes to either proliferate or stimulate in a mixed lymphocyte culture, but UVB light does not interfere with platelet function in vitro or in vivo at doses that will abrogate lymphocyte function. In a large clinical trial, UVB-irradiated platelets reduced the incidence of alloimmunization to the same extent as leukoreduced platelets [63]. Therefore, although UVB light appears to be effective in preventing alloimmunization, it is not currently used, and no systems are licensed by the FDA for routine blood bank use. Pathogen inactivation (discussed later) uses UV light and may be effective in preventing alloimmunization.

Role of thrombopoietin in platelet transfusion

It was hoped that the long-sought platelet growth factor thrombopoietin (TPO)would reduce the need for platelet transfusions. TPO could influence transfusion therapy in HCT patients in at least two ways. It could be given to the patient in hope of speeding the recovery of platelet production and shortening the period of thrombocytopenia, thus reducing bleeding complications and the need for platelet transfusions. Alternatively, TPO could be given to platelet donors to elevate the donor's platelet count, and increase the number of platelets collected and transfused, in the hope of increasing the time between transfusions and reducing the number of donor exposures that the patient received.

In patients with advanced cancer, TPO increased the platelet count in a dose-dependent manner, elevated the nadir platelet count, shortened the period of thrombocytopenia, and decreased platelet transfusion requirements [77]. However, these observations have had little impact on platelet use because these patients did not receive many platelet transfusions. The response to TPO in HCT patients receiving myeloablative therapy is even less impressive. TPO therapy has not shorted the duration of thrombocytopenia or decreased the need for platelet transfusions [77], possibly because endogenous TPO levels are already very high in these patients, TPO receptors may be downgraded or TPO may actually inhibit the release of mature platelets from megakaryocytes. Because the many pluripotent functions of TPO have not been well studied, the reasons for its role or lack thereof in the HCT setting are not completely understood.

When a TPO-like agent was administered to normal plateletpheresis donors, the level of circulating platelets increased, and thus the yield of platelets following plateletpheresis increased 2-4-fold. When these high-dose concentrates were used as a single transfusion, they provided a larger increase in platelet count than present transfusions, prolonged the interval between transfusions, and reduced the number of donor exposures [44]. However, in separate studies, TPO inhibitors occurred in normal subjects, and development of that agent has been discontinued and no further efforts to use TPO-type agents in donors are under way.

Granulocyte concentrates and granulocyte transfusion

A series of studies in the 1970s established that granulocyte transfusions provided improved survival in patients with documented Gram-negative sepsis who remained granulocytopenic for at least 10 days (reviewed in [78]). Most clinical trials of granulocyte transfusion involve patients with Gram-negative bacteremia, there is little information regarding other organisms, and patients with fever of unknown origin do not experience improvement as a result of granulocyte transfusion. Other than bacteremia, the site of infection does not seem to correlate with response to granulocyte transfusion. Prophylactic granulocyte transfusions using doses available in the 1970s were not helpful [79].

A major reason for the limited effectiveness of granulocyte transfusions was thought to be the small dose of cells [80]. Hematopoietic growth factors such as granulocyte-colony stimulating factor (G-CSF) can be used to stimulate granulocyte production in normal blood donors, increasing the level of circulating granulocytes to about 30,000/μL with G-CSF alone and, by combining the use of corticosteroids and G-CSF, the level of circulating granulocytes can be increased to approximately 40,000/μL [80]. Leukapheresis of these donors results in a dose of up to $7–8 \times 10^{10}$ granulocytes [80]. G-CSF-mobilized granulocytes have normal in vitro function and migrate to sites of infection [80]. There have been several reports of the clinical use of G-CSF-mobilized granulocytes, but the studies were small or uncontrolled, involved different definitions or patient eligibility or prophylactic transfusions, or were in children where all doses were very high due to the patient's smaller size (reviewed in [78]). With the availability of high-dose granulocyte concentrates, low-dose concentrates obtained from donors stimulated with dexamethasone only are not recommended. A large, controlled, multi-center clinical study by the National Heart, Lung, and Blood Institute-funded Transfusion Medicine/Hemostasis Clinical Trials Network did not find a benefit to high dose transfusions except that the study was underpowered and many patients did not actually receive high dose granulocyte concentrates [81].

Transfusion strategies in HCT

Pre-transplantation

For a pre-transplant transfusion strategy, patients can be thought of as being in three groups: immunocompetent but not requiring transfusion, immunocompetent and requiring transfusion, and immunocompromised. An example of the first group is patients with inborn errors of metabolism, who rarely require transfusion before HCT. The other two groups of patients have a variable degree of immunocompetence and may become alloimmunized to leukocytes or platelets following transfusion. Alloimmunization may cause transfusion reactions but, more importantly, may interfere with engraftment in some patients. The presence of HLA antibodies is associated with marrow graft rejection [82]. Pre-transplant transfusions are associated with increased graft failure rates [83,84]. For immunocompetent patients such as those with aplastic anemia, hemoglobinopathies, or myelodysplastic syndrome who require pre-transplant transfusions, efforts should be made to prevent alloimmunization. Patients with aplastic anemia should be evaluated quickly and HCT, if indicated, should be performed as soon as possible to minimize the number of pre-transplant transfusions. Many of these patients can be managed safely when prophylactic platelets are given only when the platelet count is less than 5000/μL [85]. For patients such as those with hemoglobinopathies or myelodysplastic syndrome who require red cell or platelet transfusions before HCT, the following are recommended:

- Red cells should be depleted of leukocytes.
- Platelet concentrates should be depleted of leukocytes. This can be done by filtration or by certain apheresis collection procedures.
- The use of single-donor instead of pooled random-donor platelet concentrates should be considered. However, the largest study of this issue did not show a difference in the rate of alloimmunization between single-donor and pooled random-donor platelet concentrates [63].
- Family members should not be used as blood or blood component donors (before transplant) because of the risk of alloimmunization causing an unsuccessful marrow graft [85].

Patients with malignancies who will undergo HCT usually have received multiple transfusions during the initial chemotherapy of their underlying disease. However, the effects of this on subsequent marrow engraftment are not as severe as for patients with aplastic anemia because standard chemotherapy of their malignancy is extremely immunosuppressive. Hence transfusion therapy for these patients can be that necessitated by their chemotherapy. The increasing use of reduced-intensity preparative regimens may make engraftment more problematic in heavily alloimmunized patients. Therefore, red cells and platelets should be leukocyte reduced in order to minimize the possibility of alloimmunization. Transfusions from family members probably should be avoided [85]. Although one study showed no increase in graft failure in patients with leukemia who received transfusions from family donors [86], this is not generally recommended.

Post-transplantation

Because of the severe immunosuppression caused by the pre-transplant preparative regimen, fatal GVHD can occur as a result of transfusion of viable lymphocytes that are present in blood components. Transfusion-related GVHD can be prevented by irradiating all blood components with at least 2500 cGy (see below).

During the time between HCT and marrow engraftment, the return of production of different blood cell lines varies, so that although the duration of transfusion therapy may range from a few days to several weeks, the need for different components varies with different types of transplant. Almost all patients require platelet and red cell transfusions, but since HCT does not usually interfere with the production of coagulation factors, transfusion of FFP and cryoprecipitate is necessary only if coagulopathy develops. Although HCT patients are severely neutropenic, granulocyte transfusions are usually not necessary (see above).

Regular packed red cells are used and should be leukocyte reduced to minimize the likelihood of inducing alloimmunization. Either whole blood-derived or single (apheresis)-donor platelets can be utilized. Platelets should be leukocyte reduced to prevent alloimmunization. Results of studies of leukocyte-reduced platelet concentrates in situations other than HCT have indicated that this strategy is effective [73–75]. Leukocyte reduction can decrease platelet refractoriness even in patients who were already alloimmunized [63]. Although these studies of alloimmunization have not been carried out in HCT patients, the effectiveness of leukocyte reduction in other situations indicates that leukocyte-reduced platelets should be used in HCT patients.

If patients become refractory to platelet transfusion, HLA-matched unrelated or family donor platelets can be used (see above).

Transfusion prophylaxis for invasive procedures

Most patients will undergo placement of a central venous access line, and many may undergo other invasive diagnostic procedures such as lumbar puncture, bronchoscopy, thoracentesis, bone marrow aspiration, or even liver biopsy. At the time of these procedures, the patient may have abnormal coagulation test results or be thrombocytopenic. Unfortunately, there are insufficient clinical data to determine the value of prophylactic transfusion of FFP to correct coagulopathy [87] or platelet transfusion to increase the platelet count above an arbitrary level [88,89]. However, based on an extensive summary of experience, but in the absence of firm data, the American Society of Clinical Oncology has recommended that a platelet count of 40,000–50,000/μL “is sufficient to perform major invasive procedures” [89]. Bone marrow aspiration and biopsy can be carried out safely at platelet counts of >20,000/μL, liver biopsy >50,000, gastrointestinal endoscopy >20,000, bronchoscopy >20,000, transbronchial biopsy >40,000, and dental extractions and catheter insertions >50,000/mL [89].

Prevention of transfusion-transmitted CMV infection

CMV disease is an important source of post-transplant mortality (see Chapter 87). Most CMV infections in HCT patients occur in those with CMV antibody, and probably are due to reactivation of virus from a previous infection, not to acquisition of a new strain. However, in CMV antibody-negative patients who receive unscreened blood products, the risk of developing CMV infection is high. The likelihood of these patients acquiring CMV infection is almost eliminated by the use of CMV antibody-negative blood components [90,91] and much of the remaining CMV transmission may be due to the graft and it cellularity [92].

Because a substantial portion of blood donors are CMV antibody positive, providing CMV antibody-negative blood components is sometimes difficult. Although infectious viral particles cannot be recovered from normal donors previously infected with CMV who have antibodies to CMV, blood from these donors can transmit CMV. Because it is presumed that leukocytes are the reservoir of CMV in these asymptomatic blood donors, prevention of transfusion-transmitted CMV has been attempted using leukocyte-reduced components.

Two small studies [93,94], one large controlled trial [91], and a meta-analysis [8] have established that leukocyte-reduced components are as effective as antibody-negative components in preventing transfusion-transmitted CMV. With both CMV antibody-negative and leukocyte-reduced blood, there is a small residual risk of CMV transmission of about 2–3% [95,177], which could be due to plasma viremia in seroconverting donors [96]. In the large study [91], there was no difference in the CMV seroconversion rate between patients who received leukocyte-reduced components and those who received CMV antibody-negative components. However, the few cases of clinical CMV infection occurred in the leukocyte-reduced group, compared with only seroconversion in the CMV antibody-negative group. This has caused some transplant physicians to prefer CMV antibody-negative blood components when they can be obtained. In most centers, leukocyte-reduced and antibody-negative components are considered equivalent and used interchangeably depending on the logistical considerations of each transfusion service [97].

The use of CMV antibody-negative blood components in potential HCT patients who are CMV antibody negative has been suggested. This would prevent these patients from becoming infected with CMV as a result of transfusions received earlier in their disease and before entering an HCT program. This practice is gaining in popularity, and the conversion to routine use of leukocyte-reduced blood components makes it more practical.

Other approaches that have been attempted for the prevention of CMV infection include the use of CMV intravenous immunoglobulin or ordinary intravenous immunoglobulin, although the increasing use of leukocyte-reduced blood products has obviated these other strategies.

Prevention of transfusion-associated GVHD

GVHD from blood transfusion was originally identified in immunodeficient children. It is now clear that GVHD can occur in a wide variety of immunocompromised and even some immunocompetent patients, but the more common situation in which GVHD occurs is HCT. Following HCT, GVHD is caused by the immunocompetent marrow donor cells, but viable lymphocytes contained in blood components can cause transfusion-associated GVHD in susceptible patients.

Irradiation of blood components

Warning: Hematopoietic stem cells are sensitive to irradiation. Marrow, peripheral blood, or cord blood stem cells that are intended for transplantation should *never* be exposed to irradiation.

To prevent transfusion-associated GVHD, blood components are subjected to irradiation, which interferes with the ability of lymphocytes to proliferate. Gamma irradiation or X-rays damage the lymphocytes by forming electrically charged particles or ions that alter DNA, making lymphocytes unable to proliferate [98]. UV irradiation is not an effective method of damaging lymphocytes to prevent GVHD unless it is combined with other agents as in pathogen inactivation technology (see later). Blood banks that provide large numbers of irradiated products usually irradiate with a dedicated instrument. These instruments use caesium-137 or cobalt-60 as the source of the radioactivity. Recently, relatively simple X-ray devices have been produced for use in the blood bank.

Gamma irradiation at a dose of only 500 cGy will abolish lymphocyte proliferation in mixed lymphocyte culture [99], and doses up to 5000 cGy reduce the incorporation of [^{14}C]thymidine into mitogen-stimulated lymphocytes by 85.0–98.5% [100]. Red cell survival in vivo and in certain in vitro assays are normal after up to 10,000 cGy [101]. Granulocyte chemotaxis may be slightly reduced by even 500 cGy, but this defect does not become significant until above 10,000 cGy [100]. Very high doses such as 40,000 cGy are required to interfere with phagocytosis and microbial killing. In vitro platelet function studies have generally been normal following up to a 5000 cGy dose [99]. Studies involving lower doses of irradiation such as 2500 or 3000 cGy have shown normal platelet in vivo survival [102] and post-transfusion increments [103]. hence it appears that doses of irradiation up to 5000 cGy do not have an adverse effect on red cells, platelets, or granulocytes.

One difficulty in selecting an irradiation dose is the lack of a definitive in vitro assay to establish a clinically effective dose, because of the occurrence of transfusion-associated GVHD in several individuals who received blood components supposedly irradiated with at least 1500 cGy [104–106]. The limiting dilution assay has been proposed as a good indicator of the effects of irradiation on lymphocytes because this assay detects a 5 $\log_{10}$ reduction in viable T cells, compared with a 1–2 $\log_{10}$ reduction in the mixed lymphocyte culture [98]. Studies using the limiting dilution assay showed that 2500 cGy completely eliminated T-lymphocyte growth, and, based on this experience, the FDA has recommended a minimum dose of 2500 cGy. Important practical factors are the configuration of the blood containers in the irradiated field, the distribution of irradiation within the field, and quality control methods to assure that the desired dose is actually being administered [98].

Storage of irradiated components

As the use of irradiated blood components has increased, interest has developed in irradiating the components after collection and storing them for use days or weeks later. Doses of 2000 or 3000 cGy to units of red cells result in potassium levels that are two and three times normal, respectively, after storage for 4–5 days [107]. This leakage suggests damage to the red cell membrane or the sodium–potassium pump. Some blood banks wash red cells that have been stored for several days after irradiation. However, in a thorough review, Strauss concluded that washing is not necessary for most clinical situations [108]. Based on these studies, red cells can be stored for only 28 days after irradiation. Because there is no reduction in platelet recovery and survival when previously irradiated platelets are stored [102], platelets can be irradiated and stored for the usual 5 days.

Leukocyte reduction to prevent GVHD

Because blood filters are very effective in removing leukocytes, the question has arisen as to whether blood filtration might be an alternative to irradiation for prevention of GVHD. Although, on average, filters produce components that contain fewer than 5×10^6 leukocytes, a small proportion of units may contain considerably more leukocytes. Thus, filtration is not an acceptable approach to prevention of transfusion-associated GVHD.

Pathogen inactivation and prevention of GVHD

Pathogen inactivation technologies damage nucleic acids and thus prevent cell (and also microbial and lymphocyte) multiplication. Hence blood components subjected to pathogen inactivation do not cause GVHD [109–111]. Many centers using pathogen inactivation to treat platelets no longer use irradiation [112].

Irradiation of blood products for autologous bone marrow transplantation

Although there may be some differences in the preparative regimen for autologous compared with allogeneic HCT, patients undergoing autologous bone marrow transplantation are severely immunocompromised for several weeks. The use of irradiated blood components has been adopted for autologous HCT without clinical or laboratory study.

Non-cellular blood components

Transfusion-associated GVHD has occurred in patients with congenital immune deficiency following transfusion of fresh liquid plasma [104,113,114] but has not been reported to be caused by previously frozen components such as FFP or cryoprecipitate. Plasma components contain fragments of leukocytes but few, if any, viable lymphocytes. They would not be expected to cause transfusion-associated GVHD. Although irradiation of FFP and cryoprecipitate is probably not necessary, many blood banks do irradiate these components to avoid clerical errors in which a cellular blood component might not be irradiated when necessary.

Transfusion therapy for HCT donors

Patients (autologous donors) or normal allogeneic donors of marrow may require some replacement of red cells. The volume of replacement needed can be predicted from the size of the patient and the donor [115]. It is important to anticipate whether red cell transfusion of the normal allogeneic donor will be necessary and, if so, to take steps to avoid exposure to allogeneic red cells. If red cell replacement is contemplated, the normal donor should provide autologous red cells for transfusion during marrow donation [115]. Usually, two units of red cells suffice. Erythropoietin has been used to increase erythropoiesis in autologous blood donors before elective surgery, and this step may be a helpful strategy for allogeneic marrow donors, although there are no definitive data on this point.

Various strategies using chemotherapy and/or hematopoietic growth factors are being used to mobilize blood hematopoietic cells for transplantation from either patients or normal allogeneic donors. Leukapheresis is then used to obtain an adequate dose of peripheral blood stem cells for transplantation. Donation of autologous peripheral blood hematopoietic cells by apheresis (see Chapters 36, 37, and 40) involves much less red cell loss than marrow donation, and red cell replacement is usually not necessary. Red cell transfusion may be necessary for autologous donors if the patient is anemic from the disease. Those patients/donors should receive leukocyte-reduced red cells, and the indications for transfusion are those described earlier in this chapter.

Complications of blood transfusion

The complications of transfusion can be categorized as immunologic and non-immunologic. Approximately 1–3% of transfusions result in an adverse effect during or shortly after the transfusion. These effects are considered serious in only about 0.5% of transfusions [116]. The fatality rate immediately surrounding transfusion is estimated to be about 1 per 500,000 patients transfused, or about 35 deaths annually in the United States [117]. The incidence of longer term adverse effects is difficult to establish.

Hemolytic transfusion reactions

Hemolysis can occur as a result of HCT that is incompatible for ABO or other red cell antigens (see Chapter 78). Although these create complex transfusion situations, they are not typical transfusion complications and will not be considered here. Most hemolytic transfusion reactions are due to ABO incompatibility, and a major cause of transfusion fatalities reported to the FDA is ABO-incompatible transfusion. It is estimated that a hemolytic transfusion reaction occurs following about 1 in 150,000 units of red cells transfused [118]. About half of these are of no clinical consequence, but 3–19 patients die from hemolytic transfusion reactions annually in the United States, giving a fatality rate of about 1 per 300,000 patients [17,117]. Other causes of red cell hemolysis in transfusion recipients, such as improper storage or techniques of administering the blood, are not immunologic.

ABO-incompatible hemolytic transfusion reactions are very dangerous because the patient has preformed ABO antibodies that often are immunoglobulin M (IgM) and bind complement, causing activation of the complement system with associated systemic manifestations and leading to red cell lysis. In addition, cytokines cause important biologic effects in hemolytic transfusion reactions. There is substantial production of interleukin-1 (IL-1), IL-6, IL-8, monocyte chemoattractant protein-1, and tumor necrosis factor in response to red cell incompatibility [118], and these act like a final common pathway for both the IgM and IgG red cell-incompatible systems.

The nature and severity of the symptoms do not correlate with the severity or ultimate outcome of a hemolytic transfusion reaction. Some patients may experience a severe reaction after only 20 mL of ABO-incompatible red cells, whereas others may tolerate an entire unit without signs or symptoms. The reaction may begin almost immediately upon beginning the transfusion or up to several hours after transfusion. The signs and symptoms that may accompany a hemolytic transfusion reaction are due to complement activation and release of cytokines, and include fever, chills, flushing, low back pain, hypotension, dyspnea, abdominal pain, vomiting, diarrhea, tachycardia, chest pain, or unexpected bleeding. In addition to the well-known complication of renal failure, a hemolytic transfusion reaction can lead to coagulopathy due to activation of the coagulation system and release of cytokines. Non-ABO antibodies can also cause a hemolytic transfusion reaction, but usually these antibodies are IgG immunoglobulins and cause extravascular rather the intravascular hemolysis. Extravascular hemolysis is usually a slower process with red cells removed by phagocytes and little or no complement activation. Usually, there are few, if any, symptoms.

A delayed hemolytic transfusion reaction can occur in a patient in whom no red cell antibody was detected at the time of compatibility testing but who experiences accelerated destruction of the transfused red cells after an interval during which reactivation of a previous immune response to the transfused red cells occurs. The interval after transfusion may be as little as 24 hours or up to about 1 week. A delayed hemolytic transfusion reaction may be symptomatic or asymptomatic. The most common sign is a decrease in hemoglobin after transfusion, and is the way in which most delayed hemolytic transfusion reactions are identified. Such a reaction can appear as unexplained blood loss following transfusion. In this situation, repeat red cell antibody detection testing should be done, which will usually reveal a red cell antibody that was not present before transfusion.

Febrile non-hemolytic transfusion reactions

These reactions occur in association with about 0.5–1.0% of red cell transfusions and may be common after platelet transfusions [119]. Traditionally, it was believed that they are due to leukocyte antibodies present in the patients that react with leukocytes present in the transfused components. The severity of the reaction is directly related to the number of leukocytes in the blood component. Some febrile leukocyte reactions can be prevented by removing leukocytes from the blood components; however, reactions can still occur, probably because cytokines play a major role (see the later discussion of reactions to transfused platelets). In red cell transfusions, the relative contribution of leukocyte antibody–antigen reactions versus cytokines released from contaminating leukocytes during storage is not known. Both mechanisms are probably important, and both can be prevented by leukocyte reduction of the red cells shortly after they are collected. Previously, the use of leukocyte-reduced blood components was not recommended routinely after a first febrile non-hemolytic transfusion reaction because there is only a small likelihood that a patient will experience a subsequent reaction. As the use of leukocyte-reduced red cells and platelets has become common for HCT patients, the issue of when to convert to leukocyte-reduced blood has become moot. The increased use of leukocyte-reduced blood should decrease febrile non-hemolytic transfusion reactions, and this has been documented with the use of leukocyte-reduced platelets [119].

Patients are often premedicated with antipyretics and antihistamines to prevent or modify febrile non-hemolytic transfusion reactions. Antipyretics do seem to be effective in modifying the febrile response to pyrogens, but premedication is often not effective in preventing chills and fever from platelet transfusions. There is no evidence that antihistamine modifies febrile non-hemolytic transfusion reactions in platelet recipients [120]. Therefore, although antipyretics and antihistamines can be used, expectations should be modest [17,121], and the major benefit is probably from the antipyretic [122].

Allergic and anaphylactic reactions

Allergic reactions are thought to be due to proteins in donor plasma to which the recipient was previously sensitized. Recipient mast cells and basophils containing IgE antibody become activated and release complement components, cytokines, or other vasoactive substances that cause the reaction. Reactions due to this mechanism may range from mild, involving only hives, to severe anaphylaxis with laryngospasm and shock. Allergic reactions that involve only hives with no other symptoms are probably the most frequent kind of reaction, occurring after 1–2% of transfusions. These reactions are the only situation in which the transfusion is stopped and the patient can be given an antihistamine and the transfusion can be restarted after 15–30 minutes.

Patients who are IgA deficient and have anti-IgA antibodies may experience an anaphylactic reaction if they receive blood components that contain IgA [123]. The treatment is the same as for any anaphylactic reaction. The reactions can be prevented by using red cells or platelet concentrates, washed to remove plasma IgA, and using plasma components prepared from IgA-deficient donors. Although it is a complex process, IgA-deficient patients can be managed successfully while undergoing HCT [124].

Pulmonary reactions: transfusion-related acute lung injury

Following transfusion, a very severe type of transfusion reaction is the acute, sometimes fatal, pulmonary reaction that has been termed transfusion-related acute lung injury (TRALI). TRALI is a clinical syndrome, not a single disease. It is defined as new acute lung injury occurring within 6 hours of transfusion and including hypoxemia and chest X-ray indicative of alveolar or interstitial disease but without circulatory overload [125].

Patients develop acute respiratory distress, hypoxemia, diffuse pulmonary infiltrates on X-ray, and the general clinical presentation of non-cardiogenic pulmonary edema. Fever and hypotension may also occur, and symptoms begin less than 6 hours after the transfusion is initiated. Because it is a clinical diagnosis, the incidence is difficult to determine but may be about 1 per 4500 units of blood depending on the specific component [125]. Two hypotheses for the etiology of TRALI seem to be merging. Leukocyte (HLA or granulocyte-specific) antibodies in donor plasma are one inciting cause of TRALI, being found in almost 90% of cases. An alternative hypothesis is a two-stage process in which platelet-activating factor or cytokines or bioactive lipids that accumulate in blood during storage cause general activation of neutrophils. Then a second insult such as a leukocyte antibody–antigen reaction leads to pulmonary endothelial adherence and damage. It has been suggested that TRALI is more common than currently believed and that, to avoid it, plasma from multiparous donors should not be used because of their likelihood of having HLA antibodies. Most blood centers now produces plasma products for transfusion from mostly male donors [126]. Apheresis platelets are still produced from both male and female donors because of limitations in donor availability. Screening of donors for HLA or granulocyte antibodies is not an effective strategy for TRALI prevention. The prompt recognition that pulmonary symptoms occurring during or shortly after transfusion could be due to TRALI allows prompt initiation of respiratory supportive therapy. This intervention is important because TRALI is a transient phenomenon that clears usually within 72 hours.

Reactions to platelet transfusions

Patients with platelet or HLA antibodies may have febrile non-hemolytic reactions, probably due to leukocytes contained in the platelet concentrates. The reactions usually involve chills and fever, but platelets may be trapped in the pulmonary capillaries, causing dyspnea and pulmonary edema. These febrile non-hemolytic reactions occur following 5–30% of platelet transfusions [127]. The reactions have been thought to be due to antibodies to leukocytes and have been considered similar to febrile non-hemolytic reactions from units of red cells. Because these reactions sometimes occur in non-transfused males, are more common when stored platelets are used, and are more related to the plasma than the platelets in the platelet concentrates, it has become clear that bioactive substance other than leukocyte antibodies are involved [127].

Leukocytes in the platelet concentrates produce cytokines during storage, and platelet transfusion reactions are correlated with the concentration of IL-1 and IL-6 in the plasma [128,129]. A landmark study [127] established that many (if not most) reactions to platelet transfusion are due to cytokines, not antigen–antibody reactions. Removal of the leukocytes soon after collection of the blood prevents the accumulation of cytokines in the platelet concentrate and avoids platelet transfusion reactions [76,128,130]. Removal of leukocytes by bedside filtration at the time of transfusion does not decrease the incidence of platelet transfusion reactions [130] because cytokines will have accumulated from contaminating leukocytes during storage. Therefore, platelet concentrates should be depleted of leukocytes either as part of the apheresis collection procedure or by filtration at the time of production and not later, at the time of transfusion.

Allergic reactions can also occur with platelet transfusions. Most allergic reactions are thought to be due to proteins in the donor plasma. New electrolyte-based platelet storage solutions replace most of the donor plasma and may lead to a major decrease in allergic reactions [131].

Reactions to granulocyte transfusions

Transfusion reactions are common following granulocyte transfusions. Many of these reactions are probably due to the physiologic activity of the large dose of granulocytes being administered to infected neutropenic patients, or to cytokines contained in the granulocyte concentrate. Since granulocyte concentrates do not undergo a leukocyte cross-match, leukocyte incompatibility may be present in many granulocyte transfusions and may also account for some reactions. At one time, it was believed that granulocytes transfused in association with amphotericin infusion resulted in severe pulmonary reactions with sequestration of granulocytes in the lungs. Subsequent experience has not supported this. With the large number of intravenous medications that these patients receive and the importance of transfusing granulocytes as soon as possible, it is often not practical to space the granulocyte transfusion several hours apart from amphotericin or other similar infusions. Hence a gap of several hours between amphotericin and granulocytes is suggested but is not absolutely necessary.

Other immunologic complications of transfusion

As a result of exposure to blood, patients may form antibodies to red cells, to lymphocyte, granulocyte, or platelet surface antigens, or to plasma proteins. The likelihood of antibody formation depends on the immunogenicity of the antigen and the ability of the individual to mount an antibody response. Each kind of antibody can cause a particular clinical problem later if the patient requires subsequent transfusions or organ or tissue grafts, or becomes pregnant. The first observation of transfusion-related immune modulation was the beneficial effect of transfusion on renal graft survival. In the last decade, considerable debate has developed over whether transfusion also leads to increased cancer recurrence and/or postoperative infection [6–8]. Although there is general agreement that transfusion has an immunomodulating effect, the clinical impact of this effect is not established.

Bacterial contamination of units

Bacterial contamination continues to be an important complication of blood transfusion [17,132]. The two general types of problem are bacterial contamination of platelet concentrates stored at room temperature and transmission of bacteria, especially *Yersinia enterocolitica*, from red cells stored at refrigerator temperatures [133]. Bacterial contamination probably occurs most commonly from skin flora at the venepuncture site. Other less likely possibilities are contaminated bags or solutions, contamination of ports during water-bath thawing of frozen products or during platelet pooling, or asymptomatic bacteremia in the donor. The extent of the problem is difficult to ascertain, but a positive bacterial culture has been reported in 0.3–0.4% of units of red cells and platelets [134,135], although most of these units do not cause a clinical problem. Blood banks now culture platelet units. Rates of bacterial contamination of platelets as high as 230 per 100,000 platelet units and 3 per 100,000 red cell units have been reported [17]. Clinically important transfusion-transmitted bacterial infections probably occur about 1 per 25,000 units of platelets and 1 per 250,000 units of red cells [17,136–138]. Up to eight fatalities have been reported to the FDA (Ref. 17, Chapter 14, US Department of Health and Human Services). A method is available for testing for contamination at the time of release of the platelet unit from the blood bank, but this is not yet widely used. The signs and symptoms of transfusion of contaminated platelets are variable and include chills and fever, usually beginning during the transfusion, possibly followed by nausea, vomiting, hypotension, oliguria, and respiratory symptoms. Although the problem of contaminated blood products, especially platelets, has been reduced, this potential serious adverse effect has not been eliminated. Because of the reduction in transmission of viral diseases that has occurred in the last two decades, bacterial contamination of blood components is now potentially the most serious complication of transfusion, especially in the immunocompromised recipient.

Non-immunologic complications of blood transfusion

Non-immunologic complications of transfusion include hypothermia from transfusion of large volumes of red cells that have been stored in the refrigerator, citrate toxicity that may manifest itself as hypocalcemia, and a bleeding tendency since red cells suspended in the additive preservative solution do not provide coagulation factor replacement. Proper management of fluid balance can avoid circulatory overload. Iron overload is a complication of patients who receive many transfusions, such as patients with hemoglobinopathies. It is a problem in HCT for patients with thalassemia major and may require an iron removal program following HCT (see Chapter 71) [139,140] . Air or particulate emboli that were a problem in the early days of transfusion no longer occur if proper techniques are used to administer the blood.

Transfusion-transmitted diseases

The acquired immune deficiency syndrome (AIDS) epidemic greatly increased the fear of transfusion-transmitted diseases. The risks of transfusion can be reduced by donor selection, transmissible disease testing, limiting the number of donor exposures, and reducing blood use (Table 79.3). Physicians have become much more conservative in the use of blood by changing the indications for transfusion and by using pharmacologic substitutes. Use of autologous blood became fairly popular but has decreased greatly owing to the safety of the general blood supply and more conservative use of blood. Directed donor programs in which relatives or friends donate for a specific patient are discouraged because they provide no safety advantage and are complex and costly. Donor eligibility criteria reflect the understanding of behavior that places potential donors at risk of transmitting disease. The number of donor screening questions is large and the nature of the questions is quite specific. These strategies have been remarkably successful. For instance, changes in donor selection resulted in a 90% decrease in the human immunodeficiency virus (HIV) infectivity of the blood supply in the San Francisco Bay area even before the introduction of the anti-HIV test [141]. Currently, a complex process is used to obtain the safest possible donors.

Laboratory testing of donated blood has also greatly expanded (Table 79.4). This is extremely effective in reducing the risks of blood transfusion. Original screening tests for HIV and hepatitis C virus (HCV) detected antibodies to these viruses and, thus, there was a "window phase" between exposure to the virus and the development of a positive screening test. During this window phase, the blood can be infectious. Nucleic acid amplification tests are now used to detect the infectious agent rather than antibodies to the agent [142–144]. All blood collected in the United States and most developed countries is currently being screened for HIV, HCV, and West Nile virus using nucleic acid amplification, and testing for hepatitis B virus (HBV) has been added. This testing strategy has virtually eliminated the window phase of blood donor infectivity. Estimates of disease transmission [145,146] are given in Table 79.5. A brief review of transfusion-transmitted diseases is presented below.

Table 79.3 Strategies to reduce transfusion-transmitted disease

- Careful donor selection
- Careful transmissible disease testing
- Limit donor exposure
- Minimize blood use
  - Changed indications for transfusion
  - Pharmacologic stimulation or substitution
- Modification of the blood component
- Viral and bacterial inactivation

Syphilis

Transmission of syphilis by blood transfusion was common in the early days of blood transfusion but now it is extremely rare, the last case in the United States being reported in 1966 [147]. Syphilis testing is still required of all donated blood despite the fact that the test is almost always negative when circulating spirochetes are present. Syphilis testing today probably does not contribute to blood safety, although the FDA has not agreed to calls for elimination of syphilis testing [148,149].

Hepatitis

Post-transfusion hepatitis is the most common disease transmitted by blood transfusion and is a major public health problem. It can be caused by hepatitis A, B, C, and E viruses, CMV, Epstein–Barr virus (EBV), and possibly other viruses. Prospective studies in the 1970s showed rates of post-transfusion hepatitis as high as 35%, but that was substantially reduced in the United States and other countries due to the conversion from paid donors to volunteer donors and improved testing [150].

Hepatitis A usually has a short period of viremia, symptoms occur early during the viremic phase, and there is no carrier state. Because there is no chronic viremia and symptoms coincide with the acute viremic phase, infectious donors are usually identified by the medical history. Hence post-transfusion hepatitis A is rare [151,152]. Laboratory testing of blood donors for hepatitis A is not done. Hepatitis A can be transmitted by coagulation factor concentrates or rarely by plasma derivatives. In evaluating a patient with suspected post-transfusion hepatitis, hepatitis A should be considered, but only as a remote possibility.

Most people infected with the HBV virus are asymptomatic, hence an apparently healthy individual may meet all of the donor medical history criteria and donate a unit of infectious blood. Blood is screened for HBV by testing for hepatitis B surface antigen and more recently by nucleic acid amplification [145,146,153]. A recent estimated positive rate is 1 per 450,000 donations [153]. This is a remarkable improvement in blood safety from the 35% incidence of post-transfusion hepatitis in the 1970s [150].

Acute hepatitis C is usually mild, with up to 80% of patients being asymptomatic. However, the long-term effects are more serious because the virus tends to be persistent and the condition develops into chronic liver disease [154–156]. All blood is screened for HCV using nucleic acid amplification technology. It has been estimated that the introduction of hepatitis C testing of blood donors has prevented about 40,000 cases of post-transfusion hepatitis C annually in the United States [156]. With the introduction of

Table 79.4 Laboratory testing for transfusion-transmitted diseases

Family	Pathogen	Disease	Routinely screened for in the USA	
			Yes	No
Hepatitis	HBV, HCV	Hepatitis	X	
	HEV, HGV	Hepatitis		X
Retroviruses	Human immunodeficiency virus-1 and -2	Acquired immune deficiency syndrome	X	
	Human T-lymphotrophic virus-I and -II	Malignantly lymphoproliferative disorders, neuropathy	X	
Herpesviruses	Cytomegalovirus	Cytomegalovirus retinitis, hepatitis, pneumonia	X	
	Epstein–Barr virus	Epstein–Barr syndrome		X
	Human herpesvirus-8	Kaposi's sarcoma		X
Flavivirus	West Nile	Meningitis, encephalitis	X	
	Denge	Hemorrhagic fever		X
Parvoviruses	B19	Aplastic anemia		X
Bacteria	Gram negative, Gram positive	Sepsis	X	
	Treponema pallidum	Syphilis	X	
	Borelia burgdorferi	Lyme disease		X
	Rickettsia rickettsii	Rocky Mountain spotted fever		X
	Ehrlichia chafeensis	Ehrlichiosis		X
Protozoans	*Trypanosoma cruzi*	Chagas disease		X
	Babesia microti	Babesiosis		X
	Leishmania donovani	Leishmaniasis		X
	Plasmodium sp.	Malaria	X	

HBV, HCV, HEV, HGV, hepatitis B, C, E, G viruses, respectively.

Table 79.5 Estimated risk of transfusion-transmitted infections in the United States

	United States	
	Risk donations	Estimated number of cases annually[a]
Human immunodeficiency virus	2,135,000	5.6
Hepatitis C virus	270,000	12.8
Hepatitis B virus	410,000	58.5
Bacteria	75,000	160

[a] Based on 12,000,000 units of whole blood collected annually in the United States.
Source: data from [145,146].

nucleic acid amplification testing, very little transfusion-transmitted hepatitis C remains (Table 79.5).

Despite the discovery of the HCV and implementation of screening donated blood for this virus, a few patients with post-transfusion hepatitis test negative for the known hepatitis viruses. Other hepatitis-related viruses that have been investigated and found not to be related to transfusion-transmitted hepatitis are hepatitis G or GB, TT virus and SEN virus [17,157–159]. Hepatitis E is endemic in some parts of the world and apparently can be transmitted by transfusion [17,160]. It is not established that hepatitis E virus is an important form of transfusion-related hepatitis.

Human immunodeficiency virus

Although HIV-1 infection can be transmitted by blood transfusion, only a very small proportion of AIDS cases are due to transfusion. The risk of acquiring HIV infection following transfusion with anti-HIV-1-positive blood is as high as 70–91% [161]. It has been estimated that, before the introduction of HIV-1 antibody testing in May 1985, approximately 12,000 patients were infected with HIV-1 by transfusion [162].

Because there is the interval or "window" between infection and the development of antibody to the infecting virus, HIV infection can be transmitted by anti-HIV-negative donors if they are in this window period [163–165]. Screening of blood for the HIV antigen using enzyme-linked immunoassay technology to eliminate the window phase was not effective. Nucleic acid amplification testing is now applied to all donated blood and has nearly eliminated transfusion-transmitted HIV (Table 79.5) [144–146].

Human T-lymphotrophic virus I/II

Human T-lymphotrophic virus-I (HTLV-I) is the first retrovirus that has been shown to cause malignancy, adult T-cell leukemia, in humans. The virus is also associated with a form of myelopathy referred to as HTLV-associated myelopathy or tropical spastic paraparesis. Donors with a true positive test for HTLV-I/II have approximately a 1–5% chance of developing adult T-cell leukemia during a 70-year lifespan, and another 2% may develop tropical spastic paraparesis within 5–10 years of initial infection. Although no cases of transfusion-transmitted adult T-cell leukemia or tropical spastic paraparesis have been identified, transmission of the virus by blood transfusion does occur [166]. In the United States, anti-HTLV-I is found almost exclusively in intravenous drug abusers or persons from areas that are endemic for HTLV-I [167]. Because of the potential for disease transmission, although no cases of transfusion-transmitted adult T-cell leukemia have been identified, routine testing of all donated blood for anti-HTLV-I was initiated in the United States in December 1988.

Parvovirus

The parvovirus B19 has been transmitted by blood transfusion (see Chapter 93) [168,169]. The prevalence of parvovirus in blood donors is estimated to be 1 in 3300 to 1 in 50,000 [168,169]. The low prevalence, combined with the brief period of viremia, makes transmission of parvovirus by blood transfusion rare. Transmission also occurs from coagulation factor concentrates [169] and albumin. However, the few cases of transmission by single-donor components suggest that although rare, transfusion-transmitted parvovirus infection could be a particular problem for HCT patients [169–172].

Epstein–Barr virus

Infection with EBV is followed by a lifelong carrier state, and most adults have been infected (see Chapter 90). EBV can be transmitted by transfusion [173], usually as the "postperfusion" syndrome, a virus-like illness that occurs after transfusion of fresh blood during open heart surgery. Transfusion transmission of EBV has not been a major clinical problem in HCT (see Chapter 90), although it can be transmitted to organ recipients [174] and could cause lymphoproliferative syndrome in HCT patients. No strategies are used to avoid transfusion transmission of EBV.

Parasitic diseases

It has been known for many years that some parasites are transmitted by transfusion. All species of *Plasmodium* can survive in refrigerated blood and have caused transfusion-transmitted malaria, although most cases involve *P. malariae.* All components, including RBCs, WBCs, platelet concentrates, and fresh plasma, can transmit malaria [175]. The prevention of transfusion-transmitted malaria relies on the exclusion of asymptomatic carriers based on residence or travel to an endemic area. However, in most cases of post-transfusion malaria, the interview fails to exclude the infectious donors. Laboratory tests are not used in the United States for screening donors for malaria. Thus, periodic spiking fever in a patient who has recently received a transfusion could be due to malaria, but this is very unlikely in North America.

Trypanosoma cruzi is endemic in many parts of Central and South America. Many patients with chronic *T. cruzi* infection may be asymptomatic, and therefore could pass the blood donor medical questions and donate blood. *T. cruzi* survives in refrigerated blood and can be transmitted by transfusion, although this event is rare in the United States [176]. For instance, in screening more than one million donors, none was found to have antibodies to *T. cruzi* from investigation of the recipients, and not a single case of transfusion transmission was found. Despite this, blood banks are now testing all blood donors for *T. cruzi* antibodies one time, but then not on subsequent donations.

Babesia microti and *Babesia bovi* are protozoa that occasionally infect humans by tick bites. Many infected individuals are asymptomatic, and *B. microti* can therefore be transmitted by blood donated by asymptomatic infected donors [178–180]. The ticks are prevalent in the Northeast, Mid-Atlantic, and Upper Midwest. A suitable donor screening test will be available soon and decisions regarding donor screening will need to be made soon in high prevalence areas of the Northeast and Upper Midwest.

Borrelia burgdorferi, the spirochete that causes Lyme disease, is transmitted by ticks to humans. In up to 40% of persons, the infection is asymptomatic, and the spirochetes survive in stored blood for up to 45 days [181]. Hence transmission of *B. burgdorferi* by transfusion is theoretically possible, but this has not been reported. Although a serologic test is available, it is not a suitable laboratory test for donor screening, and the widespread prevalence of the tick makes it impractical to defer donors from endemic areas.

Creutzfeldt–Jakob disease

A variant of Creutzfeldt–Jakob disease (CJD) that appears to be associated with ingestion of beef from cows affected with bovine spongiform encephalopathy or "mad cow disease" [182] raised the question of whether the variant of CJD could be transmitted by blood transfusion. CJD has been transmitted by dura, corneas, and pituitary growth hormone from CJD patients and electroencephalographic electrodes used on CJD patients [183]. Prions, thought to be involved in CJD, are highly resistant to inactivation with methods currently used in the production of plasma derivatives. Thus, even one unit of plasma contaminated with the CJD agent could render a large lot of plasma derivatives infectious, such as occurred with HIV. However, it appears that, during the manufacture of plasma derivatives, prions do not segregate with the plasma fractions that result in coagulation factor concentrates or immune globulins.

There is no evidence of blood-borne transmission or of a recent "outbreak" of CJD in the United States [184]. There have been two reports from the United Kingdom of transfusion recipients who developed CJD after receiving blood from donors who later developed CJD [185,186]. Although this does not definitively establish transmission of variant CJD, the occurrence is statistically highly unlikely, suggesting that transfusion transmission might occur. Tests of the causative agent, presumably a prion, are under development but not available for donor screening. In an effort to decrease the transfusion risk, if one exists, potential blood donors are deferred if they have spent a prolonged time in an area of high incidence of variant CJD such as the United Kingdom and parts of Europe.

West Nile virus

West Nile virus, a mosquito-borne agent, was endemic for years in Uganda and entered the United States in 1999. West Nile virus is a typical viral illness with only a few infected individuals being symptomatic with fever, chills, headache, myalgia, arthralgia, and weakness. However, viremia in asymptomatic individuals makes transfusion transmission possible. As the West Nile virus epidemic became more widespread, transfusion transmission was identified [187–189]. The existing nucleic acid amplification test systems for HIV and HCV provided a platform for introduction of West Nile virus testing, and currently all donated blood in the United States is tested for West Nile virus, at least during epidemic seasons so this risk should be nearly but not entirely eliminated.

Chickungunya and Dengue Fever Virus

These mosquito born infections are now present in the Caribbean and South Florida and may be transmitted by transfusion. No intervention steps are in place other than medical interview.

Summary of transfusion-transmitted diseases

The diseases of most concern for transmission by blood transfusion have been discussed here. However, there is a concern that emerging pathogens, the mobility of people, and continued immigration can alter the situation with transfusion-transmitted diseases. Blood safety depends on many strategies, not just laboratory tests. The donor medical history and the use of volunteer donors are excellent examples. When considering a new screening test, many factors about the infectious agent and the disease must be taken into consideration. Examples are the prevalence of the disease, infectivity of the agent, status of the epidemic, and likelihood of a carrier state. Although some of the diseases may be regional in nature, the mobility of the population may make regional screening practices unsuitable.

However, when all of the scientific discussion is completed, the issues of social policy and the public expectations of its blood supply remain. The public expects the transfusion medicine community to take steps to achieve the maximum possible safety. In the past, policy-makers and politicians have shown little tolerance for failure to take steps that would decrease risks to their constituents regardless of the cost. For instance, the costs of laboratory tests and additional regulations during the past decade have probably increased the cost of blood by 50%, and it has been estimated that introduction of nucleic acid amplification testing for HIV and HCV may have cost as much as $10,000,000 per quality-adjusted life-year saved. A more rational approach to blood safety has been sought by many; however, the decision-making process is still a complex one with no easily discernible structure for these decisions.

Three decades of work and many millions of dollars have not resulted in a safe and effective blood substitute, and it does not appear that one will be available in the near future. In contrast, there has been great progress in the development of techniques to inactivate pathogens in blood products [190–192]. Pathogen-inactivated platelets and plasma are used routinely in many parts of Europe and are recently licensed for use in the United States. Pathogen inactivation also inactivates lymphocytes, thus eliminating the need for irradiation of components [112]. With use of pathogen inactivated blood products, the paradigm for reducing transfusion-transmissible infections will shift from the present reactive to a proactive one involving treatment of blood products to render them non-infectious.

Conclusion

The kinds of blood product available for transfusion have become sophisticated, and transfusion strategies are complex. Beginning with the diagnosis and treatment of the underlying disease, transfusion therapy is designed to minimize its interference with successful engraftment. Following transplantation, patient transfusion needs can be met effectively. The blood supply is safer than ever, and although some risks of infection remain, most patients can be supported safely and effectively.

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CHAPTER 80

Vascular Access and Complications

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Introduction

Drs. Thomas Latta and Robert Lewins first attempted the administration of intravenous fluids during the cholera epidemic in England in 1832. After studying the blood and excreta of cholera patients, Dr. William Brooke O'Shaughnessy correctly deduced that the changes in composition of the blood were secondary to the fluid and electrolyte losses in the excreta, and proposed that the treatment of this disease should include the replenishment of fluid and salts intravenously. This was first attempted by Dr. Latta, who administered a hypotonic solution by direct intravenous injection through a silver needle connected to a syringe by rubber tubing; this was reported by Dr. Lewins in *The Lancet* in 1832 [1]. This practice was largely abandoned until the late 1800s, when the administration of intravenous fluids became available thanks to the advent of sterile electrolyte solutions and steel hypodermic needles. In 1945, plastic catheters for continuous vascular access were introduced following the discovery of penicillin and the need for multiple intravenous injections.

The development in the 1960s of long nylon catheters that could be placed into the central veins constituted a major technologic development [2]. These catheters were initially inserted into cancer patients via peripheral vein cutdown and later via percutaneous approaches into the subclavian and jugular veins, with the objective of limiting the chemotherapy-associated phlebitis. A major breakthrough occurred when Broviac et al. described the use of a soft, cuffed, silicone catheter for long-term central venous access in children on total parenteral nutrition [3]. This was shortly followed by the development by Hickman et al. of a similar catheter with a larger lumen for bone marrow transplant recipients [4]. These catheters could remain in place for an extended period of time, facilitating the outpatient care of these patients, and providing higher flow for chemotherapy, blood transfusions, and parenteral nutrition. These devices were further modified by the addition of a subcutaneous reservoir, which could be easily and intermittently accessed via percutaneous needles.

Later, the development of new polymers such as silicone, poly(vinyl chloride), polytetrafluorothylene (Teflon), and polyurethane led to the manufacture of a large variety of intravenous catheters. These catheters had different physical properties designed for specific applications, including chemotherapy, parenteral nutrition, hemodynamic monitoring, and hemodialysis [5,6]. The use of central venous access for treatment and support has improved the quality of life and permitted the delivery of complex chemotherapy regimens to cancer patients. Long-term central venous access has become an essential but risky aspect of the treatment of patients with hematologic malignancies [7]. In the United States, it is estimated that there are at least 250,000 catheter-related bloodstream infections (CRBSIs), resulting in a substantial increase in hospital-related morbidity and cost [8–11]. That led the Centers for Disease Control and Prevention (CDC) to lead a crusade to reduce the frequency of vascular access-related infections [12]. Hence it is imperative that all healthcare personnel understand the indications, selection, and maintenance of catheters, and the prevention, diagnosis, and treatment of catheter-related complications.

Indications and patient selection

Although there are no established guidelines for the placement of central venous access, the use of complex multidrug chemotherapy and bone marrow transplantation in cancer treatment has made the establishment of reliable long-term venous access an essential component of cancer therapy. Important considerations in deciding whether a catheter is indicated are patient preferences, the frequency and duration of therapy, the frequency of blood draws, the nature of the therapy (vesicating agents are better delivered into a central vein without risk of extravasation), the need for support therapies (total parenteral nutrition), the need for hematopoietic cell collection, plasmapheresis, and bone marrow reinfusion.

Long-term venous access is an elective procedure. The patient should be recovered from any acute infections or treatment-related complications that could prevent the safe placement of these catheters. In cases in which patients have not fully recovered and there is an absolute need for central venous access, a temporary percutaneous central line or a peripherally inserted central venous catheter (PICC) can provide access until the patient has recovered. History of prior vascular access insertions (number and location), history of previous CRBSIs, thoracic surgery, radiation, mediastinal, and thoracic disease should alert the surgeon to possible changes in the normal venous drainage. Physical examination documenting the integrity of the skin, normal venous anatomy without evidence of abnormal collaterals, changes secondary to surgical treatment and reconstruction, sites of prior catheter insertions, evidence of venous obstruction, and pulmonary reserve should be completed in every patient.

If there is any evidence of venous obstruction or history of bilateral placements of central venous accesses, the examination should be complemented with a venous imaging study. In patients with a previous history of venous thrombosis, the site can be used for access if the vein is shown to be patent by ultrasound or venogram.

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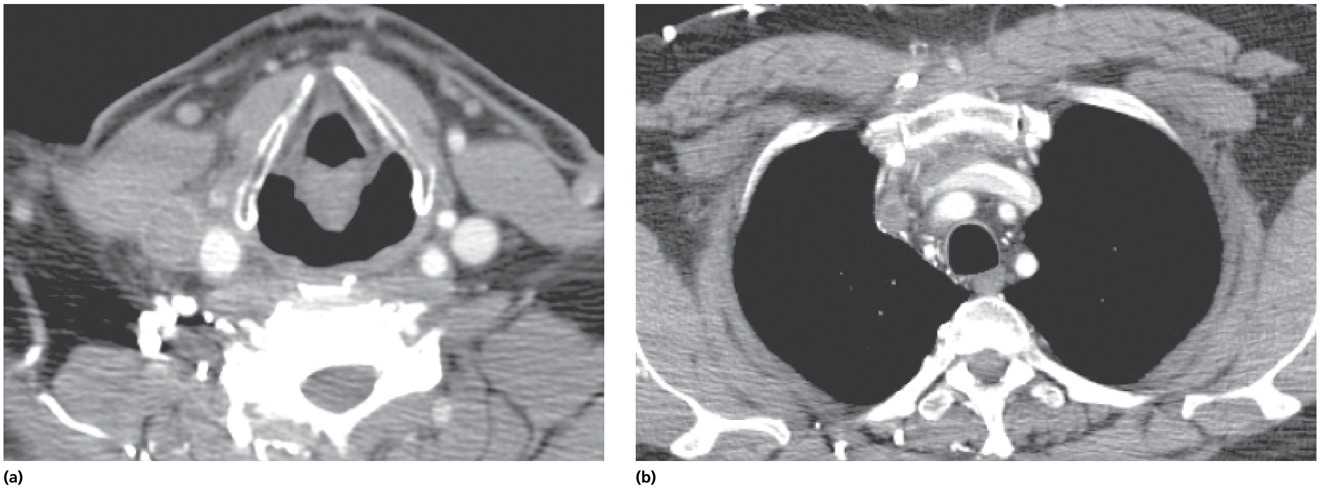

Figure 80.1 (a) Computed tomography (CT) scan showing a right internal jugular vein thrombosis. (b) CT scan showing a superior vena cava thrombosis above the entrance of the innominate vein.

Duplex Doppler ultrasound can visualize the patency and flow of the neck and arm veins. Intrathoracic veins are poorly visualized with this technique. Computed tomography and magnetic resonance imaging are very good at documenting thrombosis and patency of major intrathoracic veins (Figure 80.1). Venous angiogram is still considered the "gold" standard in the study of venous anatomy and should be performed whenever non-invasive examinations fail to provide a definitive diagnosis. Chest radiography can reveal important information such as the presence of pleural effusions, lung metastases, and mediastinal tumors, which can modify the site selection for the placement of central venous access. Table 80.1 provides a list of conditions that should be considered in patient selection.

A relative contraindication to the placement of a permanent central venous access is a granulocyte count less than 1500/mm^3. Such patients have a higher incidence of septic episodes that might prompt early catheter removal. Thrombocytopenia and platelet dysfunction are frequently encountered in cancer patients. Preoperative platelet transfusion to 50,000/mm^3 allows for the safe placement of catheters in the majority of patients without bleeding complications. In those patients with refractory thrombocytopenia to platelet transfusions, venous cutdown might be the safest approach for catheter placement. Many cancer patients have abnormalities in their clotting factors secondary to malnutrition or chemotherapy treatments. Vitamin K or fresh frozen plasma is usually necessary for the correction of these abnormalities. Patients who cannot tolerate the recumbent or Trendelenburg position can frequently have their catheters placed through a cutdown approach under local anesthesia.

Except for those patients who require a line for long-term antibiotic treatment, the only absolute contraindication for the placement of a long-term indwelling catheter is the presence of an active infection.

Table 80.1 Patient and treatment factors that affect catheter selection

Catheter-related factors:
- Duration of therapy (short or long term)
- Frequency of treatment (intermittent or continuous)
- Number of lumina required

Therapy for which the catheter is intended:
- Transfusions of blood products
- Parenteral nutrition
- Chemotherapy administration (types of chemotherapy agent)
- Hematopoietic cell collection
- Supportive care

Patient-related factors:
- Previous history of chest or neck surgery or radiation (mastectomy, neck biopsy)
- Presence of a chest, neck, or mediastinal tumor
- Pulmonary function (asthma, pleural effusion)
- Cardiac function (arrhythmias, pacemakers)

Venous access factors:
- Current venous access
- Previous history of venous access
- Previous history of catheter-related complications
- Previous or current history of veno-occlusive disease or lymphedema

Hematologic and coagulation status:
- Red cell blood count
- Platelet count
- History of bleeding disorders
- Anticoagulation (Coumadin, aspirin, Plavix)

Infection risk:
- White blood cell count
- History of recent infections
- History of catheter-related infections

Allergies:
- Anesthetics
- Cleansing solutions
- Dressings
- Latex

Catheter selection

Long-term venous catheters are designed for external access, such as the Hickman, Broviac, Groshong, and Quinton catheters [13], or for access through a subcutaneous port, such as a Port-a-Cath and Infusaport [14]. These catheters are available with different lumen diameters and as single- and multi-lumina devices [15]. Central venous catheters peripherally placed through an antecubital vein (PICC lines) [16] have become increasingly popular owing to their ease of placement and their ability to be placed by trained nursing personnel. Unfortunately, these devices are associated with a higher incidence of catheter-related complications [9]. In general, treating physicians based on their preferences and the catheter's intended use make the catheter selection. There are very few studies that document patient preferences. In a study of acute leukemic patients randomized to have either dual-lumina subcutaneous ports or dual-lumina cuffed tunneled catheters, patients' perceptions were prospectively documented [17]. Not surprisingly, patients preferred the subcutaneous port, but the study was discontinued owing to a higher incidence of bleeding at the insertion port site.

An important general consideration in the selection of the appropriate catheter is that catheter resistance to infusion depends on the catheter length and diameter of the lumen. During laminar flow, the flow-rate through a catheter is governed by Poiseuille's law:

$$\text{Flow} = \frac{\pi \times (P_1 - P_2) \times R^4}{8 \times n \times L}$$

where P_1 and P_2 are the fluid pressures at the proximal and distal end of the tube, respectively, R is the radius of the tube, n is the viscosity of the fluid, and L is the length of the tube. Flow is then directly proportional to the fourth power of the radius of the tube and inversely proportional to the length [18]. Therefore, variations in the lumen diameter have a greater effect on the flow rate than do changes in the length of the catheter [18]. There is a paucity of recent studies comparing the different catheter materials in the clinical setting. The advantage of a polyurethane catheters is that at equal external diameter, they have a larger inner diameter than silicone catheters. This gives silicone catheters greater strength and bending and break resistance, and gives polyurethane catheters higher flow [6]. From the point of view of thrombogenicity and infection, there does not seem to be a clear advantage of one material over the other [19]. In a randomized study of 228 patients comparing three different subcutaneous ports connected to either silicone or polyurethane catheters, there was a significant higher incidence of catheter breakage with the polyurethane catheters (7.8% vs. 1.3%) [20].

Peripherally inserted central venous catheter (PICC)

The use of PICC lines has become widespread in healthcare since they were first introduced in the late 1970s. Factors that have influenced the popularity of the PICC are its ease of insertion with low complication rate, the ability to be inserted by a trained nurse at the bedside without the need for patient sedation, and the high level of patient satisfaction [21]. As a result, the PICC is the device of choice for patients who require a long-term venous access for antibiotic therapy, parenteral nutrition, pain management, and fluid replacement, but do not require frequent blood samples or infusion of blood products [16]. They are made of either silicone or polyurethane and are available in various sizes, single- or dual-lumina, and can remain in situ for up to 1 year. The use of PICC lines in oncology was initially controversial owing to reported higher CRBSIs and catheter-related venous thrombosis than tunneled or implanted catheters [10,11]. Mollee et al. recently conducted a prospective study involving 1127 lines in 727 patients with hematologic malignancies (54%) and solid tumors (44%) [8]. This study had strict definitions for catheter-associated and related bloodstream infections; the incidence of catheter-associated bloodstream infections (CABSIs) for PICC lines was 4.22 per 1000 days in patients with aggressive hematologic malignancies and 1.1 per 1000 days in patients with other hematologic malignancies. This compared favorably with patients with aggressive hematologic malignancies and tunneled devices who had a CABSI incidence of 7.26 per 1000 days and was essentially the same as the infection rate for patients with implanted subcutaneous ports of 3.89 per 1000 catheter days. In a recent study of 57 patients who underwent autologous bone marrow transplant for hematologic malignancies, the incidence of CRBSI was 1.5 per 1000 PICC days [22]. Recent studies have also documented similar low incidences of PICC-related events in children with hematologic malignancies and solid tumors with an overall incidence of 1.11 complications per 1000 catheter days [23]. The marked decrease in the incidence of CRBSI associated with PICC lines is due to the introduction of rigorous prevention guidelines for catheter-related complications. The use of hand-held Doppler ultrasound increases the insertion success rate, especially in patients with several previous peripheral access insertion attempts. Ultrasound guidance is the preferred method for PICC placement in patients with no clinically identifiable peripheral vein and is an important factor in reducing the trauma associated to the insertion, which has been associated with an increase in PICC-related venous thrombosis (PRVT). Other factors associated with PRVT are the catheter diameter (gauge) and the tip location near the atrial–caval junction. The incidence of PRVT in cancer patients has also decreased and varies widely among studies [21]. Tran et al. reported an incidence of 7.8% symptomatic PRVT among 498 patients with hematologic malignancies; this prompted them to change to a tunneled device placed in the internal jugular vein for the next 667 patients. The incidence of symptomatic venous thrombosis dropped to 0.4% [24]. The retrospective nature of this study did not allow for the control of other known predisposing factors for PRVT limiting the validity of their findings.

Implantable subcutaneous ports

An implantable port is a totally implanted vascular access device that is inserted on the chest wall or in the antecubital area. It is made up of a subcutaneous port containing a silicone septum that is implanted in a subcutaneous pocket, and is connected to a catheter that is inserted in the vein via cutdown or venous puncture using the Seldinger technique. The catheter can be open-ended or have a distal valve (Groshong tip) to prevent blood reflux into the catheter. Randomized studies have failed to show an advantage of the Groshong tip in preventing port-related complications. Furthermore, the inability to draw blood samples increased from 2% for open-ended catheters to 12.5% for the Groshong tip [25]. The port can only be accessed with the use of a non-coring (Huber) point needle, which is inserted through the skin into the septum. The needle can only remain in situ for up to 7 days at a time. The advantage of this port is that it does not interfere with daily activities, does not require local care when it is not accessed, and has a less significant impact on body image. The port requires monthly flushing and is associated with a lower infection risk. Disadvantages of ports include the requirement for a needle for access, the flow rate being limited by the needle diameter, low reliability for obtaining blood draws, higher frequency of catheter occlusion, and the possibility of soft tissue chemotherapy extravasations if the needle is misplaced or migrates out of the port after insertion. Subcutaneous ports are primarily indicated in patients who require intermittent therapy and do not need continuous intravenous access. Subcutaneous ports consistently have the lowest incidence of CRBSI of all the central venous access devices [8,9]. The number of lumina and the flow rate limit its use in patients requiring multiple concomitant intravenous therapies.

Externally tunneled catheter

The Broviac and Hickman catheters were developed in the mid-1970s and constituted the first examples of the skin-tunneled catheter. The tunnel provides a distance between the vein entry site and the skin exit site, and this, along with a Dacron cuff, has been shown to reduce infection risk. The cuff is usually placed about 2 cm from the entry site; the local tissue reaction and granulation tissue entering the cuff prevent infections and catheter dislodgement. These devices provide reliable long-term access and are made of either silicone or polyurethane. Single-, double-, and triple-lumina catheters are available. These devices are usually placed by surgeons, radiologists, or other trained personnel under fluoroscopic guidance via either cutdown or percutaneous venous access. Externally tunneled devices are preferred for those patients who require continuous or frequent catheter access for treatment, blood samples, or support treatment (parenteral nutrition or blood product transfusion) or are receiving potentially toxic therapy if the infusion extravasates into the subcutaneous tissue.

The choice of number and size of lumina should be determined based on the intensity and complexity of the therapy. Pooled studies vary significantly with respect to patient characteristics, catheter maintenance schedules, and diagnosis criteria for infection. Maki and Safdar report an overall mean infection rate of 1.1 per 1,000 catheter days for tunneled catheters and of 0.01 per 1,000 days for subcutaneous ports in a review of over 200 studies [9]. Other important differences between these devices are outlined in Table 80.2.

The hematopoietic cell transplant patient merits special attention. In recent years, the use of peripheral blood progenitor cells has nearly supplanted bone marrow as the preferred autologous hematopoietic progenitor cell source for reconstitution after high-dose chemotherapy. In the past, peripheral blood progenitor cells were collected via a large-bore, stiff, temporary, dual-lumina central venous catheter that provided flow rates of at least 50 mL/min. The apheresis procedure is lengthy and time consuming, since large blood volumes must be processed during multiple sessions until enough progenitor cells have been collected. On the other hand, the transplant process also requires long-term multi-lumina venous access, but only requires a smaller, soft, and flexible catheter for chemotherapy administration, supportive care management (including frequent blood sampling, intravenous antibiotics, analgesics, antiemetics, blood components, and parenteral nutrition), and reinfusion of hematopoietic cells.

Since different catheter properties are required, the transplant procedure traditionally involved the insertion of two venous access devices, one for apheresis and the other for the treatment and supportive care. Over the last few years, the advent of soft silicone and polyurethane catheters specially designed for apheresis treatment has permitted the use of a single catheter for the entire transplant process. These catheters are shorter and have large lumina, staggered at the tip to prevent recirculation during the hematopoietic cell collection [26]. The design of these catheters makes them more likely to kink during placement if special precautions are not taken to maintain adequate flow rates. They also have a very short distance from the cuff to the proximal connectors, which makes them uncomfortable for the frequent venous access required in this patient population. A compromise between these catheters is to place a large-bore (12 Fr.) Hickman catheter with two round lumina of 1.3 mm, each of which provides comfortable access while allowing a 50 mL/min flow rate for a successful apheresis.

Table 80.2 Differences between devices for central venous access

	Peripherally inserted central venous catheter lines	Externally tunneled devices	Subcutaneous ports
Lumen	Single and dual lumina	Single, dual, and triple lumina	Single and dual lumina
Maintenance	Daily; training required	Daily; training required	Monthly; no training required
Activity	Some restrictions	Some restrictions	No restrictions
Blood sample	Low reliability	High reliability	Moderate reliability
Uses	Chemotherapy, TPN, antibiotic therapy, supportive therapy, transfusions	Hematopoietic cell collection and infusion, photopheresis, plasma/red cell exchange, transfusions, supportive therapy, chemotherapy	Chemotherapy, TPN, antibiotic therapy, supportive therapy, transfusions
Cost	Low insertion cost	High maintenance cost	High insertion cost
Access	Painless	Painless	Needle access
Flow	Determined by the lumen	Determined by the lumen	Determined by the needle
Complications	High	High	Low
CRVT	3.4–7.8%	8.6%	0.4–6.1%
CABSI	1.81–7.71 per 1000 catheter-days	1.0–7.26 per 1000 catheter-days	0.1–3.89 per 1000 catheter-days
Placement	Trained nurses; at bedside	Physician, in operating room or radiology	Physician, in operating room or radiology
Removal	Easy	Easy; in the office	Second procedure required

TPN, total parenteral nutrition.

Insertion site selection

The main veins used for central venous access are the internal and external jugular and subclavian veins, which are used for all types of central venous access device. Occasionally, the femoral veins are selected when the above sites are not available or for short-term access. The cephalic and basilic veins are the preferred sites for PICC venous access. The right side of the patient is usually preferred for access from the internal [27] and external jugular vein, and the left side is preferred for access from the subclavian vein [28] because the vessel anatomy allows easier access to the superior vena cava (SVC) and provides the easiest route for the practitioner inserting the device.

Regardless of the insertion site, it is important to pay special attention to the tunneling of the catheter to avoid kinking, and to the location of the tip of the catheter (Figure 80.2). An increased risk of venous thrombosis is associated with the tip of the catheter being located in the upper third of the SVC or in the innominate vein. Even though the lower third appears to be the favored location for the SVC tip, there is still some debate over the optimal location for the SVC tip. A good point of reference under fluoroscopy in the patient in the Trendelenburg position is the carina of the main bronchus bifurcation (Figure 80.3) [29]. Even though right atrial placement is associated with arrhythmias, atrial thrombus, and potential cardiac or valve perforations when the catheter is kept for an extended period of time, it is still performed in some centers.

Several studies have attempted to evaluate the merits of the different venous access sites. Biffi et al. conducted a study in 403 cancer patients who were randomized to have their venous port placed through a percutaneous access in the subclavian or internal jugular vein or by cutdown in the cephalic vein [30]. With 1-year median follow-up, the port-related infection was 0.8, 2.4, and 2.5% for the internal jugular, subclavian, and cephalic vein access, respectively ($p = 0.464$). Likewise, there were no significant differences in the incidence of venous thrombosis. A recent Cochrane Review that included the above-mentioned study failed to show evidence of catheter-related complications (infection, thrombosis, and mechanical complications) for long-term catheters placed in the internal jugular versus the subclavian vein in cancer patients [31]. The same

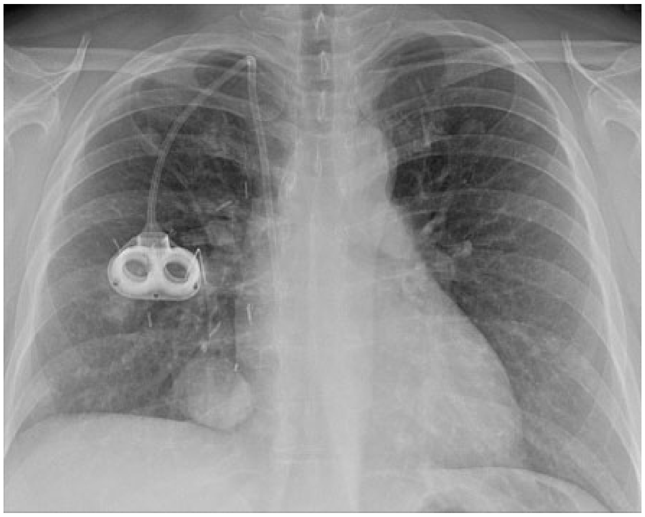

Figure 80.2 X-ray showing kinking of the catheter at the internal jugular vein insertion site.

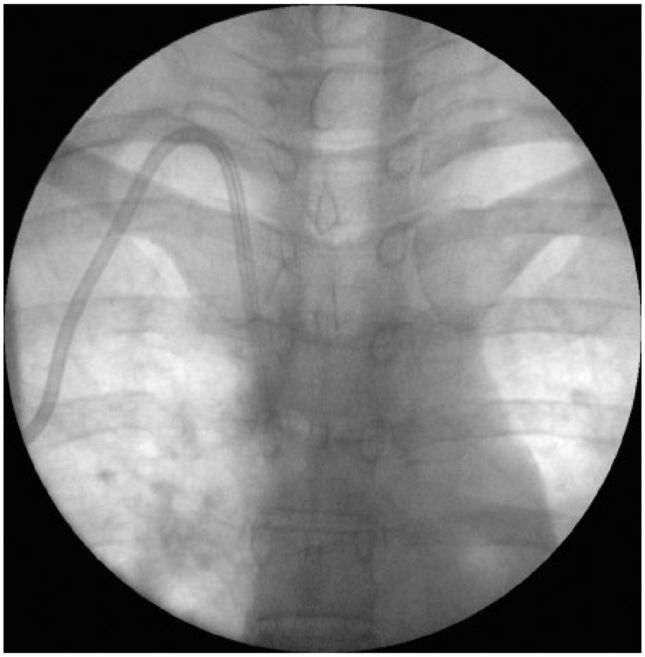

Figure 80.3 Intraoperative fluoroscopy showing the tip of a dual-lumina silicone catheter at the carina. Also note the soft curve at the internal jugular vein entry site.

study showed that for short-term non-tunneled venous access, the femoral vein route had higher colonization (14.2%) rates than the subclavian (2.2%) [relative risk (RR) 6.43; 95% confidence interval (CI) 1.95–21.21] route but not higher CRBSI (1.49% vs. 0.74%). There was also a significant increase in the incidence of catheter-related thrombosis with femoral catheters (21.6%) and with subclavian access (1.9%) (RR 11.53; 95% CI 2.80–47.52).

Insertion technique

Long-term intravenous catheters are better placed in a surgery or radiology suite or a similar outfitted procedure room in order to minimize the incidence of infections. Most procedures are performed on an outpatient basis or immediately prior to chemotherapy admission. Local anesthesia with monitored care and short-acting barbiturates, or conscious sedation with short-acting agents, provides safe and excellent patient sedation [32]. The use of prophylactic antibiotics during the insertion of long-term venous access is controversial; a systematic review of several randomized studies failed to show a significant benefit for this practice [33]. The realization that CRBSI is the leading cause of systemic infection in hematology-oncology patients has led to the development of strict guidelines from the CDC, from the Center of International Blood and Marrow Transplant Research, and from the Bone Marrow Transplant Associations in Amethe United States and Europe [34]. The most important element is the implementation of a CABSI prevention bundle: hand hygiene, full barrier precautions, careful selection of the insertion site to avoid sites with higher infection rates (femoral and internal jugular vein for short-term non-tunneled venous access), use of chlorhexidine for skin preparation, and early removal of unnecessary central venous access [12]. A meta-analysis showed a 50% reduction in catheter-related infections when chlorhexidine gluconate–alcohol-based solutions rather than povidone–iodine solutions were used for entry site care [35].

The use of intraoperative fluoroscopy to document appropriate catheter placement and to prevent complications is highly recommendable. In a prospective study carried out by experienced operators, placement of catheters without fluoroscopic guidance resulted in a 6% incidence of catheter misplacement that required repositioning under fluoroscopy at a later time [36]. The most common technique used in catheter placement is the percutaneous method of Seldinger, which uses the subclavian or internal jugular vein [37,38]. Special care should be taken during the introduction of the wire [39]. In most cases, the guidewire should not be introduced without fluoroscopic guidance for more than 18 cm, which is the average distance in adults from most puncture sites to the atrial–caval junction in adults. Alternatively, a cutdown over the cephalic, external, or internal jugular vein can provide venous access.

For the percutaneous approach, the patient is placed supine, in the Trendelenburg position. The anterior chest, neck, and shoulders are prepared in a sterile manner. The site for venous access is infiltrated with anesthetic, and the right side is most commonly preferred for the internal jugular vein and the left side for the subclavian vein [27,28,40]. The use of ultrasound can aid the placement of the needle, especially when an internal jugular vein approach is attempted [41,42]. The consistent use of ultrasound guidance has decreased the incidence of immediate and long-term catheter-related complications[43]. A systematic review of 18 randomized trials showed that ultrasound guidance for internal jugular vein cannulation was more effective and less expensive than the landmark method, resulting in a faster catheter placement and a higher rate of success in both children and adults [42]. The needle is advanced bevel up carefully and slowly into the vein, while aspirating with the attached syringe (without Luer-lock). No more than three attempts to puncture any given vein should be tried, and the bilateral percutaneous approach should not be attempted in the same session without the confirmation of absence of complications. Vein penetration is confirmed by easy blood flow into the syringe, the syringe is disconnected, preventing air penetration with the thumb, and a flexible J-guidewire is advanced through the needle for no more than 18 cm for most insertion sites and 16 cm for the right internal jugular vein; fluoroscopy confirms the placement of the wire at the atrial–caval junction. Placement of the catheter without fluoroscopic guidance should be discouraged. Pulsatile flow indicates an arterial puncture. When in doubt, the needle should be connected to a pressure transducer before proceeding. After an arterial puncture, the needle needs to be withdrawn, and local pressure should be applied for 5–10 minutes.

Occasionally, in patients with an existing Port-a-Cath, this can be converted to a tunneled device by wiring the existing catheter. This can usually be accomplished if the previous catheter was placed by venous puncture rather than cutdown. This procedure is contraindicated in patients who might have a catheter-related infection and should include routine culture of the removed catheter [44].

Resistance to the advancement of the wire is usually secondary to either misdirection of the wire into a secondary vein or migration outside the vein. Fluoroscopy confirms the wire position; if the wire is outside the vessel, the needle needs to be removed with the wire to prevent its shearing. If the wire requires to be redirected, the needle should be replaced with a 2.5-inch 16-gauge Angiocath, prior to withdrawing the wire. After the successful placement of the wire, the exit site for the catheter, and also the subcutaneous tunnel between this site and the vein access, are anesthetized, and the puncture site is enlarged to admit the catheter and introducer sheath. Placement of the sutures at the catheter exit and vein access site prior to tunneling the catheter prevents potential needle catheter punctures. The catheter is tunneled, measured, and custom-cut to reach the SVC–right atrium junction (approximately the fourth anterior intercostal space) or the tracheal bifurcation under fluoroscopy. The catheter cuff is placed within 2 cm of the exit site.

The dilator and the peel-away introducer sheath are advanced slowly over the wire under frequent fluoroscopy control, gently withdrawing and advancing the wire to confirm that the dilator and sheath are threading over the wire and not migrating outside the vessel. The dilator and wire are withdrawn, and the catheter is advanced through the introducer to the SVC–right atrium junction. Catheter tip placement within the right atrium is associated with a high incidence of right atrial thrombus and occasional supraventricular arrhythmias [45]. Prior to the removal of the introducer, the catheter position should be confirmed by fluoroscopy. Failure to position the beam perpendicular to the patient will provide a false impression of the catheter position. Difficulties in advancing the catheter through the introducer usually imply that the introducer is bent, in which case the introducer should be peeled away slowly while advancing the catheter. This is a frequent occurrence if the catheter is placed through the costoclavicular ligament. The catheter should never be handled with sharp instruments, and only non-toothed forceps should be used if needed (Figure 80.4). The port placement is similar except for the creation of the subcutaneous pocket. The pocket needs to be placed over a hard surface (chest, arm, thigh or pelvis) and should be easily palpable and large enough to allow a no-tension wound closure. The port is sutured to the muscular fascia to prevent port migration or turning.

When the catheter placement has been completed, the catheter position should be examined with fluoroscopy to rule out any kinking that would prevent normal functioning. Catheter infusion (no resistance) and withdrawal (no turbulence) should be tested in the surgical suite prior to heparinization of the lumina. In the recovery room, a chest X-ray should be obtained to rule out complications (Figure 80.5).

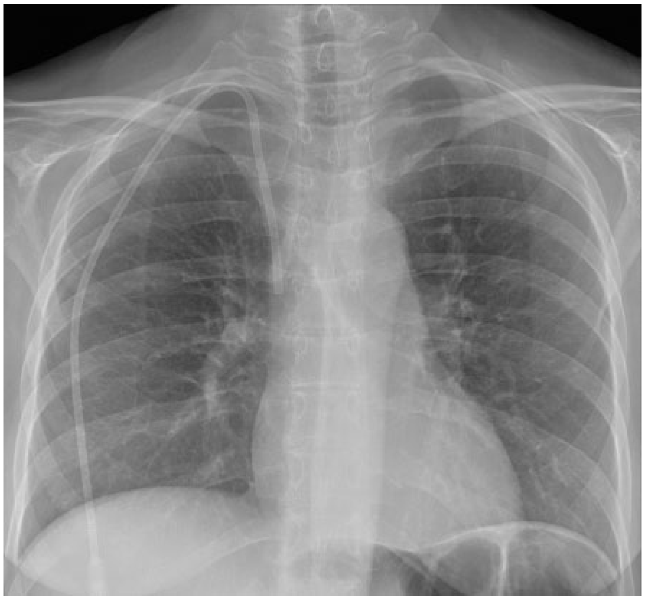

Figure 80.4 X-ray showing a dual-lumina silicone catheter inserted into the internal jugular vein.

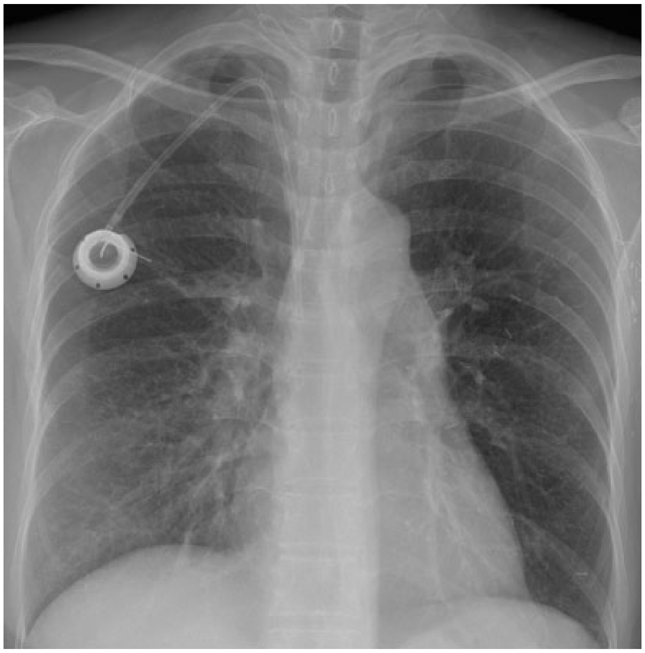

Figure 80.5 X-ray showing placement of a subcutaneous single-lumen venous port into the right internal jugular vein.

Catheter care

Most of the catheter-related clinical care protocols are the product of the individual centers observations, resulting in significant variability among centers in the guidelines used for catheter care and the management of their related complications. In a study of 23 Belgian and Dutch centers for the treatment of patients with hematologic disease, the authors documented great variability in the catheter care guidelines and catheter-related complications treatment protocols among the centers [46]. These findings underscore the need for the systematic implementation of evidence-based catheter-care guidelines and for the ongoing monitoring and reporting of catheter-related outcomes. The CDC has issued an evidence-based guideline for the prevention of catheter-related infections [47]. This comprehensive guideline covers all aspects of catheter selection, insertion technique, and subsequent care. Adherence to these recommendations is essential to achieve a decrease in the incidence of catheter-related infection. Several studies have demonstrated the importance of establishing a hospital-wide program to implement these guidelines systematically and monitor the incidence of catheter-related infections, obtaining sustained CRBSI reductions in the intensive care unit in excess of 66% [48,49]. Neutropenia poses additional challenges in the prevention of CRBSI. To determine the impact of an infection surveillance and catheter-care intervention program, Chaberny et al. studied 268 patients with hematologic malignancies during their neutropenic phase, with 202 of them undergoing bone marrow transplantation [50]. The study was divided into two phases of 18 months each; during the surveillance period, CABSIs and nosocomial infections were collected during the neutropenic phase according to a standardized protocol. After this period, the CABSI rate was calculated and presented to the healthcare personnel. The clinical staff went through an intensive training course to improve catheter-care infection control measures according to established evidence-based guidelines. This included dressing changes, routine catheter-care guidelines, and the use of chlorhexidine–silver sulfadiazine-impregnated catheters. The investigators achieved a 35.8% reduction in the incidence of CABSI episodes per 1000 neutropenic days, but the overall rate of CABSI remained high with a reduction from 38.4% to 26.2%. By multivariate analysis, other factors that contributed to the high incidence of CABSI were the diagnosis of acute leukemia [odds ratio (OR) 1.92; 95% CI 1.12–3.32] and bone marrow transplantation (OR 2.11; 95% CI 1.10–4.26). The implementation of patient education has also been well documented as an important factor in the reduction of CRBSI. In a randomized study of 82 patients with hematologic malignancies with tunneled central catheters, Møller et al. showed a 50% reduction in catheter-related infections [a CRBSI decrease from 4.22 per 1000 catheter days in the control group to 1.92 per 1000 catheter days in the intervention group ($p < 0.05$)] with a patient education and training program [51]. Furthermore, patients in the interventional group assumed ownership of the catheter care and displayed more independence and self-reliance in interviews focusing on clinical and psychosocial outcomes [52]. The M.D. Anderson Cancer Center has had a patient education program in place since 1970, and reported a steady decline in CRBSI per 1000 catheter days from 0.42 in 2006 to 0.12 in 2010 [53].

There is considerable controversy regarding the best occlusive dressing. A systematic review of all randomized trials failed to show a difference in the incidence of infection between gauze and tape versus transparent polyurethane film dressings on CRBSI, catheter dislodgement, tolerance, and reaction to dressing in hospitalized patients [54]. The guideline calls for the use of sterile gauze or transparent or semitransparent dressing to cover the catheter entry site (Category IA). Topical antibiotic ointments should not be used at the insertion site; dressings should be changed if they become moist, loose, or soiled (Category IB). The catheter should not be submerged in water and the connectors should be protected during showering (Category IB). For tunneled cuffed devices, dressing changes are recommended weekly until the insertion site has healed. It is not clear if a dressing is required after the insertion site has healed and the sutures have been removed. A chlorhexidine-impregnated sponge is currently recommended only for short-term non-tunneled catheters at the insertion site. A 2% chlorhexidine wash is used for daily skin cleansing.

Implanted ports require flushing with heparin solution (2–3 mL, 100 U/mL) every 4 weeks or after each use. During treatment, the non-coring (Huber) needle is replaced every fifth to seventh day. Hickman catheters need to be flushed daily with heparin solution (2–3 mL, 100 U/mL) or after each use, and protective caps need to be replaced biweekly. PICC lines require flushing twice per day and after each use with heparin solution, 3–5 mL per lumen. Several studies, especially in children, have documented that the use of vancomycin (25 μg/mL) in the lock solution reduces the incidence of catheter-related infections without increasing the incidence of vancomycin-resistant infections [33,55]. The CDC guidelines recommend the use of prophylactic antimicrobial lock solution in patients with history of recurrent CRBSI in spite of adherence to the rest of guidelines. Studies have shown high levels of catheter patency with the use of non-heparin-containing solutions when a positive-pressure catheter cap is used [56]. Catheters with Groshong tips require weekly flushing with 5 mL of saline solution.

Complications

Complications related to catheter insertion

Complications during catheter placement can be related to the method of insertion or to the experience of the operator. The overall incidence of major complications is approximately 2–5% [57]. The use of imaging during catheter insertion has significantly reduced the incidence of complications, especially during insertion. In a study of 1978 consecutive central venous catheter placements under ultrasound guidance, the vein was accessed on the first attempt in 98.5% of the patients. There was an incidence of arterial puncture of 0.3%, and operators failed to access the vessel in 0.9% of cases [43]. Most importantly, there were no instances of major bleeding, pneumothorax, or nerve puncture. The incidence of complications with the subclavian or internal jugular approach is similar in prospective randomized studies [58]. In general, the incidence of catheter malposition is more frequent with the subclavian approach, and the incidence of arterial puncture is more frequent with internal jugular insertion.

Pneumothorax

This is the most frequent major complication when using the percutaneous technique. A higher incidence of up to 17% is seen in nutritionally compromised and emaciated patients. This complication is also related to the number of attempts to access the vessel and the experience of the operator. Several steps can be taken in order to avoid this complication. The use of a cutdown technique should be encouraged in high-risk patients. Using an ultrasound-guided percutaneous internal jugular vein approach can also effectively avoid this complication. The withdrawal of air during the insertion of the needle is usually secondary to a loose connection between the syringe and the needle rather than a lung puncture.

Pneumothorax can be due to a tear of the pleura or the lung parenchyma. This complication is usually recognized in the postoperative upright expiration chest film, but in some cases is not seen until a few hours or days after insertion. Patients that complain of shortness of breath or pleuritic pain hours or days after the procedure should have a repeat chest X-ray to rule out a delayed pneumothorax or other catheter-related complication. A pneumothorax of less than 30% in an otherwise asymptomatic patient can be observed provided that the pneumothorax does not increase in subsequent films. Symptomatic patients or those with a larger pneumothorax are best treated with a small anterior chest tube thoracostomy connected to a Heimlich (one-way) valve, water seal, or suction [59,60].

Arterial punctures

This frequent and under-reported complication is seen more commonly with the internal jugular than the subclavian vein approach. This complication can be avoided with the use of ultrasound guidance to access the internal jugular vein [61]. The presence of pulsatile flow from the needle confirms the arterial puncture; removal of the needle and compression of the vessel for 5 minutes are usually all that is necessary. If initially missed, the placement of the guidewire to the left of the thoracic spine should be evidence of an arterial puncture (in patients with persistent left vena cava, the wire will also be to the left of the spine; an intraoperative venogram will confirm the diagnosis). If unsure, an Angiocath needs to be placed over the wire and connected to a blood pressure transducer. The introducer sheath should not be inserted until the operator is sure that the guidewire is in the vein.

Hemothorax

This complication, usually due to the perforation of one of the major vessels with the introducer, the needle, or the wire during the catheter placement, is fortunately rarely seen (Figure 80.6) [58,62]. Careful attention to the technique and use of fluoroscopy during advancement of the introducer should prevent this complication. Special attention should be given to the length of the introducer sheath. In the majority of trays, this sheath is 20 cm long; usually no more than 16 cm from the right side and 18 cm from the left side are required to reach the desired catheter tip location. Furthermore, the dilator should only be introduced until the peel-away sheath is in the vein. It can then be withdrawn into the sheath to prevent potential perforations with the tip of the dilator. Most of these patients can be treated with a large lateral tube thoracostomy connected to a closed thoracic suction system. Many of these systems have a blood reinfusion collecting system. Thoracotomy is indicated in patients with persistent bleeding (more than 500 mL/hour) or with massive hemothorax (more than 1500 mL).

Catheter tip malposition

This problem is usually recognized and corrected at the time of surgery with the use of fluoroscopy. Catheters placed in the azygos or internal mammary veins can mimic an SVC placement. These catheters frequently do not withdraw blood easily, and the tip of the catheter does not move with the heart rate; a lateral view of the thorax or a venogram will confirm the position. Placement of the catheter in the proximal vena cava or brachiocephalic trunk usually results in venous thrombosis (Figure 80.7). The ideal tip location is at the atrial–caval junction, with a free-floating tip. Catheter migration after the placement can usually be corrected with an angiographic guidewire placed through the femoral vein.

Catheter occlusion

Placement of the catheter medial and close to the clavicle may cause a compression of the catheter between the clavicle and the first rib. This complication, which may cause fracture and distal embolization of the catheter, can be recognized by the compression of the lumen (pinch-off sign) (Figure 80.8). Selecting a more lateral and lower puncture site can prevent it. If recognized during placement, the catheter should be repositioned through a different venous access site.

Catheter entry site bleeding and hematomas

These complications, which can be prevented with the correction of clotting factors, are frequent in thrombocytopenic patients. Compression over the tunnel and the catheter entry site while the coagulopathy is corrected usually prevents the problem.

Air embolization

This is an infrequently recognized complication during catheter placement that can result in a life-threatening complication. This is especially true in children and patients with spontaneous breathing. The best method to avoid this complication is to ask the patient to do a Valsalva maneuver, or by requesting the anesthesiologist to provide positive ventilation while introducing the guidewire into the needle or the catheter into the peel-away sheath [63].

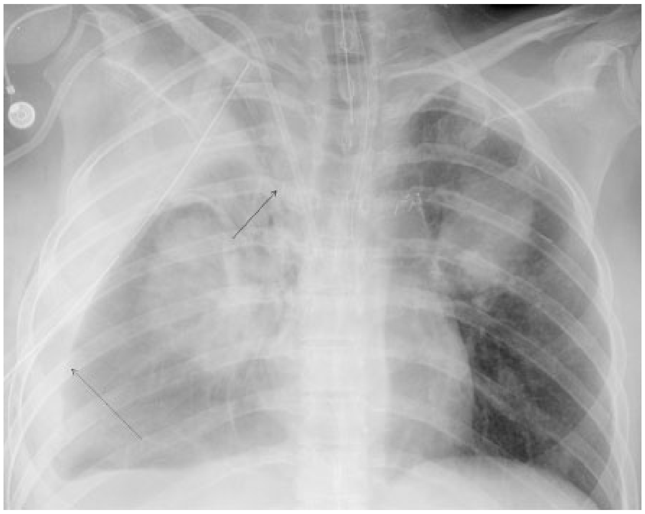

Figure 80.6 X-ray showing a large extrapleural hemothorax (arrows) following placement of a dual-lumina external silicone catheter in a patient with a mediastinal tumor. Notice the placement of a large chest tube.

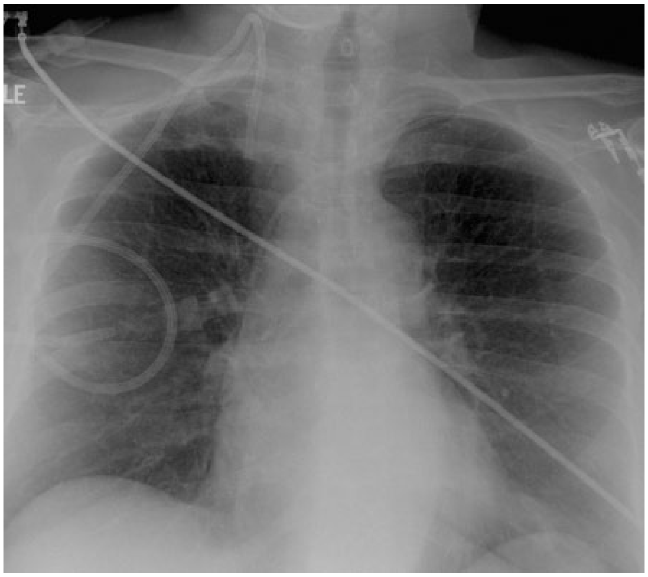

Figure 80.7 X-ray showing right internal jugular vein insertion in the upper third of the superior vena cava. Short catheters usually result in venous thrombosis and catheter dislodgement.

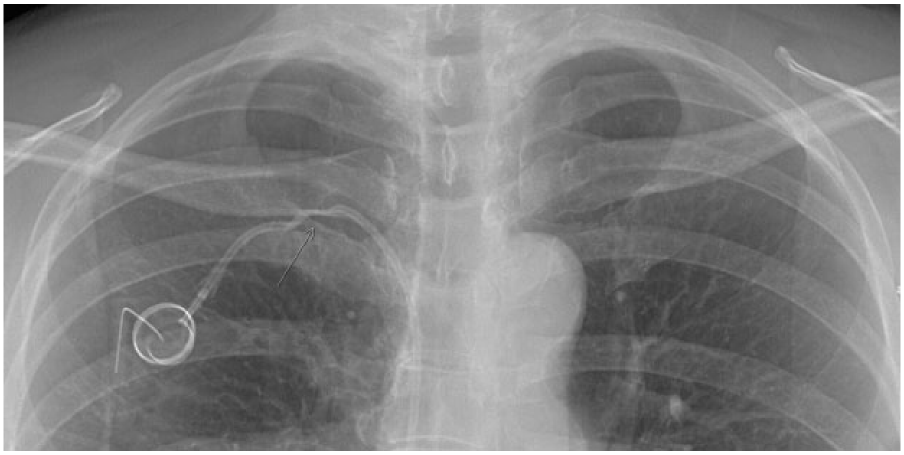

Figure 80.8 X-ray showing subclavian insertion of subcutaneous port with compression of the catheter between the first rib and the clavicle ("pinch-off" sign).

Cardiac tamponade

This insertion complication usually occurs secondary to the guidewire or the dilator. It can be avoided if neither is advanced beyond the atrial–caval junction. The use of fluoroscopy during the procedure helps to prevent this usually fatal complication. This complication, which is sometimes not immediately recognized, can be the result of the migration of the catheter into the pericardium after its placement.

Cardiac arrhythmias

This complication is usually secondary to the wire placement, and some surgeons use the presence of arrhythmias to confirm the placement of the wire into the right atrium. This can usually be avoided if the wire is not advanced beyond 18 cm. Usually; these arrhythmias are limited to a few extra systoles or a short run of supraventricular tachycardia that stops after withdrawing the wire. This complication can also occur after placing the catheter if the tip stimulates the atrial node. This can be avoided by not placing the catheter beyond the atrial–caval junction. Occasionally, this is not recognized until the patient is in recovery or might be diagnosed days after the catheter placement. Patients describe a rapid heart rate usually when they adopt certain positions. To establish the diagnosis, they should be connected to a monitor while they change positions. In rare occasions, a 24-hour Holter monitor is necessary. The presence of a new atrial arrhythmia after surgery usually requires repositioning of the catheter.

Nerve injury

This infrequent complication is due to the needle placement, and is more common with supraclavicular approaches. It can be avoided by using ultrasound during the needle placement. Rarely, it occurs secondary to a hematoma or other local complication. The brachial plexus is the most commonly affected site. Even though the most common symptom is pain, occasionally the patient might also present motor symptoms. Ordinarily, these symptoms are detectable immediately after the procedure. Failure to recognize them immediately is usually due to the effects of the local or general anesthesia. The presence of severe pain that persists days after the procedure, especially if it radiates to the arm or is associated with motor deficits, should be suspicious for a nerve injury. Imaging studies to rule out a hematoma and nerve conduction studies are sometimes needed to confirm the diagnosis. Patients with persistent and out of the ordinary pain days after the procedure sometimes require the removal and repositioning of the catheter in another site. Motor symptoms may require months to recover.

Catheter-related complications

Catheter occlusion or malfunction

Inability to withdraw or infuse through a catheter can be secondary to catheter tip malposition, or kinking, thrombosis of the vein, or catheter occlusion due to clotting or intraluminal precipitation of medications. In a review of 141 cases, Petersen et al. demonstrated that the incidence of catheter malfunction was mainly related to the location of the tip of the catheter in relation to the SVC and right atrium (RA) junction [29]. In this study, catheters located at or below the SVC–RA junction malfunctioned in 2.3% of cases, whereas catheters located above 2 cm from the SVC–RA junction malfunctioned in one-third of cases. In a subsequent series in which they paid attention to the location of the tip of the catheter during placement, they were able to reduce catheter malfunction from 42% to 8%.

Whenever there is problem with infusion or withdrawing from a catheter, 5000 U in 2 mL of urokinase should be injected into the catheter. After 30 minutes, the urokinase should be withdrawn and the catheter tested again. This can be repeated twice and left for longer periods (24 hours) if not effective [64]. Alternatively, 2 mg of recombinant tissue plasminogen activator (TPA) can be used instead of urokinase; the TPA should be left for 30–120 minutes and the dose repeated for a second and third time if catheter patency is not established [65]. This protocol restores catheter flow in 87% of patients [66]. In a double-blind placebo-controlled randomized study that did not required radiographic contrast injection or catheter position confirmation, TPA was shown to be effective in restoring catheter patency in 74% and 90% after the first and second dose, respectively, without reported adverse effects [67]. When compared with regular heparin flushing, the use of prophylactic urokinase failed to prevent these complications [68]. The frequency of catheter occlusions requires the institution of standardized nursing practices in every chemotherapy infusion unit [69].

Inability to withdraw from a catheter might also be due to the development of a fibrin sheath that excludes the catheter from the lumen of the vein [70]. This complication, which occurs in 50–80% of the catheters, does not always result in catheter occlusion. In these patients, a Valsalva maneuver or repositioning of the patient can sometimes allow a successful blood draw. A venogram can usually confirm this etiology (Figure 80.9). These patients rarely respond to a urokinase or TPA bolus. An infusion of 200 U/kg/hour of urokinase, not to exceed 100,000 U/hour, for 6–12 hours is usually more effective. Patients with contraindication for thrombolytic therapy should not be treated with this protocol; fibrinogen levels should be checked and kept above 100 mg/dL. Chemical occlusion can be treated with instillation of a mixture of 0.01 N hydrochloric acid and heparin for calcium–phosphorus precipitation or low-pH medications and sodium bicarbonate for high-pH medications [71]. The solution is irrigated in and out for 2 minutes, clamped for 1 hour, then aspirated. A suggested algorithm for the management of catheter occlusions is outlined in Figure 80.10.

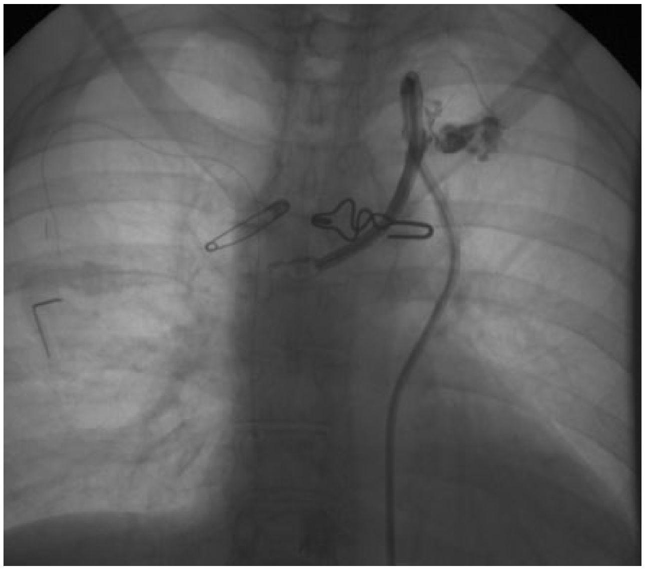

Figure 80.9 X-ray showing catheter tip placed in the innominate vein resulting in venous thrombosis. The injection of contrast through the catheter in this single frame from a video-fluoroscopic study shows no distal flow but contrast reflux around the catheter (fibrin sheath) to small collaterals in the supraclavicular region.

Catheter damage/fracture

External catheters can be damaged at the site of the clamp or the suture. The use of needleless connections for infusions and irrigations should prevent needle damage. Most catheters have kits to replace the external portion of the catheter. Repair of the catheter has not been associated with an increased incidence of infection. Intravascular catheter fractures usually occur as a consequence of compression between the first rib and the clavicle (Figure 80.11). This usually can be recognized on a chest X-ray by the "pinch-off" sign [72]. Patients with this syndrome usually have intermittent and positional occlusion of the catheter flow. A segment of the catheter that embolizes into the heart or the pulmonary circulation can usually be retrieved with a snare through the femoral vein by an interventional radiologist (Figure 80.12) [73].

Drug extravasation

Accidental dislodgement of the needle from the port may lead to extravasation of the chemicals into the subcutaneous tissue. This may lead to chemical cellulitis, tissue damage, necrosis, or loss of soft tissue. Clinical signs of extravasation are changes at the infusion site with pain, burning, swelling, or erythema. If these are suspected, the infusion should be stopped and the needle withdrawn. Management of extravasation injuries depends on the type of drug infusing and on the amount extravasated. Occasionally, the patient will complain of pain along the tunnel or at the insertion site during infusion. The catheter should not be used until a contrast study has been obtained to document the catheter integrity and patency. On rare occasions, the extravasation is due to a fibrin sheath that excludes the catheter from the main lumen of the vein, causing a backflow of the solutions around the catheter to the entry site.

Catheter-related venous thrombosis

Catheter-related venous thrombosis (CRVT) can occur as a direct effect of the insertion procedure, the access site, the effect of the catheter, the location of the tip, the underlying disease, or the effect of the substances being infused. During insertion, endothelial damage initiates the coagulation cascade, which is perpetuated by the

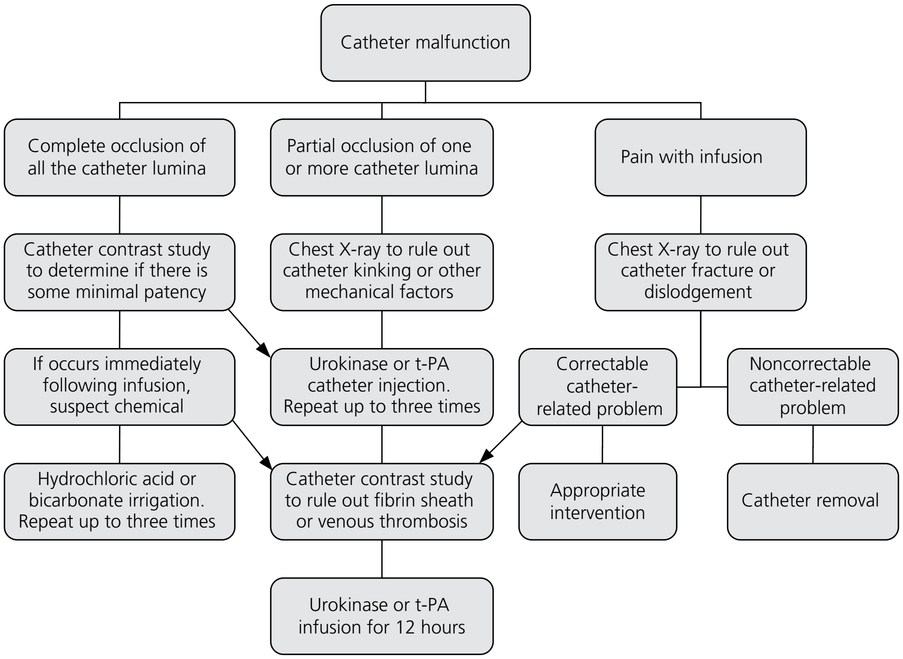
Catheter malfunction
Complete occlusion of all the catheter lumina
Partial occlusion of one or more catheter lumina
Pain with infusion
Catheter contrast study to determine if there is some minimal patency
Chest X-ray to rule out catheter kinking or other mechanical factors
Chest X-ray to rule out catheter fracture or dislodgement
If occurs immediately following infusion, suspect chemical
Urokinase or t-PA catheter injection. Repeat up to three times
Correctable catheter-related problem
Noncorrectable catheter-related problem
Hydrochloric acid or bicarbonate irrigation. Repeat up to three times
Catheter contrast study to rule out fibrin sheath or venous thrombosis
Appropriate intervention
Catheter removal
Urokinase or t-PA infusion for 12 hours

Figure 80.10 Catheter malfunction algorithm. t-PA, tissue plasminogen activator.

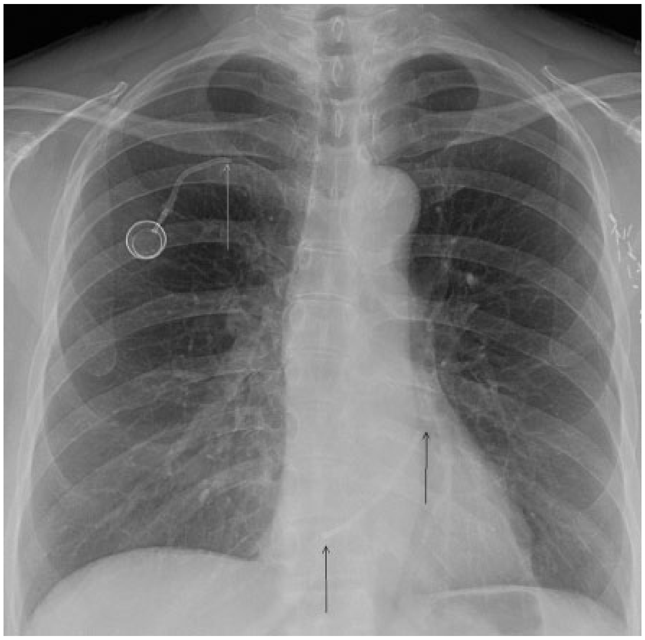

Figure 80.11 X-ray showing catheter fracture at the junction between the first rib and the clavicle with the free catheter segment in the right ventricle.

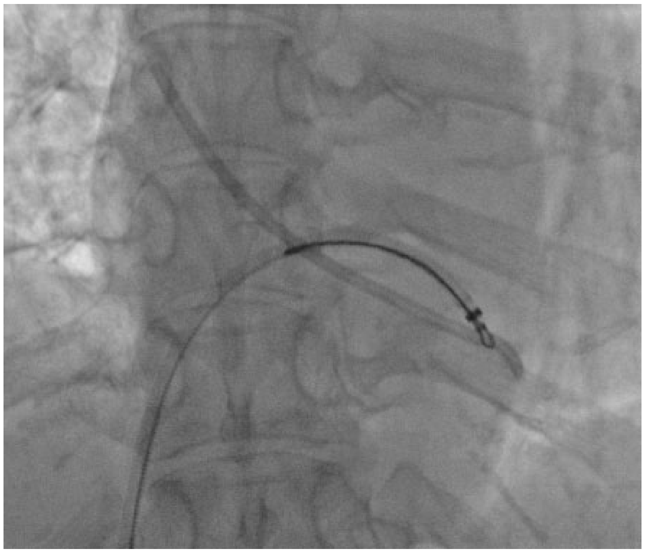

Figure 80.12 Single frame from a continuous fluoroscopy scan showing retrieval of a catheter fragment from the right ventricle using an endoloop inserted through the femoral vein.

chronic effect of the catheter in the lumen. After insertion, a mural thrombus and a fibrin sheath form around the catheter, often creating a sleeve that excludes the catheter from the true venous lumen, causing catheter malfunction [74]. Other contributing mechanical factors include the relationship between the size of the catheter and the lumen of the vein, which is particularly important in children and women. Most of the CRVTs are asymptomatic; venograms obtained in prospective studies 6 weeks after catheter placement have shown evidence of venous thrombosis in up to 36% of the patients, with only 6% of the patients reporting symptoms. The incidence of CRVT seems to be higher in older studies, which is most likely due to new insertion techniques, improvements in the compatibility of the catheter materials, and the selection of the access sites [46]. Pulmonary embolism occurs in 15–25% of patients with symptomatic CRVT and 15% develop postphlebitic syndrome. The incidence of catheter-related infections increases the incidence of catheter-related venous thrombosis in hematology patients (RR 17.6; 95% CI 4.1–74.1) [75]. Ipsilateral arm swelling, pain, and development of collaterals should alert the physician to the possibility of venous thrombosis. Venogram, computed tomography scans, and duplex Doppler venous imaging can establish the diagnosis and the site of the obstruction (Figure 80.13). The treatment should be directed at the prevention of pulmonary embolisms, avoidance of clot propagation, prevention of the postphlebitic syndrome, and preservation of the catheter whenever possible. With these considerations in mind, the catheter should be removed only if it is no longer necessary or after failure of the initial therapy. In a prospective study of 74 cancer patients with acute symptomatic deep venous thrombosis (DVT) treated with subcutaneous dalteparin 200IU/kg/day for 5–7 days and warfarin with a target international normalized ratio (INR) of 2.0–3.0, there were no episodes of recurrent venous thromboembolism and three patients (4%) had major bleeds [76]. No catheters required to be removed because of infusion failure or recurrence or extension of DVT. It is also important to note that up to 20% of patients with catheter-related venous thrombosis may have a hypercoagulable state due to inherited conditions such as a factor V Leiden gene mutation [77]. The 60-day mortality of upper extremity or internal jugular DVT is reported to be up to 30%, usually secondary to the underlying disease rather than the vein thrombosis; pulmonary embolism is seen in 3–5% of patients [78,79].

In a large meta-analysis study that included 5636 subjects from five randomized clinical trials and seven prospective studies, Saber et al. reported that the use of implanted ports decreased the incidence of CRVT compared with PICC lines (OR 0.43; 95% CI 0.23–0.80), whereas past history of DVT (OR 2.03; 95% CI 1.05–3.92), subclavian venous puncture insertion site (OR 2.16; 95% CI 1.07–4.34), and improper catheter tip location (OR 1.92; 95% CI 1.22–3.02), increased the risk of CRVT [80]. In this meta-analysis, the number of catheter lumina, the technical difficulties with catheter insertion, the use of thromboprophylaxis, current or recent exposure to estrogens, and the site of catheter insertion were not independent predictors of CRVT by multivariate analysis. There was no significant difference in the incidence in CRVT in patients with hematologic malignancies (7.7%) and solid tumors (7.5%).

There are insufficient data regarding the effectiveness of prophylactic low-dose Coumadin (warfarin sodium) or low molecular weight heparin (LMWH) in the prevention of CRVT [81]. Based on the lack of benefit in three randomized trials published in 2005 comparing LMWH or Coumadin with placebo in the prevention of CRVT, the Seventh American College of Chest Physicians Conference on Antithrombotic and Thrombolytic Therapy recommended against the use of prophylactic anticoagulation in patients with malignancy and an indwelling central venous catheter. It is also important to note that the use of heparin flush and other techniques to maintain catheter patency has not been shown to decrease CRVT. Mitchell et al. conducted a systematic review of the nonpharmacologic interventions for the prevention of CRVT [82]. Ten randomized studies met the inclusion criteria for the review; the inclusion criteria, study methodology, and objectives of the studies did not permit data meta-analysis. A Cochrane review of anticoagulation for patients with cancer and central venous catheters included

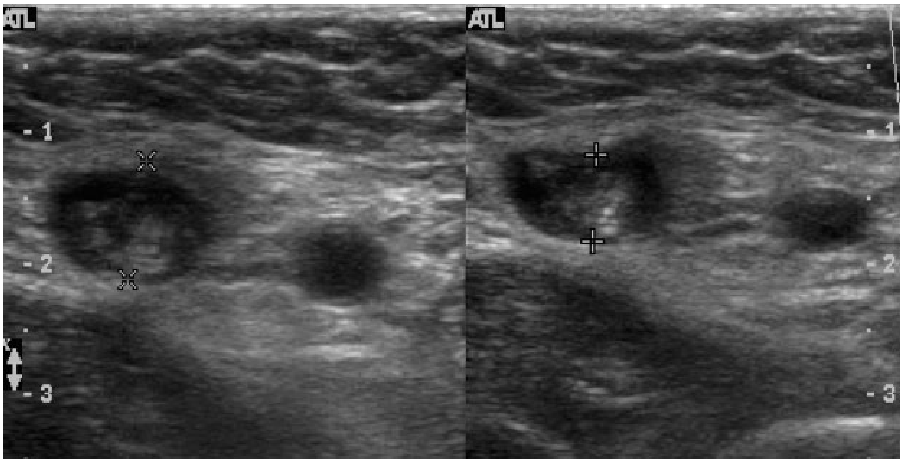

Figure 80.13 Venous ultrasound scan showing subclavian vein thrombosis on the left. Note the venous thrombus in the lumen. On the right, there is external compression of the vein with minimal change.

3611 patients enrolled in 12 randomized clinical trials that used prophylactic LMWH, unfractionated heparin, or vitamin K antagonist versus placebo. The review did not show any benefit of the treatment arm in the prevention of CRVT, the incidence of symptomatic DVT, major bleeding, thrombocytopenia, infection, or death [83].

Patients should be treated with systemic heparin or subcutaneous LMWH for 5–10 days prior to changing to oral anticoagulation. Continuation on LMWH should be considered for patients with a high risk for recurrent thrombosis [84]. Fibrinolytic agents are also effective in the management of this complication and seem to be associated with less postphlebitic syndrome. The incidence of catheter-related venous thrombosis [49] is associated with the catheter tip position (the more proximal, the more likely the development of venous thrombosis), the size of the catheter (especially triple lumina), and the access site (percutaneous access having a higher incidence of thrombosis than vein cutdown). The use of low-dose daily Coumadin (1 mg/day) or LMWH as a prophylaxis for catheter-related DVT remains controversial. The use of low-dose Coumadin prophylaxis has been shown to be safe in patients treated with high-dose chemotherapy and stem cell transplantation provided that the platelet count is above 50,000/dL [85]. A systematic review of the evidence showed that there is no conclusive evidence to recommend the routine use of venous thrombosis prophylaxis [86,87]. A suggested algorithm for the management of catheter-related venous thrombosis is outlined in Figure 80.14.

Catheter-related infection

CABSI and CRBSI should not be considered an unavoidable complication of cancer care. Active prevention, education, and early recognition of these complications have resulted in a steady decrease in incidence and its consequences. The Health Care Infection Control Practices Advisory Committee of the CDC has established a goal of a 100% compliance with the “central line bundle” that includes five recommendations: hand hygiene, maximum sterile barrier, chlorhexidine skin preparation, optimal site selection. and daily evaluation of the necessity for lines. Catheter-related systemic infections are potentially the most life-threatening complications, especially when they occur in the immunocompromised patient. The incidence of this complication can be significantly diminished by strictly following the catheter care protocols discussed in the previous sections. As discussed before, establishing a continuous quality improvement program to monitor and ensure compliance with established evidence-based catheter care guidelines significantly reduces the incidence of CABSI, CRBSI, and their complications [49,50]. In addition to the implementation of strict catheter care guidelines, catheter prevention efforts have focused in the use of prophylactic antibiotics at the time of insertion, the use of antibiotic–heparin lock solutions, and the use of modified catheters impregnated with antibiotics or other substances that prevent catheter colonization. The use of prophylactic antibiotics at the time of catheter insertion does not reduce the incidence of CABSI except in neutropenic patients and in patients with a previous history of CRBSI. The use of antibiotic–heparin lock solutions has been mostly studied in children; a meta-analysis of five randomized trials showed a significant reduction in CABSI but not CRBSI; it is not clear if these results can be extrapolated to the adult population and this approach is not currently recommended [88]. In recent years, considerable effort has been put into the modification of catheters by impregnating antibiotics or silver in the catheter wall or by bonding or coating the catheter with heparin, chlorhexidine, or silver sulfadiazine. Unfortunately, there is a paucity of data on the effect of antimicrobial coating or impregnation on long-term catheters in neutropenic patients. A prospective randomized study utilizing new catheter technology with silicone-impregnated catheters with minocycline and rifampin showed that these non-cuffed, non-tunneled catheters were as likely to be colonized as standard cuffed, tunneled silicone catheters [89]. In the same study, CRBSI was four times less likely to originate from antimicrobial-impregnated than from tunneled catheters (0.36 versus 1.43 per 1000 catheter-days), but the antimicrobial-impregnated catheters remained in place for a shorter period (mean 30.2 versus 43.8 days). A meta-analysis of 11,586 patients included in 37 randomized studies involving short-term placement of impregnated non-tunneled catheters showed a consistent reduction in the incidence of CRBSI with antibiotic-impregnated (RR 0.28; 95% CI 0.15–0.54) and heparin-bonded catheters (RR 0.16; 95% CI 0.06–0.43) compared with standard catheters; chlorhexidine–silver sulfadiazine-impregnated catheters failed to show a reduction in CRBSI (RR 0.80; 95% CI 0.62–1.04) [90]. The CDC recommends the use of impregnated catheters only when the implementation of a comprehensive strategy to reduce CABSI and CRBSI, including education of the healthcare personnel who insert and maintains the catheters, and the use of maximal sterile barrier precautions and chlorhexidine–alcohol skin antisepsis during central line placement fails [12].

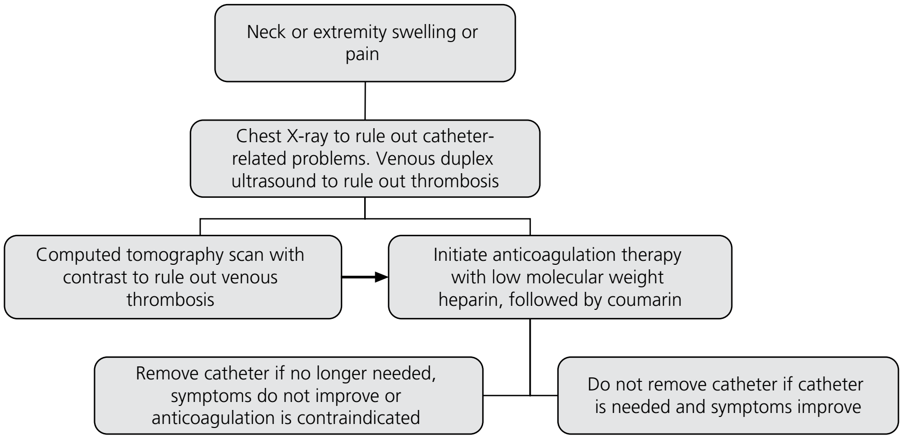
Neck or extremity swelling or pain
Chest X-ray to rule out catheter-related problems. Venous duplex ultrasound to rule out thrombosis
Computed tomography scan with contrast to rule out venous thrombosis
Initiate anticoagulation therapy with low molecular weight heparin, followed by coumarin
Remove catheter if no longer needed, symptoms do not improve or anticoagulation is contraindicated
Do not remove catheter if catheter is needed and symptoms improve

Figure 80.14 Algorithm for venous thrombosis.

The diagnosis of a CABSI and CRBSI is very complex and varies considerably among publications [91]. The CDC defines CABSI as a bacteremia or fungemia with at least one positive peripheral blood culture in a patient with systemic clinical manifestation of infection, without another apparent infection source other than the catheter. To make the diagnosis of CRBSI, the CDC requires systemic clinical manifestations of infection with at least one positive peripheral blood culture and no apparent other source of infection. In addition, one of the following must be present:

a a positive semi quantitative [>15 colony-forming units (CFU) per catheter segment] or quantitative (>10^3CFU per catheter segment) culture, with the same organism (species and antibiogram) isolated from the catheter segment and the peripheral blood sample;

b simultaneous quantitative cultures of blood samples with a ratio of ≥3:1 (catheter versus peripheral);

c a differential period of positive blood culture from the catheter versus peripheral blood of 2 hours.

The advantage of the last two methods is that they do not require removal of the catheter to establish the diagnosis of CRBSI. Simultaneous quantitative cultures have a high specificity but are labor intensive and costly. The use of a time differential for positive blood culture between the catheter and peripheral blood of more that 2 hours has a reported 91% specificity and 94% sensitivity for the diagnosis of CRBSI [92]. The accuracy of this method requires that the same volume of blood sample is obtained from both sites and inoculated into the culture bottle.

The catheter may become colonized by the skin flora at the insertion site, from contamination of the catheter hub during external manipulation, from bacteremia from other site of infection, or from contaminated infusions. Therefore, the incidence of CABSI is related to the method and site of catheter insertion, the type of catheter, the period and degree of immunosuppression, the skin colonization, the frequency of use, the number of lumina, the type of dressing, and patient age (with a higher incidence of infections among younger patients). The incidence of CRBSI among patients with hematologic malignancies varies widely among the published studies (0–21%), depending on the definition of CRBSI used, the duration of the neutropenia, and the intensity of the treatment [46]. In a study of 213 hematology patients with short-term central venous catheters, it was shown that age was an independent risk factor for baseline skin colonization; male gender and baseline skin colonization were independent risk-predicting factors for subsequent skin colonization and positive catheter tip culture at the time of removal [93]. Several randomized studies comparing external tunneled catheters with implantable ports have failed to show differences in the incidence of infections among these devices. In a systematic review of 200 prospective studies, Maki et al. [9] reported that the incidence of catheter-related infections with external cuffed tunneled catheters was 1.6 per 1000 catheter days compared with 0.1 per 1000 catheter days for implanted ports, and 1 per 1000 catheter days for PICCs. Mollee et al. studied 1127 central venous accesses in 727 adult patients with hematologic and non-hematologic malignancies [8], and the incidence of CABSI among patients with aggressive hematologic malignancies was 7.26 per 1000 catheter days for tunneled catheters, 3.89 per 1000 days for implantable ports, and 4.22 per 1000 days for PICC lines; multivariate Cox regression identified the catheter type (higher CASBI for tunneled catheters than ports and PICC), patient diagnosis (higher CASBI for hematologic than non-hematologic malignancies), and the number of previous lines as the independent variables predictors of CASBI. This study established PICC as a reasonable option to tunneled cuffed catheters for venous access in patients with hematologic malignancies. The high incidence of CABSI in patients with hematologic malignancies compared with patients with solid tumors is not surprising, given the degree of immunosuppression, the duration of their neutropenia, and the frequency and number of catheter manipulations.

Most of the infections are secondary to Gram-positive cocci (coagulase-negative *Staphylococcus* 62%, *Staphylococcus aureus* 4%) and Gram-negative bacilli (GNB) (29%) (Enterobacteriaceae and *Pseudomonas aeruginosa*); only 4% of the infections are fungal related (*Candida*) [94]. Sometimes it is difficult to identify the source of GNB bacteremia in cancer patients. In a study of 266 patients, it was determined by multivariate analysis that infections due to *Stenotrophomonas maltophilia*, polymicrobial bacteremia, and >1000 CFU from the central line blood culture were more likely to be CRBSI, whereas in neutropenic patients, GNB bacteremia was most likely not catheter related [95]. Whenever an infection is suspected, appropriate antibiotic coverage should be instituted after the blood cultures are drawn, and antibiotic selection needs to be reassessed after the cultures are back. There are no randomized studies documenting the catheter salvage rates following antibiotic treatment. Several studies report catheter salvage rates of up to 50% following antibiotic treatment, at the expense of higher infection recurrence [96,97]. Indications for catheter removal are the persistence of symptoms or positive blood cultures after 3 days of appropriate antibiotic coverage, hypotension, severe systemic

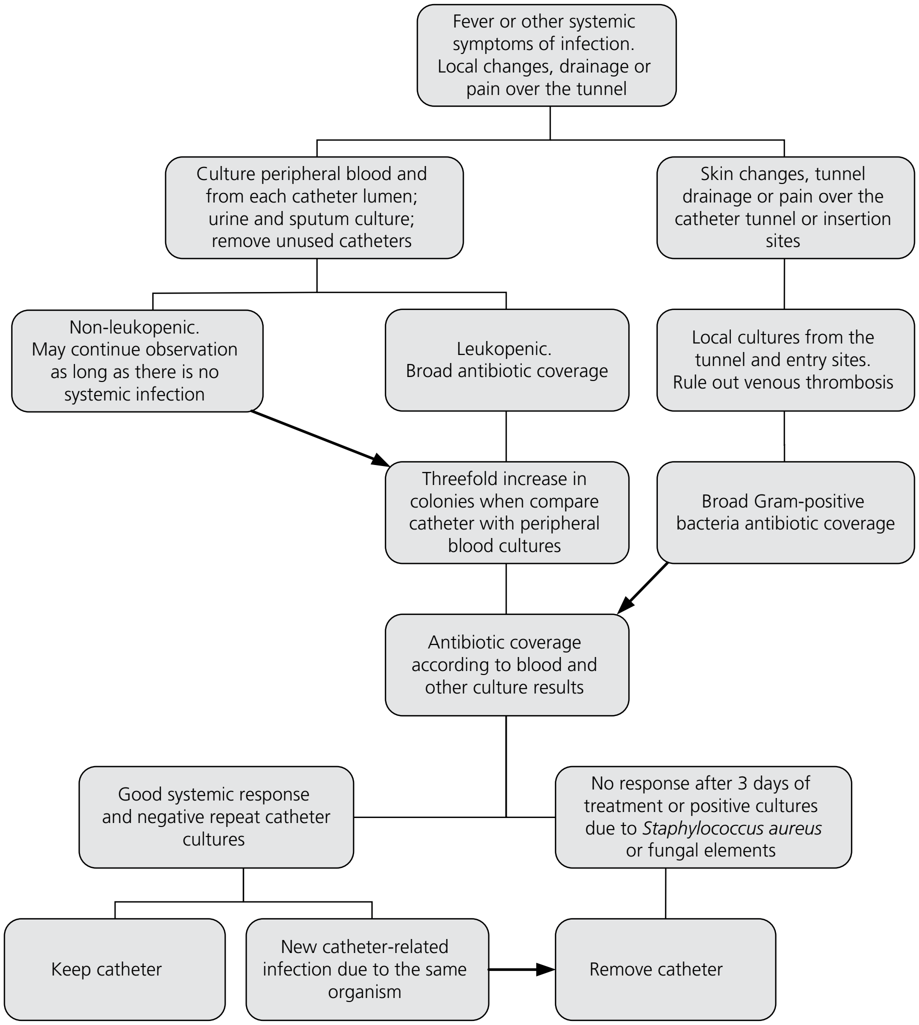
Fever or other systemic symptoms of infection. Local changes, drainage or pain over the tunnel
Culture peripheral blood and from each catheter lumen; urine and sputum culture; remove unused catheters
Skin changes, tunnel drainage or pain over the catheter tunnel or insertion sites
Non-leukopenic. May continue observation as long as there is no systemic infection
Leukopenic. Broad antibiotic coverage
Local cultures from the tunnel and entry sites. Rule out venous thrombosis
Threefold increase in colonies when compare catheter with peripheral blood cultures
Broad Gram-positive bacteria antibiotic coverage
Antibiotic coverage according to blood and other culture results
Good systemic response and negative repeat catheter cultures
No response after 3 days of treatment or positive cultures due to *Staphylococcus aureus* or fungal elements
Keep catheter
New catheter-related infection due to the same organism
Remove catheter

Figure 80.15 Algorithm for catheter-related infection.

compromise, infections secondary to fungi or *S. aureus* (these organisms being rarely eradicated with antibiotic treatment), and recurrence of infections secondary to the same organism after successful treatment. There are limited data on what to do with patients with positive blood cultures from the catheter and negative peripheral blood cultures. Park et al. reported a retrospective review of 112 instances in 103 patients with hematologic malignancies who had only positive Hickman catheter blood cultures; 83% of them had temperatures higher than 38°C [98]. All patients received empiric antibiotic therapy; in those patients for whom the antibiotic therapy was appropriate, only 4% developed a CRBSI and 77% of the Hickman catheters were salvaged. In the patients for whom the empiric therapy did not cover the initial microbial isolate, 19% developed a CRBSI and 74% of the catheters were salvaged. A suggested algorithm for the management of catheter-related infections is outlined in Figure 80.15.

Tunnel or pocket infections are less common. The presence of swelling, tenderness, and warmth over the area is usually an indication of a local infection. In leukopenic patients, pain and fever are frequently the only symptoms. These infections are usually secondary to Gram-positive cocci. Culture of the exit site or the skin around the Huber needle will normally isolate the microorganism. Local care, removal of the Huber needle, and systemic antibiotics are measures needed in the treatment of most of these infections. *S. aureus* and fungal infections usually require removal of the catheter. The use of a second antimicrobial cuff in the external devices failed to reduce the incidence of systemic or local infections [99].

Catheter removal

There are several methods for the removal of a long-term intravenous cuffed catheter. In general, removal of the catheter and the cuff is preferred and recommended by the catheter manufacturer. This can be accomplished by catheter traction when the cuff is close to the insertion site [100]. Anesthetic infiltration around the cuff and gentle freeing of the cuff through the tunnel with a curved hemostat clamp usually help with cuff removal. When the cuff is high in the tunnel, a cutdown technique is usually necessary to remove the cuff [101]. Alternatively, a traction technique can be used, which often results in leaving the cuff behind [102]. The incidence of retained cuff infections is low and requires cuff removal [103,104]. The traction technique for catheter removal can result in fracture of the catheter at the cuff, requiring a cutdown to remove the cuff and the remainder of the catheter. The length of the fractured segment helps to determine the location of the cuff. This complication is more frequent with small-lumen and non-silicone catheters. Compression of the tunnel prevents bleeding or air embolism during the procedure.

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CHAPTER 81

Pharmacologic Prevention of Acute Graft-versus-Host Disease

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Historical development

In 1955, a description of graft-versus-host disease (GVHD) in animals, then known as "secondary disease," was reported by Barnes and Loutit [1]. Named to differentiate it from the primary disease of radiation sickness, fatal secondary disease was observed in irradiated mice given allogeneic spleen cells but not in recipients of syngeneic cells. By the late 1950s, it was apparent that the skin abnormalities and diarrhea of secondary disease and runt disease (a wasting syndrome in unirradiated newborn mice given allogeneic spleen cells) were the result of immunologically competent cells introduced into an immunoincompetent host. The term graft-versus-host was introduced to describe the vector of this immunologic assault [2,3]. Early human transplants of allogeneic marrow were often complicated by GVHD [4–6]. Features were remarkably similar to those seen in animal studies of GVHD [7] and to reports of GVHD developing in immunodeficient children who had received blood transfusions [8]. Because it has been difficult to separate the illness caused by immunologic attack from the consequences of this assault (including immunodeficiency, organ dysfunction and infection), both aspects have been considered a part of human GVHD. Historically, the term *acute* GVHD describes a distinctive syndrome of dermatitis, hepatitis, and enteritis developing within 100 days of allogeneic hematopoietic cell transplantation (HCT). However, with the use of reduced-intensity conditioning (RIC) regimens, the clinical picture of acute GVHD can occur after 100 days, thus making the day 100 cut off less relevant. This distinction is not merely semantics because of the need to define these syndromes clearly in clinical trials.

Pathogenesis

In a remarkably perceptive summary in 1966, Billingham [9] defined the criteria for the development of GVHD as follows:

1. The graft must contain immunologically competent cells.
2. The host must possess important transplantation alloantigens that are lacking in the donor graft, so that the host appears foreign to the graft and is, therefore, capable of stimulating it antigenically.
3. The host itself must be incapable of mounting an effective immunologic reaction against the graft, at least for a sufficient time for the latter to manifest its immunologic capabilities; that is, it must have the security of tenure.

These criteria now require some modification to incorporate current understanding of the biology of GVHD (see Chapters 13 and 14). The occurrence of autologous GVHD suggests that inappropriate recognition of host self-antigens may occur, and transfusion-associated GVHD (T-GVHD) may develop in certain immunocompetent individuals. A multistep model of antigen expression, cytokine production, T-cell activation, and tissue injury has been described and Figure 81.1 depicts this interactive construct [10].

Alloreactivity

Abundant animal data demonstrate that T-lymphocytes contained in the donor inoculum proliferate and differentiate in vivo in response to disparate histocompatibility antigens on host tissues and directly, or through secondary mechanisms, attack recipient cells, thus producing the signs and symptoms of acute GVHD (see Chapter 13) [7,9,11]. The afferent arm of acute GVHD consists of antigen presentation, activation of T cells, clonal proliferation, and differentiation [12]. Cell death of host targets results from alloreactivity of donor-derived T cells. In the efferent phase, release of cytokines from activated lymphocytes contributes to cell death, either directly or through recruitment of secondary effectors, such as natural killer (NK) cells [13]. Based on mechanistic roles postulated for interleukin (IL)-1, IL-2, lipopolysaccharide (LPS), and proinflammatory cytokines such as IL-6, interferon (IFN)-γ, and tumor necrosis factor (TNF), immunomodulatory agents have been administered in vivo to control GVHD. The microbial environment, especially of the gastrointestinal (GI) tract, is tightly associated with this alloreactivity. Compared with conventionally housed irradiated mice, enteric GVHD was significantly reduced in germ-free mice given incompatible marrow [14]. Human studies confirmed this gnotobiotic effect [15,16]. Microorganisms likely act as triggers of GVHD by stimulating Toll-like receptors (TLRs), activating the innate immune system, sharing antigenic epitopes with gut epithelial cells, or by activating latent virus-inducing antigens on cell surfaces to become targets of alloreactivity [17]. Gut damage could also lead to leakage of LPS into the circulation, thereby releasing cytokine effectors of GVHD [18]. The research into the human microbiome is an exciting field, especially with recent data from murine and human transplantation demonstrating significant changes in specific bowel flora when measured by ribosomal 16S RNA sequencing [19]. These data suggest the potential for manipulating the bowel flora as one mechanism for reducing intestinal inflammation and GVHD.

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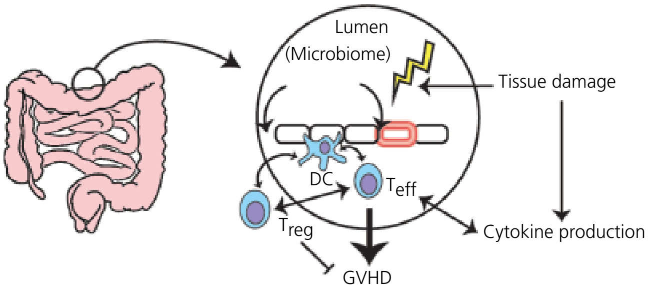


Figure 81.1 Interactive events leading to graft-versus-host disease (GVHD). T-cell activation and tissue injury release cytokines that produce the clinical manifestations of GVHD. IDC, dendritic cell; Teff, T effector; Treg, regulatory T cell.

Predictive factors

Some of the predictive factors have been utilized as targets for agents in the prevention of GVHD. HLA disparity between the hematopoietic cell donor and recipient is the most powerful factor governing the severity and kinetics of GVHD. The importance of class I and II allele matching in reducing the risk for GVHD has been widely demonstrated in unrelated donor HCT cohorts [20]. In a National Marrow Donor Program (NMDP) analysis of individuals undergoing HCT from HLA-A and -B-matched unrelated donors, HLA-DRB1 allele disparity was independently associated with an increased risk of severe acute GVHD [21]. Further studies have determined the effects of HLA matching (low or high resolution or both) on engraftment, GVHD, and mortality in 1874 donor–recipient pairs retrospectively typed at high resolution for HLA-A, -B, -C, -DRB1, -DQ, and -DP. Mismatches at HLA-A, -B, -C, and -DRB1 each had similar adverse effects on mortality. These adverse effects on outcome were more evident in transplants with low-resolution versus only high-resolution mismatches. Mismatches for HLA-DQ or -DP did not significantly affect outcome. When high-resolution mismatches at HLA-A, -B, -C, and -DRB1 were considered together, adverse effects on survival and GVHD were observed [22]. Minor HLA peptides have been identified and sequenced, and minor histocompatibility antigen (mHA) disparities appear correlated with the risk for GVHD in adults given HLA-identical donor grafts [23,24]. The importance of mHA matching is further supported by the observed low rate of GVHD in populations that feature genetic homogeneity [25]. Sex mismatching and donor parity have been associated with an increased risk of acute GVHD [26]. Female donor T cells could recognize H-Y minor antigens on host Y chromosome-containing cells. Moreover, previously parous donors could have experienced alloimmunization as a result of unshared minor antigens of the fetus [27].

Age is another key factor, with younger patients experiencing less acute GVHD [26,28]. Moreover, using older matched unrelated donors has led to a higher incidence of GVHD in the recipients. The source of allogeneic hematopoietic cells may also influence the development of GVHD. For more than two decades, experience was derived from marrow transplant cohorts describing rates and risk factors for GVHD [6,26]. Virtually all marrow grafts were infused as fresh products, although one group reported an apparent reduction in rates of GVHD with cryopreserved marrow [29].

Of considerable interest to stem-cell biologists, umbilical cord blood cells appear less immunologically reactive than bone marrow cells [30]. When utilized in HCT, there is a lower rate of GVHD, especially since many are donor–recipient pairs are not HLA matched [31,32]. Allogeneic peripheral blood progenitor cells (PBPCs) have become a more common source for HCT and associated with an increased risk for chronic GVHD appears [33]. Faster engraftment, fewer hospitalizations, less donor morbidity, and economic advantages are noted with the use of peripheral blood. A meta-analysis of 16 studies (five randomized and 11 cohort) found that the relative risk (RR) for acute GVHD after PBPC transplantation was 1.16-fold greater ($p = 0.006$) compared with bone marrow transplantation (BMT) [34]. In a cohort study of PBPC compared with BMT from unrelated donors, GVHD rates were similar but non-relapse mortality (NRM) was lower and survival was higher after PBPC transplantation (see Chapter 41) [35]. A prospective randomized study conducted by the Bone Marrow Transplant Clinical Trials Network (BMT CTN) demonstrated an increase in chronic GVHD with the use of PBPC with no survival advantage [36]. One interpretation of these data suggests that patients with a lower risk of relapse would benefit from marrow rather than PBPC since there would be less chronic GVHD.

Studies of CD34$^+$ cell dose have shown advantages in resource utilization and cost in autologous HCT. In allogeneic PBPC transplantation, a higher hematopoietic cell dose ($6.3–10 \times 10^6$ CD34$^+$ cells/kg) was associated with a sharp increase in GVHD risk among cyclosporine (CSP) recipients. In contrast, CD34$^+$ cell dose did not appear to influence the occurrence or severity of acute or chronic GVHD in allogeneic BMT [37]. Of interest, a low PBPC dose ($<3 \times 10^6$ CD34$^+$ cells/kg) was associated with slower hematologic recovery, increased rates of fungal infections and NRM, and decreased overall survival.

As shown in Figure 81.1, activated donor T cells release proinflammatory cytokines which upregulate HLA expression (see Chapter 13). One report found that the genotype in the donor of IL-1 receptor antagonist (allele 2) was protective against severe GVHD developing in the recipient [38]. Single nucleotide polymorphisms were determined in sibling transplants and recipient genotypes for IFN-γ intron 1, IL-10^{-1064} and TNF d3 were found to be associated with acute GVHD, whereas IL-6^{174} genotype was associated with chronic GVHD [39]. In a similar study of genetic and clinical factors, polymorphisms of IL-10 in both donor (RR 3.5) and recipient (RR 7.9) predicted for acute GVHD along with donor cytomegalovirus (CMV) seropositivity and mHA mismatching. More recently, polymorphisms in the innate immunity gene (*NOD2/Card15*) have also been associated with chronic GVHD. A recent genome-wide scan of 40 previously reported candidate single-nucleotide polymorphisms (SNPs) demonstrated only a few associations with GVHD [40]. Use of imputed genotypes to replicate previous IL-10 associations was validated, as was the IL-6 donor genotype. The donor genotype was associated with a 60% increase in risk for grade III–IV acute GVHD after related HCT (adjusted $p = 0.028$).

The incidence of acute GVHD after matched sibling HCT varies inversely with the efficacy of post-transplant immunosuppressive prophylaxis and with the intensity of the preparatory regimen. Multivariate analysis revealed that an increased dose of pre-transplant total body irradiation (TBI) was associated with an increased risk of GVHD. Among HLA-identical HCT recipients who received GVHD prophylaxis with methotrexate (MTX) and CSP, acute GVHD developed in 48% of patients given 1575 cGy TBI, compared with 21% of patients given 1200 cGy TBI ($p = 0.02$) [41]. One hypothesis is that more intensive myeloablative conditioning might eliminate persisting host cells and restrict the development of mixed donor–host chimerism, which has been associated with a decreased probability of GVHD [42]. Alternatively, more intensive conditioning could increase GI damage, modify the microbial environment, release cytokine mediators, and/or attenuate the dose of administered GVHD prophylaxis. Some studies have indicated that donor or host infection (as denoted by seropositivity for the herpesviruses) predicts the subsequent development of GVHD [43], whereas others found no predictive association [44].

Prophylaxis of GVHD had consisted of the most commonly utilized immune suppressive drugs. These drugs were initially used as single agents and then in combination. These drugs are discussed individually below; however, the efficacy for prophylaxis of GVHD relies on combination of these agents. Table 81.1 summarizes the available randomized studies using these drugs.

It is important to point out that the diagnosis of GVHD is a clinical one with the pathology helping sometimes in confirming the diagnosis. Therefore, the overall grading based on clinical findings is a potential methodologic problem in GVHD prevention trials. There can be significant inter-observer differences at a single institution. Moreover, there are significant differences between institutions as to what is determined to be, for example, grade II acute GVHD. Efforts to find biomarkers for GVHD are ongoing and would help considerably in future trials. For example, the elevation of REG3α seems to be associated with lower GI and liver acute GVHD [45]. The drugs utilized for the prevention of GVHD are discussed individually below and are broadly outlined in Figure 81.2.

Non-specific immunosuppressive drugs

Corticosteroids

This class of drug has been combined with other immunosuppressants in prophylaxis against GVHD, but is most frequently used as front-line therapy in the treatment of established GVHD.

Pharmacology

The most commonly utilized corticosteroid is methylprednisolone (MP), which differs from prednisolone and prednisone only by the addition of a 6α-methyl group. The 6α-methyl group blocks the specific binding of this corticosteroid to transcortin, the plasma protein that carries steroids. Instead, MP is primarily bound to albumin. The frequent side effects from MP may be dependent on the albumin level in the host. The lack of transcortin binding leads to a higher partition coefficient and the result is a significantly greater penetration into bronchial alveolar fluids, which may provide a therapeutic advantage for the treatment of pulmonary inflammatory states.

Signaling by the steroid molecules is achieved through binding to a specific cytosolic receptor, thereby initiating its pharmacologic effects (Figure 81.3). This binding of steroid to receptor activates the complex, enabling it to translocate rapidly into the nucleus. There it is associated with specific sequences known as the glucocorticoid response elements (GREs). These response elements regulate the mRNA expression of certain proteins, leading to the known biologic effects. Generally, larger doses produce greater intensity and duration of effects. The utility of corticosteroids in GVHD is thought to be related to its lympholytic activity. The presumed activity is that corticosteroids destroy many but not all lymphoid cells. Hence other mechanisms, such as down-modulation of cytokines, are also likely to be responsible [46]. There are data to suggest that the major effect of corticosteroids is the suppression of proinflammatory cytokines rather than direct cellular cytotoxicity of lymphocytes [47]. In clinical trials that measured cytokines, the levels of TNF-α decreased after the initiation of corticosteroids used for the therapy of GVHD [48].

Toxicity

There are many adverse effects associated with corticosteroids. In addition to their immunosuppressive effects, which set the stage for infectious complications, most of the adverse effects are related to Cushingoid features, hyperglycemia, fluid retention, hypertension, occasional psychosis or neurotoxicity, and myopathy. Other chronic effects such as osteoporosis, cataract formation, and aseptic necrosis also occur. Corticosteroids are sensitive to a variety of drug interactions, specifically with anticonvulsants, which may increase the total clearance of unbound corticosteroids.

Clinical use

The drug of choice has been MP followed by oral prednisone when patients are able to eat sufficiently or when a patient's diarrhea has resolved so that one can be confident that they will absorb the medication without difficulty. One regimen, which combines CSP, MTX, and prednisone (beginning after all MTX doses have been administered), is among the most potent combinations for the prevention of acute GVHD, resulting in only 9% grade II–IV acute GVHD in the best transplant candidates (patients in first remission of acute leukemia or first chronic phase of chronic myeloid leukemia) for allogeneic BMT [49]. The doses of MP given for prophylaxis have varied and most GVHD prevention regimens do not include corticosteroids. In support of the delayed use of corticosteroids are the results noted in a prospective randomized study comparing CSP, MTX, and MP versus CSP and MTX in patients receiving BMT from an HLA-identical sibling [50]. MTX was given in four doses (on days 1, 3, 6, and 11), and MP was not begun until day 14. Acute GVHD was more common in the patients treated without MP. This study also noted no difference in relapse or in chronic GVHD. However, a second randomized study found no difference between these two regimens in preventing either acute or chronic GVHD [51]. Based on these data, our current recommendation is to save the use of corticosteroids for the treatment of acute GVHD.

Most prophylactic regimens taper the steroid dose to zero by +180 days. When steroids have been used for the treatment of acute GVHD, the usual methylprednisolone dose is 1–2 mg/kg administered in divided doses with a tapering schedule after about 2 weeks of 10% every 3–5 days. Occasionally higher pulse doses of up to 10 mg/kg have been used. Chronic GVHD treatment dose is started in the region of 1 mg/kg on a daily basis. The goal thereafter is to switch patients to an alternating day regimen to spare the side effects of corticosteroids [52]. For older patients, a small dose of prednisone, similar to those used in autoimmune diseases, may be necessary to suppress reactivation of GVHD.

Corticosteroids have also been used as local treatment for GVHD using oral beclomethasone 17,21-dipropionate (BDP), a synthetic diester of beclomethasone, a corticosteroid analog that has appeal in the treatment of GI GVHD by virtue of its ability to direct therapy to inflamed GI mucosa [53]. Patients were randomized to receive prednisone for 10 days plus either BDP (8 mg/day) or placebo for 50 days. The results of this study demonstrated that oral BDP was more effective than placebo in restoring oral intake in patients with grade II GVHD with GI symptoms, and allows prednisone to be rapidly tapered without a flare of intestinal symptoms. Importantly, there was a survival advantage for those on BDP that lasted past the first year of randomization. Unfortunately, a second confirmatory randomized study did not replicate the initial data.

Table 81.1 Randomized trials of single-agent and combination immunomodulation for prevention of acute GVHD

Study	Disease groups	Patients (*n*)	Regimens compared	Median age (years)	Acute GVHD (%)	Chronic GVHD (%)	Overall survival (%)
HLA-identical sibling donors							
Ramsay et al. (1982) [120]	Non-malignant and malignant diseases	32	MTX + ATG + PSE	16	21	6	52
			vs.		(p = 0.01)	(n.s.)	(n.s.)
		35	MTX	16	48	9	44
Storb et al. (1983) [15]	Severe aplastic anemia	22	MTX + CSP	23	18	58	73
			vs.		(p = 0.01)	(n.s.)	(n.s.)
		24	MTX	23	53	36	58
Storb et al. (1986, 1989) [57,121]	AML in CR1 and CML in CP	43	MTX + CSP	30	33	26	65
			vs.		(p = 0.01)	(n.s.)	(n.s.)
		50	CSP	30	54	24	54
Forman et al. (1987) [122]	Acute leukemia and CML	53	MTX + PSE	26	47	?	53
			vs.		(p = 0.05)	?	(n.s.)
		54	CSP + PSE	26	28	?	57
Santos et al. (1987) [65]	Non-malignant and malignant diseases	42	CSP + MP	23	32	40	38
			vs.		(p = 0.05)	(n.s.)	(p = 0.03)
		40	CY + MP	24	68	18	20
Sullivan et al. (1989) [28]	Advanced-stage malignant diseases	25	Long MTX + BC	19	82	44	24
			vs.		(p = 0.016)	(n.s.)	(n.s.)
		40	Short MTX	18	59	51	30
			vs.		(p = 0.0016)	(n.s.)	(n.s.)
		44	Long MTX	19	25	33	41
Storb et al. (1990) [123]	Non-malignant and malignant diseases	59	MTX + CSP + PSE	32	46	62	52
			vs.		(p = 0.02)	(p = 0.01)	(n.s.)
		63	MTX + CP	28	25	40	46
Chao et al. (1993) [49]	Malignant diseases	74	CSP + PSE	32	23	60	59
			vs.		(p = 0.02)	(n.s.)	(n.s.)
		75	MTX + CSP + PSE	28	9	57	46
Deeg et al. (1997) [124]	Hematologic malignant diseases	60	CSP	36	73	21	26
			vs.		(p = 0.01)	(n.s.)	(n.s.)
		62	CSP + MP	39	60	44	23
Ratanatharathorn et al. (1998) [71]	Hematologic malignant diseases	164	MTX + CSP	40	44	49	57
			vs.		(p = 0.01)	(n.s.)	(p = 0.02)
		165	MTX + TACR	40	32	56	47
Chao et al. (2000) [51]	Hematologic malignant diseases	96	MTX + CSP	34	20	52	51
			vs.		(n.s.)	(n.s.)	(n.s.)
		90	MTX + CSP + PSE	34	18	46	60
Ruutu et al. (2000) [125]	Hematologic malignant diseases Severe aplastic anemia	55	MTX + CSP	41	56	48	72
			vs.		(p = 0.001)	(p = 0.06)	(n.s.)
		53	MTX + CSP + PSE	42	19	36	65
Locatelli et al. (2000) [126]	Severe aplastic anemia	37	MTX + CSP	20	30	44	94
			vs.		(n.s.)	(n.s.)	(p = 0.05)
		34	CSP	18	38	30	78
Bolwell et al. (2004) [127]	Hematologic malignant diseases	19	CSP + MTX	46	37	64	68
			vs.		(n.s.)	(n.s.)	(n.s.)
		21	CSP + MMF	49	48	63	52
Blazar et al. (2006) [128]	Hematologic malignant diseases	31	Placebo	46	40	NA	84
			vs.		(n.s.)		(n.s.)
		69	palifermin	46	38	NA	82
HLA-non-identical donors							
Nash et al. (2000) [76]	Non-malignant and malignant diseases	90	MTX + CSP	35	74	70	50
			vs.		(p = 0.001)	(n.s.)	(n.s.)
		90	MTX + TACR	34	56	76	54
Bacigalupo et al. (2001) [101]	Hematologic malignant diseases	25	No ATG	29	36	65	52
			vs.		(n.s.)	(n.s.)	(n.s.)
		29	ATG 7.5 mg/kg	28	41	38	55
		28	No ATG	28	50	59	46
			vs.		(p = 0.001)	(n.s.)	(n.s.)
		27	ATG 15 mg/kg	32	11	41	41
HLA-identical and -non-identical donors							
Sullivan et al. (1990) [129]	Non-malignant and malignant diseases	191	Control	NA	51	NA	35
			vs.		(p = 0.0051)		(n.s.)
		191	IVIg	NA	34 (if ≥20 yrs)	NA	33
Feinstein et al. (1999) [130]	Non-malignant and malignant diseases	121	Control	NA	73	70	50
			vs.		(n.s.)	(n.s.)	(n.s.)
		120	Short IVIg	NA	65	76	42
Antin et al. (2002) [131]	Hematologic malignant diseases	3	Placebo	3	33	NA	66
			vs.		(n.s.)	(n.s.)	(n.s.)
		10	rIL-11	11	25	NA	44
Antin et al. (2002) [132]	Hematologic malignant diseases	90	Placebo	44	59	NA	45
			vs.		(n.s.)	(n.s.)	(n.s.)
		91	IL-1RA	41	61	NA	35
Ruutu et al. (2002) [133]	Hematologic malignant diseases	119	Control	40	10	NA	55
			vs.		(p = 0.006)	(n.s.)	(p = 0.01)
		123	ursodeoxycholic acid	38	2 (grade 3–4)	NA	71

AML, acute myeloid leukemia; ATG, antithymocyte globulin; BC, donor buffy coat cells; CML, chronic myeloid leukemia; CP, chronic phase; CR, complete remission; CSP, cyclosporine; CY, cyclophosphamide; GVHD, graft-versus-host disease; MP, methylprednisolone; MTX, methotrexate; PSE, prednisone, TACR, tacrolimus; n.s., not significant.

Intracellular	Antimetabolites	purine synthesis inhibitors: Mycophenolate mofetil		
	Macrolides	Calcineurin inhibitors: Cyclosporine Tacrolimus		
	mTOR inhibitor	Sirolimus		
Extracellular	Antibodies	Monoclonal	Serum Target (non cellular)	Infliximab
			Cellular target	CD3 (OKT3) CTLA-4 (Ipillimunab) Interleukin-6 receptor (Tocilizumab) IL-2 receptor/CD24 (Basiliximab/ Daclizumab)
		Polyclonal	Anti-thymocyte globulin (horse or rabbit)	
	-cept (Fusion)	CTLA-4 TNF	Abalacept Belatacept TNF inhibitor (Etanercept)	

Figure 81.2 Immunosuppressive drugs for GVHD.

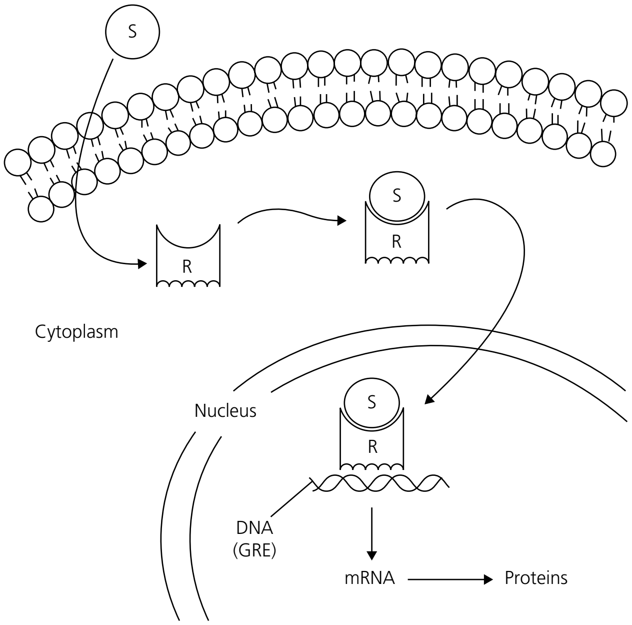


Figure 81.3 Diagram of the mechanisms of action of steroids and the intracellular receptor. The complex of the drug and receptor translocates efficiently into the nucleus where it interacts with glucocorticoid response elements (GREs), leading to the generation of messenger RNA and protein synthesis.

Methotrexate (MTX)

MTX is an analog of aminopterin, the folic acid antagonist introduced in 1948 for the treatment of acute leukemia**.** The precise mechanism by which MTX prevents GVHD is not understood; however, it is likely related to MTX's ability to inhibit cellular growth and division. Therefore, antigen-activated T cells that are rapidly proliferating would be particularly sensitive to this antimetabolite. MTX can induce tolerance following marrow transplantation. In canine transplantation, MTX given after the graft was effective in controlling GVHD and in inducing tolerance when donor and recipient were matched for the dog leukocyte antigen system [54].

Pharmacology

At the cellular level, MTX exerts its cytotoxic effect by inhibiting dihydrofolate reductase (DHFR). DHFR is the intracellular enzyme responsible for converting folic acid to reduced folate cofactors. The reduced state of the folates, namely the tetrahydrofolates, are responsible for the transport of single carbon groups that are required for purine and thymidylate synthesis. Once the single carbon group has been delivered, the oxidized folates must be converted back to tetrahydrofolates by DHFR. MTX binds to DHFR, thereby preventing its ability to reduce the oxidized folates to tetrahydrofolate and blocking further purine or thymidylate synthesis.

Following the administration of MTX, approximately 50% is bound to plasma proteins, especially albumin, with the highest tissue-plasma equilibrium reported in the kidney, liver, GI tract, and muscle. The GI tract is an important site of distribution and metabolism of MTX, perhaps accounting for one of the primary sites of toxicity. One concern in using MTX is its clearance in those patients who have pleural or ascitic fluid since these reservoir spaces can have a substantial impact on the disposition of MTX. These spaces can be a reservoir from which MTX can be continuously released, leading to severe toxicity. At the low intravenous (IV) doses, MTX clearance correlates with glomerular filtration, and renal excretion of unmetabolized drug is the major route for elimination.

Polyglutamate formation is also important to MTX metabolism [55]. The formation of MTX polyglutamates intracellularly may account for its retention within the cell and its cytotoxic effects. The enzyme folyl polyglutamate synthetase adds glutamate residues to folates or antifolates such as MTX. The polyglutamate derivatives of MTX appear to be as toxic to DHFR as the native compound. If the retained MTX polyglutamates are produced differentially in lymphocytes compared with other normal cells, the effective concentrations of the drug would differ. For example, these differences in intracellular concentrations may account for selective toxicities and may selectively inhibit certain subpopulations of cells [56].

Toxicity

The most common adverse effects of MTX when used for GVHD prophylaxis are hematopoietic, renal, hepatic, and GI mucosal toxicity. Patients may have an elevation of creatinine and bilirubin levels following the administration of MTX. As many of these are also the side effects of CSP or tacrolimus, dose attenuation of MTX and CSP or tacrolimus may be necessary. Full target doses may not be achievable in patients with grade III–IV mucositis resulting from the preparatory regimen because of airway obstruction and severe oropharyngeal bleeding.

Clinical use

The use of MTX following allogeneic BMT has been exclusively with the IV formulation. The drug doses administered have been relatively small, given the inability to dose escalate MTX significantly (Table 81.2). Initial studies in Seattle tested MTX as a single agent on a weekly basis up to day +100 but, because of the toxicity of weekly administration of MTX, the dose was changed to four doses and combined with CSP [57]. MTX, when used with CSP or tacrolimus, generally is injected IV on days 1, 3, 6, and 11 following BMT. This schema is currently the most commonly utilized prophylaxis regimen. Another schema that is commonly used following unrelated stem-cell transplantation has been named “mini-dose MTX,” usually used in conjunction with tacrolimus [58]. In this regimen, MTX is given on days 1, 3, 6, and 11 at 5 mg/m^2. The overall prevention of GVHD was similar to that of standard dose MTX with lower systemic toxicities. In general, patients should be very well hydrated, have their urine alkalinized with sodium bicarbonate until the urine pH is 8 or above. Moreover, dose attenuation should be considered for elevations of creatinine, bilirubin, and grade III–IV mucositis. One suggested schema for dose attenuation of MTX is shown in Table 81.3. There has been one report of leucovorin rescue after MTX [59]. In this small series, the use of leucovorin was not associated with an adverse outcome, although this practice is not commonly used. Leucovorin circumvents the blocked tetrahydrofolate synthesis by supplying reduced folates directly, therefore bypassing the blocked DHFR.

Specific T-cell immunosuppressive drugs

Cyclosporine (CSP)

CSP, also known as cyclosporin A, has led to a considerable expansion of the field of allogeneic transplantation given its potent immunosuppressive activity. CSP is a cyclic peptide, first extracted in 1969 from two strains of fungi isolated from soil samples. CSP was tested as an antifungal agent and demonstrated only limited effects, but it was highly effective as an immunosuppressive agent. These studies led to it being administered to patients in 1978 [60].

Pharmacology

CSP is a neutral hydrophobic cyclic peptide composed of 11 amino acids (Figure 81.4). Over the past decade, much work has been devoted to understanding the mechanism of CSP’s activity. Moreover, CSP, as well as tacrolimus, has been used as a chemical probe to dissect the pathways of T-cell activation. Although CSP and tacrolimus are distinct in their molecular structures (Figure 81.4), their mechanisms of action will be discussed together since they are almost indistinguishable in their cellular functions. What is known thus far is that CSP, and tacrolimus, block the calcium-dependent signal transduction pathways distal to engagement of the T-cell receptor (TCR) (Figure 81.5). This block interrupts the activation of T cells since the signaling from the TCR is prevented. In dissecting the signaling pathway, several critical intracellular molecules have been identified and include calmodulin, calcineurin, and the nuclear factor of activated T cells (NF-AT). The activation cascade includes calcium binding to calmodulin, which leads to binding calcineurin. The activated calcineurin may dephosphorylate the cytoplasmic unit of NF-AT, which allows translocation of the NF-AT from the cytoplasm into the nucleus to form a competent transcriptional activator of the *IL-2* gene [61].

Table 81.2 Outline of some regimens used for graft-vs.-host disease (GVHD) prophylaxis

CSP			MTX[a]		
Day	**Dose (mg/kg)**	**Route**	**Day**	**Dose (mg/m^2)**	**Route**
−2 to +3	5	IV QD over 20 h	+1	15	IV
+4 to +14	3	IV QD over 20 h	+3	10	IV
+15 to +35	3.75	IV QD over 20 h	+6	10	IV
+36 to +83	5	PO BID	+11	10	IV
+84 to +97	4	PO BID			
+98 to +119	3	PO BID			
+120 to +180	2	PO BID			
+181	Off				

Tacrolimus/MTX[b]		
Day	**Dose**	**Route**
−2 to +30 (or until good oral intake) (maintain level of 10–30 ng/mL)	0.03 mg/kg	IV QD over 24 h
+30 to +120 (maintain level of 10–30 ng/mL)	Convert IV to PO at a ratio of 1:4	PO BID
+120 to +180 (maintain level of around 10 ng/mL)		
+181	Off	

Tacrolimus			Sirolimus		
Day	**Dose**	**Route**	**Day**	**Dose**	**Route**
−2 to +30 (or until good oral intake) (maintain level of 10–30 ng/mL)	0.02 mg/kg	IV QD over 24 h	−3 −2 to + 100 (maintain level of 3–12 ng/mL)	12 mg 4 mg	PO PO
+30 to +120 (maintain level of 10–30 ng/mL)	Convert IV to PO at a ratio of 1:4	PO BID	+101 to +180	Taper to off by +180	
+120 to +180 (maintain level of around 10 ng/mL)					
+181	Off				

BID, twice daily; QD, four times daily; IV, intravenous; PO, oral.

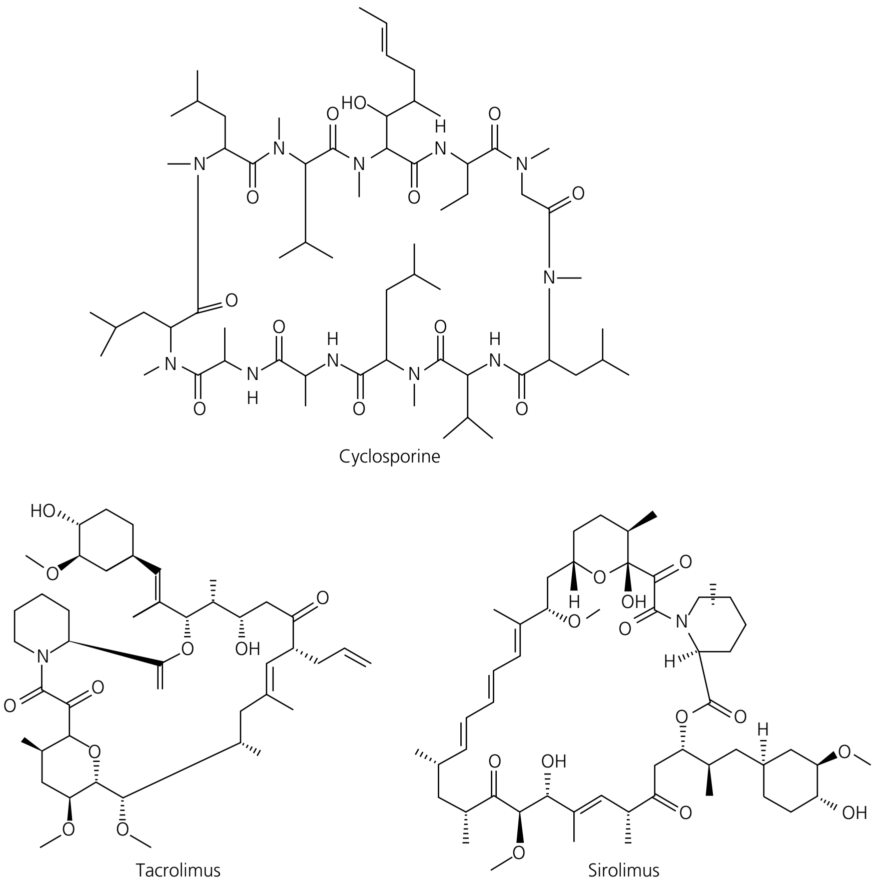
[a] If a “mini” dose MTX is used, the doses are 10 mg/m^2 day +1 and 5 mg/m^2 days +3, 6.

[b] MTX dosing is similar to the regimen with CSP above.

Table 81.3 Suggested dose adjustments for methotrexate (MTX)

	Dose (%)
Bilirubin(mg/dL)	
<2.0	100
2.1–3.0	50
3.1–5.0	25
>5.0	Hold dose
Creatinine (mg/dL)	
<1.5	100
1.5–1.7	75
1.8–2.0	50
>2.0	Hold dose

The cytosolic binding protein for CSP was isolated in 1984 from lymphocytes and named cyclophilin for its high affinity for CSP [61]. The analogous major isoform of the binding protein



Cyclosporine

Tacrolimus

Sirolimus

Figure 81.4 Structures of cyclosporine (CSP), tacrolimus, and sirolimus.

for tacrolimus has been termed tacrolimus binding protein (FKBP). Both cyclophilin and FKBP are highly basic and very abundant in the cytoplasm. Although these two proteins do not have sequence homology, they both function as the enzyme prolyl *cis–trans*-isomerase (or rotamase) [62]. Rotamase accelerates the conversion of *cis* and *trans* rotamers of proline-containing peptides or proteins. This conversion step is thought to be the rate-limiting step during normal protein folding. Both of these molecules are potent inhibitors of rotamase activity. The binding of CSP or tacrolimus to these rotamases suggests that inhibition of this enzyme may be an important step leading to immunosuppression. The mechanism of action of CSP is exerted through binding to cyclophilin (as tacrolimus is bound to FKBP). This complex of drug and binding protein interrupts the activation cascade at the level of NF-AT dephosphorylation, and therefore NF-AT is not able to enter the nuclear domain and activate the IL-2 gene (Figure 81.5).

CSP is a highly lipophilic drug that is extensively metabolized with subsequent biliary and, to a lesser extent, urinary secretion. Over 15 metabolites have been isolated and identified. Some metabolites also have immunosuppressive activity while others are nephrotoxic. Thus, the clinical efficacy and interactions between CSP and the other drugs may be confounded by its metabolites. However, the most commonly reported inhibition or induction of CSP metabolism is by cytochrome p450 enzymes, specifically HLp and PCN1 enzymes that are members of the cytochrome P450 IIIA gene family [63].

Toxicity

The major clinical toxicities that need to be monitored with CSP administration are renal insufficiency and elevation of bilirubin levels. Both of these complications appear to be dose related. The acute nephrotoxicity is due to vasoconstriction and ischemia of the afferent arterioles in the kidney. These acute changes are reversible but if present for too long can lead to an irreversible interstitial injury with glomerular thrombosis resulting in permanent azotemia. A major difficulty clinically is to differentiate specific side effects of CSP from those of other drugs such as amphotericin, MTX, and aminoglycoside or from an underlying disease process such as GVHD. Other side effects that are commonly encountered are hypertension, hyperglycemia, headaches, and hirsutism. Rarer events include gum hypertrophy, brittle nails, acne, nausea, and vomiting. One specific clinical concern is the apparent association of hypomagnesemia and seizures in patients on CSP. Many patients who have a seizure while on CSP may also have concurrent hypomagnesemia and hypertension. Correction of these two abnormal findings is important in the management of patients. Hypertension alone can occasionally become a significant problem. Usually nifedipine is the drug of choice since it is an effective antihypertensive, and it has not been associated with changes in the CSP levels. A rare but potentially reversible toxicity associated with CSP is posterior reversible encephalopathy syndrome (PRES) [64]. The clinical syndrome is characterized by headaches, confusion or decreased consciousness, visual complaints, and seizures. There are commonly associated findings of posterior cerebral white matter

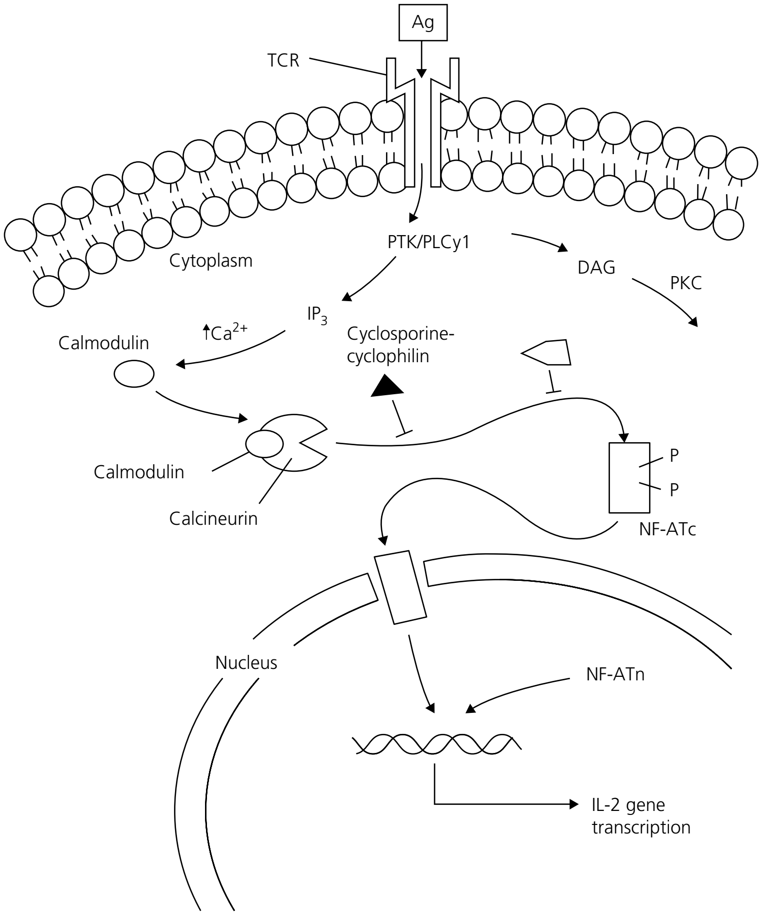


Figure 81.5 Simplified diagram of the signal transduction found in the activation of a T cell through the T-cell receptor (TCR) and the point where cyclosporine (CSP) and tacrolimus interact with its binding proteins to block the generation of nuclear factor of activated T cells (NF-AT) and its subsequent generation of interleukin 2 (IL-2). Sirolimus, although similar to tacrolimus in structure, does not inhibit the signaling through the TCR but rather inhibits signaling through the CD28/B7 family of molecules and prevents the egress of cells from the G_1 phase. DAG, diacylglycerol; IP3, inositol triphosphate; PKC, protein kinase C; PLC, phospholipase C; PTK, protein tyrosine kinase.

vasogenic edema in studies such as magnetic resonance imaging (MRI). Prompt recognition is important, so that the findings can be reversed. CSP should be stopped and treatment of the hypertension and seizures (if present) instituted. A different immunosuppressant should be used in lieu of restarting CSP. Another rare but important toxicity of CSP (and tacrolimus) is the induction of a hemolytic uremic syndrome (HUS)/thrombotic thrombocytopenic purpura (TTP) syndrome. Patients present with schistocytes and elevated creatinine with or without mental status changes. In some circumstances, this toxicity will abate if one calcineurin inhibitor is substituted for the other.

Clinical use

CSP can be administered IV or orally. It is initially administered IV since in the early phase following transplantation many patients develop mucositis and GI damage from the preparatory regimen and also from MTX given for GVHD prophylaxis. Once the patient is eating and drinking without significant difficulties CSP may be switched to an oral preparation. CSP is available as an olive oil solution and as soft gelatin capsules in addition to the microencapsulated form. CSP is erratically and incompletely absorbed after oral administration, with many factors reported to influence oral CSP bioavailability. For example, food intake may impact its absorption, especially if the meal is high in fat content. The absorption kinetics of CSP have been described as either of zero order or a series of first-order processes. CSP dosage usually is governed by research protocols, beginning the drug at a higher dose as a "loading dose," with a gradual taper over time. However, there are several variations of dosing schedules. An example of a CSP regimen in given in Table 81.2.

There have been several studies attempting to correlate the efficacy of prophylactic CSP with its level in either whole blood, plasma, or serum. Although the specific analytic methods are clearly more precise and preferable to the non-specific methods for therapeutic drug monitoring, the technical difficulties with current high-performance liquid chromatographic methods make it less practical to utilize it widely. Moreover, there are limited data to suggest that there is a correlation between CSP levels and clinical outcome. The results from these studies suggest that there may be a complex relationship between dose, blood level, and occurrence of GVHD [65,66]. These studies failed to demonstrate a clear direct correlation between drug level and occurrence of GVHD.

From a practical standpoint, CSP is administered to achieve a "therapeutic level" but, beyond that, it is important to deliver the planned dose per protocol. It is also important to be certain that the level drawn is a trough level in the steady state, otherwise the values will not be interpretable. CSP levels are used to increase the dose if it is below the therapeutic threshold. In general practice, the dose is not decreased for elevated levels if no toxicity is present, but if the level is above twice the upper limit of the therapeutic range, then it is reasonable to decrease the dose to avoid potential neurotoxicity (i.e. seizures). There are also concerns about the possible interactions between CSP and other medications. Many different drugs have been implicated in increasing or decreasing CSP levels in patients. Such medications and their effects are listed in Table 81.4 [67,68].

Table 81.4 Cyclosporine (CSP) drug interactions

Decrease CSP levels	Increase CSP levels[a]
Phenytoin	Erythromycin
Phenobarbital	Ketoconazole
Carbamazepine	Itraconazole
Primidone	Fluconazole
Trimethoprim	Voriconazole
Rifampicin	Diltiazem
Nafcillin	Verapamil
Octreotide	Nicardipine
Sulfonamides	Acetozolamide
	Alcohol
	Colchicine
	Fluoroquinolones
	Imipenim

[a] Not all macrolides are identical in effects because some do not form stable complexes with cytochrome P450 enzymes.

Tacrolimus (FK506)

Tacrolimus is a macrolide antibiotic extracted from the soil fungus *Streptomyces tsukubaensis* [69]. It is completely different from the cyclic peptide CSP, but it does exhibit a very similar selective immunosuppressive activity (Figure 81.4). Its mechanism of action is also through the inhibition of signaling through the TCR (Figure 81.5).

Pharmacology

Tacrolimus is highly lipophilic, and its method of administration is similar to that of CSP. Its mechanism of action has been reviewed above in conjunction with CSP. It is generally given IV in the early phases following allogeneic transplantation and then switched to the oral formulation. There is rapid distribution of the drug to the central compartment after a short IV infusion. Following oral administration, tacrolimus is erratically and incompletely absorbed [70]. The major difference from CSP is that the absorption of tacrolimus does not seem to depend on the presence of bile salts. Tacrolimus undergoes extensive distribution with the highest levels found in the lung, kidney, heart, and spleen. While in the blood, it is distributed into red blood cells. Less than 1% of the IV or oral dose appears unchanged in the urine, indicating that the drug is almost completely metabolized in the liver prior to its elimination. It is eliminated primarily through monodemethylation, dimethylation, and hydroxylation. The half-life of tacrolimus is approximately 9 hours and longer in those patients who have hepatic dysfunction. Tacrolimus is monitored in a similar fashion to CSP. Appropriate doses and levels of tacrolimus have not yet been clearly defined in patients undergoing allogeneic BMT, but the general blood levels have been 10–20 ng/dL.

Toxicity

Tacrolimus is moderately well tolerated in humans. Most of the data on adverse events can be categorized into neurotoxicity, renal toxicity, and hyperglycemia and generally have been reported in solid organ transplantation recipients. Tacrolimus can cause an increase in serum creatinine concentration similar to that of CSP, probably secondary to a decrease in glomerular filtration. Hypertension in previously normotensive patients has also been described. Occasionally hypertension can be refractory to antihypertensive medication. The most commonly utilized antihypertensives have included the calcium channel blockers. Central nervous system (CNS) effects have also been found, including headaches, tremors, parathesias, photophobia, mental status changes and PRES. Other findings include pulmonary symptoms such as dyspnea, musculoskeletal pain, itching, GI complaints (including anorexia, nausea, vomiting, and abdominal pains), and fatigue. Although the adverse effects of CSP and tacrolimus appear to be similar, there may be some advantage of tacrolimus in hypertensive patients. Hyperkalemia has also been observed following tacrolimus administration.

Clinical use

Tacrolimus has been evaluated in combination with MTX for the prevention of GVHD. In a multicenter trial, 329 recipients of HLA-identical marrow transplants were randomized to receive a short course of MTX plus either tacrolimus or CSP [71]. The incidence of grade II–IV acute GVHD was significantly lower in patients who received tacrolimus (32% vs. 44% with CSP), even though more unfavorable patients were enrolled in the tacrolimus group. This difference was largely due to a reduction in grade II disease. The incidence of chronic GVHD was similar in the two groups (56% and 49%), but severe chronic disease was more likely with CSP. There were, however, surprising adverse findings in the tacrolimus group:

1. There was inferior 2-year survival (41% vs. 50% with CSP) and overall survival (47% vs. 57% with CSP) that was largely due to poorer survival of patients with advanced disease (25% vs. 42%).
2. There was a higher incidence of regimen-related adverse events, which predominantly occurred in patients with advanced disease.

The conclusion of the study was that in patients without advanced disease, tacrolimus plus MTX was more effective than CSP plus MTX in the prevention of acute GVHD, with no difference in disease-free or overall survival. The survival disadvantage in patients with advanced disease warrants further study.

Another randomized trial also compared tacrolimus plus MTX with CSP plus MTX [72]. Tacrolimus was more effective than CSP for the prophylaxis of acute GVHD but there was no difference in patient survival or relapse. Although these trials were performed in patients with HLA-identical marrow transplants, uncontrolled [73–75] and controlled [76] observations indicated that tacrolimus also has activity against acute GVHD in recipients of matched unrelated donor transplants. The doses used in GVHD prophylaxis trials begin at 0.03–0.04 mg/kg/day as a continuous infusion. When patients are able to sustain near normal oral food and fluid intake, the drug is given orally at a dose of 0.15 mg/kg/day administered in two divided doses.

Sirolimus (rapamycin)

Sirolimus is a lipophilic macrolide that was identified more than 20 years ago during an antibiotic screening [77]. This molecule is produced by a strain of *Streptomyces hygroscopicus* isolated from a soil sample from Easter Island (Rapa Nui). Although lacking antibacterial activity, it inhibited yeast growth and growth of filamentous fungi. The first demonstration of sirolimus's immunosuppressive activity was obtained from studies demonstrating the inhibition of immunoglobulin E (IgE) production and also efficacy in experimental allergic encephalomyelitis and adjuvant arthritis [77]. The interest in sirolimus as an immunosuppressive agent coincided with the discovery of the immunosuppressive activity of tacrolimus since the structures of both of these molecules contained a distinctive hemiketal-masked α,β-diketopipecolic acid amidic component (Figure 81.4).

Pharmacology

Sirolimus binds to the same family of intracellular receptors as tacrolimus, termed tacrolimus binding proteins (FKBPs). In a similar fashion to tacrolimus, sirolimus has two domains, a domain bound by FKBP and an effector domain forming a composite surface that interacts with the mammalian target of sirolimus. The intracellular target of sirolimus [termed the mammalian target of rapamycin (MTOR)] is ubiquitously expressed in human tissue, with the highest level found in the testes and skeletal muscle.

Although sirolimus and tacrolimus bind to the same family of intracellular binding proteins, the mechanisms of immunosuppression are clearly distinct. Sirolimus inhibits the growth of hematopoietic and lymphoid cells in vitro. The most striking observation is that it suppresses cytokine-driven growth of these cells. The data demonstrate a significant increase in the proportion of G_1 phase cells when sirolimus is added [78]. The mechanism of action of sirolimus is to inhibit specifically the progression of cells from G_1 into the S phase, suggesting that it may interfere with the signaling that is critical for the cyclin/cyclin-dependent kinase (CDK) complex required for cellular proliferation. Data using human peripheral blood T cells demonstrate that the block of G_1 may be related to an increase in a species of titratable inhibitor of G_1 cyclin/CDK [79]. In addition to regulation of the cell cycle, sirolimus may also exert an effect in signal transduction pathways that mediates specific cytokine responses. For example, sirolimus has been shown to inhibit IL-1-driven IFN-γ production [80]. Moreover, the secondary signals required for T-cell activation and proliferation are dependent on ligation of the B7 family with CD28. Lack of the secondary signal has been shown to result in anergy in the responding T cell. While signaling through the TCR is not affected by sirolimus, signaling through CD28 is inhibited by sirolimus [81]. CD28 signaling leads to a sustained downregulation of the inhibitor I kappa B (IκB). CD28-mediated downregulation of IκB-α is prevented by sirolimus. These data suggest that sirolimus affects the CD28 signaling pathway, which may be a significant mechanism of its action and distinct from either CSP or tacrolimus. Moreover, in murine experimental models sirolimus has been shown to expand selectively naturally occurring $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) in vivo [82]. Sirolimus has also been used to expand these cells ex vivo for cellular therapies directed against activated T cells. This approach is being tested in patients as a method to prevent GVHD.

Toxicity

Sirolimus appears toxic to the GI system, including elevation of liver function tests and diarrhea. In contrast to the renal side effects that are common with CSP and tacrolimus, nephrotoxicity is rarely encountered with sirolimus. However, sirolimus may potentiate the nephrotoxicity of CSP [83]. Other toxicities of sirolimus include hypertriglyceridemia, a decrease in platelets and leukocytes, epistaxis, blood pressure changes, headaches, nausea, mucous membrane irritation, and infections. The use of sirolimus at high doses may also increase the risk of hepatic sinusoidal obstruction syndrome (SOS) [84]. Adverse reactions previously observed with other macrolide antibiotics are also of concern. Testicular atrophy has been observed in both mice and non-human primates. These early observations suggest that liver function abnormalities, thrombocytopenia, and neutropenia may be its dose-limiting toxicity.

A retrospective review of 488 patients studied the effect of treatment with sirolimus on the incidence of SOS [84]. When used with cyclophosphamide/total body irradiation (CY/TBI) conditioning, sirolimus was associated with a significantly increased incidence of SOS [odds ratio (OR) 2.35]. The concomitant use of MTX further increased this rate (OR 3.23), whereas sirolimus without MTX was not associated with a significantly increased risk of SOS (OR 1.55). Overall treatment-related mortality was lowest when sirolimus was used without MTX. When used with busulfan-based conditioning, sirolimus was associated with an even higher rate of SOS (OR 8.8). These data suggest that myeloablative doses of busulfan should not be used with sirolimus-based immunosuppression [85].

Clinical use

Sirolimus has been used as the front-line regimen for the prevention of GVHD in combination with tacrolimus and low-dose MTX for GVHD prophylaxis in mismatched related donor and mismatched unrelated donor HCT [86]. All evaluable patients engrafted. An absolute neutrophil count of 500/µL was achieved on day +18 (range, 11–32 days). Sustained platelet counts of more than 20,000/µL were attained on day +29 (range, 14–98 days). Grades 0–I acute GVHD occurred in 75% of patients. Grade II, III, and IV acute GVHD occurred in 13, 8, and 5%, respectively (total grade II–IV GVHD, 26%). It was concluded that sirolimus was tolerable, adequate blood levels were achievable, and there was a low rate of acute GVHD compared with historical data in this high-risk population.

A second study explored sirolimus and tacrolimus without MTX following matched related donor (MRD) and matched unrelated donor (URD) HCT [87]. No overall differences between the 53 MRD and 30 URD transplants were noted. Engraftment was rapid, with median times to neutrophil and platelet engraftment of 14 and 12 days, respectively. The incidences of grade II–IV and grade III–IV acute GVHD were 20 and 5%, respectively; the cumulative incidence of chronic GVHD was 59%. The 30- and 100-day treatment-related mortalities were 0 and 4.8%, respectively.

This regimen was tested formally in a randomized phase III study in the MRD population comparing tacrolimus/sirolimus with tacrolimus/MTX in a trial conducted by the Bone Marrow Transplant Clinical Trials Network (BMT CTN). A total of 304 patients were randomized, comparing sirolimus/tacrolimus with tacrolimus/MTX. The overall data suggested less mucositis with sirolimus/tacrolimus but no differences in GVHD or survival [88].

Mycophenolate mofetil

Mycophenolate mofetil (MMF) is a morpholinoethyl ester of mycophenolic acid (MPA). MPA is produced by several species of *Penicillium* molds and this agent possesses antibacterial, antifungal, antiviral, antitumor, and immunosuppressive properties. MMF's immunosuppressive activity occurs after hydrolysis to MPA. Therefore, MPA is the active moiety but it is formulated as MMF to enhance its bioavailability. MMF inhibits the proliferation of T and B cells and the production of antibodies [89]. Most of the clinical data are in the solid organ literature. The developmental work on MMF has utilized the dog model in BMT. Studies in the dog model of allogeneic transplantation by Storb et al. [90] demonstrated that the combination of MMF and CSP was highly effective at the prevention of host-versus-graft rejection and graft-versus-host reaction following a reduced-intensity conditioning regimen. These studies led to a marked increase in this form of transplantation worldwide. The contribution of MMF to the development of this approach was significant.

Pharmacology

Purines are essential for the growth and survival of cells. Cells have two pathways to produce purines: de novo and salvage pathways. Lymphocytes are highly dependent on the de novo synthesis whereas other cells can utilize both (Figure 81.6). In the de novo pathway, the ribose-phosphate portion of the purine nucleotide is derived from 5-phosphoribosyl-1-pyrophosphate, which is derived from adenosine triphosphate and the sugar ribose-5-phosphate. Therefore, 5-phosphoribosyl-1-pyrophosphate is an essential intermediate in the synthesis of purines. Following T-cell stimulation, there is a brisk and sustained increase in 5-phosphoribosyl-1-pyrophosphate and guanosines, and deoxyguanosines activate 5-phosphoribosyl-1-pyrophosphate synthetase. Therefore, depletion of guanosines would lead to a decrease in 5-phosphoribosyl-1-pyrophosphate synthetase and inhibition of purine synthesis, which is required for T-cell activation. MPA action is through the inhibition of inosine monophosphate dehydrogenase (IMPDH). This enzyme catalyzes the oxidation of inosine monophosphate to xanthine monophosphate. Xanthine monophosphate is a required intermediate metabolite in the synthesis of guanosine triphosphate. The enzyme IMPDH is a critical enzyme for the de novo biosynthesis of purine nucleotides, specifically guanosine monophosphate (GMP). Blockade of GMP synthesis leads to a negative feedback inhibition of 5-phosphoribosyl-1-pyrophosphate and prevention of T-cell activation. The therapeutic index of MMF depends on the lymphocytes' reliance on de novo synthesis of purines, allowing for greater immunosuppressive activity with less toxicity.

Toxicity

MMF does not seem to deplete GTP in neutrophils, in contrast to lymphocytes and monocytes. Its selective action on lymphocytes seems to spare the higher risk of acute infections. The major side effect of MMF has been suppression of the hematopoietic system with neutropenia. GI side effects including GI bleeding have also been reported occasionally. Different preparations differ in the degree of GI toxicity. Doses of up to 1.5 g twice daily had no nephrotoxicity, myelosuppression, or other serious side effects in patients with rheumatoid arthritis. The use of MMF has also been associated with progressive multifocal leukoencephalopathy (PML), and a "black box" warning to this effect has been issued by the US Food and Drug Administration (FDA). Methods for monitoring drug levels of MMF are available, although it is not yet clear how these tests should be utilized. One study demonstrated that the median bioavailability of MMF was 72%, with eightfold variability [91]. There was also the suggestion from this study that three times daily dosing may be more advantageous.

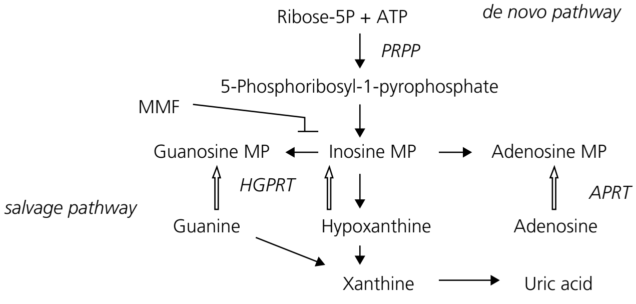


Figure 81.6 The salvage and de novo pathway of purine synthesis. Inositol monophosphate (IMP) is at a central position and mycophenolate mofetil (MMF) inhibits IMP dehydrogenase, necessary for the conversion of inosine MP to guanosine MP. HGPRTase, hypoxanthine–guanine phosphoribosyltransferase; PRPP, 5-phosphoriboxyl-1-pyrophosphate, APRT, adenine phosphoribosyltransferase.

Clinical use

MMF has been used as part of the front-line regimen for the prevention of GVHD especially within the setting of reduced-intensity preparatory regimens. This drug is commonly used as part of the post-transplant immune suppression as there are data in the dog model that it supports donor cell engraftment. It is possible that the prolonged administration of MMF helps to delay the onset of GVHD since the use of MMF instead of MTX as immunosuppressive therapy led to a delay of the onset of GVHD after reduced-intensity HCT. However, preliminary results of a prospective trial in which MMF was given twice daily for 27 days in combination with CSP suggested that this combination is not superior to the standard regimen of MTX plus CSP for preventing GVHD after ablative HCT from related and unrelated donors [92]. In this study, the median time to engraftment after hematopoietic cell transplantation was 15 days (range, 10–20 days). The incidence of acute GVHD was 62%, which was comparable to that in a group of historical controls receiving CSP and MTX for GVHD prophylaxis. Although a significant improvement in the prevention of GVHD was not suggested, compared with CSP and MTX, MMF in combination with CSP could be considered in cases in which MTX is contraindicated.

Cyclophosphamide

Cyclophosphamide and its nitrogen mustard-derived alkylating agent ifosfamide were developed by Norbert Brock and ASTA-Werke after synthesizing more that 1000 candidate compounds in the 1950s. The goal was to convert the base nitrogen mustard into a non-toxic prodrug form (also called latent drug) so that it could be given intravenously with less toxicity. Once inside the cells, the prodrug would be converted into the active toxic form. It is chemically an oxazaphosphorine and was the first of the prodrugs designed for cancer therapy.

Pharmacology

Given that cyclophosphamide is a prodrug, it is converted by mixed-function oxidase enzymes found in the liver. The main metabolite is 4-hydroxycyclophosphamide (historically used in purging studies of the bone marrow in autologous HCT), which is the active moiety. Another active metabolite is phosphoramide mustard, which can be formed from the tautomer of 4-hydroxycyclophosphamide, aldophosphoramide. This metabolite is only formed in cells that have low levels of aldehyde dehydrogenase (ALDH). Aldophosphoramide is converted to phosphoramide mustard and acrolein (the cause of hemorrhagic cystitis). Phosphoramide mustard forms DNA cross-links both between and within DNA strands at guanine N-7 positions leading to inter- and intrastrand cross-linkages. Since these cross-links are irreversible, the cell is unable to divide and cell death occurs.

Toxicity

Cyclophosphamide has relatively little typical chemotherapy toxicity as ALDHs are present in relatively high concentrations in bone marrow stem cells, liver, and intestinal epithelium. ALDHs protect these actively proliferating tissues against toxic effects by converting aldophosphamide to carboxyphosphamide rather than the toxic metabolites phosphoramide mustard and acrolein. It is the high levels of ALDH in the marrow that allow its use after an ablative regimen for HCT. Adverse drug reactions include chemotherapy-induced nausea and vomiting, bone marrow suppression, diarrhea, darkening of the skin/nails, alopecia or thinning of hair, changes in color and texture of the hair, and lethargy. At high doses of cyclophosphamide, hemorrhagic myocarditis can occur with high mortality. Hemorrhagic cystitis is a frequent complication, but this can be prevented by adequate fluid intake and mesna (sodium 2-mercaptoethane sulfonate), a sulfhydryl donor that binds acrolein.

Clinical use

Earlier studies had shown that high-dose cyclophosphamide given post-transplantation along with tacrolimus and MMF was safe and effective in preventing graft rejection and GVHD after non-myeloablative conditioning and transplantation of T-cell-replete bone marrow from partially HLA-mismatched related donors [93,94]. These results suggested the feasibility of a phase I/II study to evaluate the effectiveness of single-agent high-dose cyclophosphamide in reducing the incidence of acute and chronic GVHD following myeloablative allogeneic HCT. All patients (78 HLA-matched related and 39 HLA-matched unrelated donors) received conventional myeloablation using busulfan plus cyclophosphamide conditioning and T-cell-replete bone marrow, followed by 50 mg/kg per day of cyclophosphamide on days 3 and 4 post-transplantation [94]. The incidences of grade II–IV and grade III–IV acute GVHD were 43 and 10%, respectively, and the day-100 and 2-year non-relapse mortalities were 9 and 17%, respectively. At a median follow-up of 26 months among surviving patients, the cumulative incidence of chronic GVHD was 10%. These data suggest that, in the majority of patients, this use of cyclophosphamide after transplantation results in selective removal of alloreactive donor T cells, with the most dramatic effect being the low incidence of chronic GVHD. These results are being expanded and repeated in other centers for a more definitive conclusion.

Antibodies

Many antibodies have been used for the prevention and treatment of GVHD. Most of the promising data have come from studies using selected monoclonal antibodies directed at specific epitopes. However, these antibodies are not generally available and most of the studies have involved small groups of patients. Several antibodies will be discussed, namely intravenous immunoglobulin (IVIg), anti-CD3, antithymocyte globulin (ATG), daclizumab or basiliximab (anti-IL-2 receptor), infliximab (anti-TNF-α receptor), etanercept (soluble TNF receptor), and alemtuzumab (Campath 1H).

Anti-CD3

OKT3 is an anti-CD3 monoclonal antibody that is commercially available. This antibody has been used to treat GVHD, but because it is a stimulatory antibody to the T cell, the results have not been uniformly successful. Carpenter et al. showed that soluble antihuman CD3 monoclonal antibodies induce apoptosis of activated T cells [95]. They evaluated different anti-CD3 antibodies and demonstrated that the ability to induce apoptosis varied among them. Compared with OKT3, one monoclonal antibody termed HuM291 induced more sustained phosphorylation of ERK-2, greater release of IFN-γ, and more activation-induced T-cell death. The use of HuM291 (visilizumab) in a phase I study in patients with steroid refractory acute GVHD was highly encouraging. This antibody is not capable of cross-linking with Fc receptors on accessory cells and therefore does not activate proliferation of T cells. In one study, of the 17 enrolled patients, five achieved a complete resolution of GVHD, eight had partial improvement, two showed no change, and two progressed. Eight of the 13 responders had sustained responses, but the estimated 1-year survival was only 24%. Epstein–Barr virus (EBV) reactivation was also a problem and therefore monitoring for EBV is important with early intervention with rituximab if necessary.

Intravenous immunoglobulin (IVIg)

Polyclonal human immunoglobulins purified and sterilized from serum pooled from many donors have been used to manipulate the immune system. There are many preparations of IVIg with different titers of anticytomegalovirus activity. To date there are no known clinical differences with the use of any of these commercial preparations. Occasionally, one preparation may be preferable to another based on the need to conserve the volume that a patient receives. The initial studies focused on its use to prevent infectious complications following BMT [96,97]. However, in a large randomized trial of IVIg, this product was also associated with a decrease in the incidence of GVHD, especially in BMT recipients under the age of 20 years. Moreover, there was a decrease in the overall mortality in favor of those who had received IVIg [98]. Although this agent is effective in the early phase following BMT, its prolonged use, in the absence of hypogammaglobulinemia after day +100, does not reduce late complications or chronic GVHD and may be associated with impairment of humoral immune recovery. Moreover, serious issues such as the extremely high costs of these products and occasional limited availability have led to a decrease in their use.

Antithymocyte globulin

Antithymocyte globulin (ATG) is a polyclonal immunoglobulin prepared by injecting horses (Atgam) or rabbits (Thymoglobulin) with human thymocytes or with a T-cell line (Fresenius). These antibodies are capable of destroying human leukocytes. The half-life of these antibodies differs significantly between the horse and rabbit ATG and the functional half-life of these antibodies is not well defined, although rabbit ATG is more potent than horse ATG. ATG has been used as part of the preparatory regimen prior to infusion of donor cells to decrease the incidence of graft rejection or following BMT to prevent GVHD. In two prospective studies using ATG as part of GVHD prophylaxis, ATG was not effective [99,100]. Prospective and retrospective studies have had mixed results regarding the efficacy and safety of various ATG preparations for the prevention of GVHD. In a report of 109 patients enrolled in two randomized trials from Italy, use of rabbit ATG in patients undergoing unrelated donor transplants reduced the incidence of both severe acute and extensive chronic GVHD [101]. However, this did not translate into a reduction in treatment-related mortality because of an increase in fatal infections in the ATG treatment arm.

Another trial randomly assigned 202 patients undergoing allogeneic HCT from matched unrelated donors to receive GVHD prophylaxis with CSP plus MTX, with or without rabbit ATG [102]. The addition of ATG significantly reduced the overall incidence of acute and chronic GVHD, without an increase in relapse or non-relapse mortality, and without compromising overall survival. However, addition of ATG did not significantly reduce the incidence of grade III–IV acute GVHD or death within 100 days, the primary end-points of the study. A subsequent report of this study demonstrated a lower incidence and severity of chronic GVHD without increasing the risk of relapse [103]. Patients treated with ATG were more likely to have undergone successful taper of other immunosuppressants.

A retrospective analysis of 1676 adults undergoing reduced-intensity HCT for hematologic malignancy compared outcomes among patients who underwent in vivo T-cell depletion with ATG (584 patients) or alemtuzumab (213 patients) with those of patients who did not undergo T-cell depletion [104]. When compared with T-cell-replete transplantation, ATG resulted in similar rates of acute GVHD; lower rates of chronic GVHD (40% versus 52%), higher rates of relapse (49% versus 38%), and lower overall survival (38% versus 50%) at 3 years.

These studies suggest that ATG is effective in decreasing the rate of acute and chronic GVHD in patients undergoing HCT, but that these benefits do not translate as yet into a survival benefit. The efficacy and safety of ATG are likely affected by the intensity of the preparative regimen. Since ATG is a foreign xenogeneic protein and an antibody, serum sickness can occur. Corticosteroids, acetaminophen, and H1 and H2 blockers are frequently used to prevent or treat the symptoms associated with the infusion of ATG. The use of ATG after BMT has been associated with an increase in lymphoproliferative disorders [105].

Anticytokine therapy

Several cytokines have been implicated in GVHD. IL-2 is a critical molecule in T-cell survival and stimulation. There are two monoclonal antibodies in clinical use directed against the IL-2α receptor (daclizumab) and also a chimeric anti-IL-2 monoclonal antibody (basiliximab) [106]. The bulk of the data have been obtained from solid organ allograft rejection studies. The use of anticytokine agents in stem-cell transplantation is limited. The initial results of the use of dacluzimab are encouraging, although these are phase II data and further studies are necessary to confirm these results. As with all antibodies, toxicities associated with such proteins are common and include myalgia, fevers, rash, pruritus, arthritis, and headaches. Further caution is warranted in targeting the IL-2 receptor since CD25 is also expressed in regulatory T cells. In a prospective study with contemporaneous controls, the use of an immunotoxin directed against this epitope resulted in a higher incidence of acute GVHD [107]. The data suggested that CSP blocked the induction of CD25 expression on alloreactive T cells but had no detectable effect on CD25 expression on regulatory T cells, thus enhancing the loss of regulatory T cells.

TNF is another cytokine that has been implicated in acute GVHD. Use of a polyclonal neutralizing antibody against TNF-α resulted in a 70% reduction in GVHD-associated mortality and also in diminished lesions in the skin and the intestines in an experimental model [108]. Similar findings were observed using a neutralizing antibody against murine TNF with reduction of splenomegaly in GVHD models. Higher TNF levels have also been described in those patients who develop GVHD, although this finding has not been universally observed [109]. There are two anti-TNF molecules. Infliximab is a chimeric monoclonal antibody that binds to TNF. There is also a fusion molecule, etanercept, that consists of the soluble TNF receptor (p75) linked to the Fc portion of human IgG_1 (TNFR:Fc) [110]. These molecules are successful in the therapy of Crohn disease and rheumantoid arthritis and could be equally useful following allogeneic BMT, but well-designed studies will be necessary. There are preliminary data for the use of infliximab in the therapy of acute GVHD, but these data have not been reported in the peer-reviewed literature.

Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody directed against CD52. This antibody was formerly known as Campath-1H and is active against early- and late-stage chronic lymphoid leukemia and also T-cell prolymphocytic leukemia. Alemtuzumab targets the 21–28-kDa cell surface glycoprotein CD52, a protein present on the surface of mature lymphocytes but not on lymphoid progenitors. The function of CD52 is unknown, but it is found on >95% of peripheral blood lymphocytes and monocytes. Upon binding to CD52, alemtuzumab initiates its cytotoxic effect by complement fixation and antibody-dependent cell-mediated cytotoxicity mechanisms [111].

Alemtuzumab has been associated with frequent infusion-related events, including hypotension, rigors, fever, shortness of breath, bronchospasm, chills, and/or rash. These side effects necessitate close follow-up of the patient, especially in their first dose infusion. Other serious infusion-related events have been reported: syncope, pulmonary infiltrates, adult respiratory distress syndrome, respiratory arrest, cardiac arrhythmias, myocardial infarction, and cardiac arrest. An important and potentially fatal complication of therapy with alemtuzumab is the increased risk for opportunistic infections, in particular, reactivation of cytomegalovirus.

Several groups have utilized this monoclonal antibody as a backbone to a preparatory regimen in reduced-intensity conditioning to decrease GVHD. The first report of using alemtuzumab in a reduced-intensity conditioning regimen was investigated in 44 patients with hematologic malignancies [112]. Forty-two of the 43 evaluable patients had sustained engraftment. There were no cases of grade III–IV acute GVHD. Only two patients developed grade II acute GVHD, and only one had chronic GVHD. The estimated probability of non-relapse mortality was low at 11%. These data suggested that inclusion of alemtuzumab in a reduced-intensity preparative regimen is associated with durable engraftment, minimal toxicity, and low incidence of GVHD. The use of this monoclonal antibody has been extended for use in haploidentical hematopoietic cell transplantation [113]. In a standard-risk group of 19 patients with aplasia or in remission at transplantation there was a 1-year survival rate of 63% [95% confidence interval (CI), 38–80%] and median overall survival time of 2.9 years (95% CI, 6.2–48 months). While some groups have used this monoclonal antibody as a backbone of their preparatory regimen, it is not used universally because of concerns regarding infections and relapses.

Other agents

Induction of anergy

An exciting approach for the prevention of GVHD is to induce tolerance by inhibition of costimulatory molecules. To this end, the use of a chimeric molecule, CTLA4-Ig, has been investigated. Data generated from studies of T-cell activation have clearly demonstrated that there is a critical need for a second costimulatory signal for a positive immune response. Lack of secondary signals results in specific T-cell anergy. CTLA-4-Ig is a fusion protein designed in an attempt to prevent costimulation signals. Variable results have been observed using the CTLA-4-Ig fusion protein to block GVHD across major histocompatibility barriers [114]. These results may vary according to the model of GVHD selected (i.e. major histocompatibility differences) or the activation state of T cells. Methods to block costimulation hold significant promise in achieving antigen-specific unresponsiveness or anergy and therefore a state of tolerance [115]. In a clinical trial of tolerance induction, haploidentical bone marrow was treated ex vivo with CTLA-4-Ig in the presence of recipient peripheral blood [116]. The laboratory parameters such as T-cell precursor frequency against allogeneic targets were markedly reduced. The preliminary clinical data were encouraging, with a low incidence of GVHD and encouraging overall survival. Such new approaches may circumvent the need for prolonged immunosuppression after BMT and may be clinically beneficial for patients who require an allogeneic BMT.

Photopheresis/pentostatin

The use of extracorporeal photopheresis or pentostatin is a choice for patients with chronic GVHD. This combination approach has been explored as a means to induce tolerance in a reduced-intensity preparatory regimen. Patients received a preparative regimen of photopheresis, pentostatin, and TBI followed by allogeneic stem-cell infusion from 6/6 or 5/6 HLA-matched related donors or 6/6 HLA-matched unrelated donors. GVHD prophylaxis consisted of cyclosporin A and a short course of MTX. Sixteen of 18 patients developed full donor chimerism with no day-100 transplant-related mortality. Grade II–IV acute GVHD and extensive chronic GVHD developed in 19% and 18% of patients, respectively. At a median follow-up of 14 months (range, 1–35 months), the 1-year failure-free and overall survival were 64% and 65%, respectively [117].

Conclusion

This chapter has focused primarily on the most frequently used drugs for immunosuppression or immunomodulation for GVHD (Table 81.5). As we learn more about the impact of the human microbiome, especially of the gut and skin, effector cell trafficking to target organs of GVHD, more directed therapy to impact the gut flora, adhesion molecules, and homing receptors may be developed [118]. Because GVHD and solid organ rejection remain such a difficult clinical problem, there is a need for continued efforts to develop novel immunosuppressive or immunomodulatory agents. As shown in Table 81.1, the number of randomized controlled trials for the prevention of acute GVHD has been modest over the past 30 years. Moreover, there is only one agent approved by the FDA for GVHD prevention (Gamimune). Well-designed randomized trials in this complex disorder are critical for advances in the control of GVHD [119].

Table 81.5 Immunosuppressive drugs, targets, and mechanisms of action discussed in this chapter

Drug	Target	Mechanism of action
Corticosteroids	Steroid receptor	Multiple, including cytokine expression, lymphocyte activation, and cell depletion
MTX	DHFR	Blocks DNA synthesis
CSP	Calcineurin	Blocks lymphocyte activation
Tacrolimus	Calcineurin	Blocks lymphocyte activation
Sirolimus	MTOR	Blocks costimulatory signal and cell division
MPA	IMPD	Blocks DNA synthesis
Dacluzimab/basiliximab	IL-2 receptor	Blocks lymphocyte activation
IVIg	Multiple	Unknown
ATG	Multiple	Blocks lymphocyte activation
Infliximab	TNF receptor	Blocks lymphocyte activation
Etanercept	TNF	Blocks lymphocyte activation
Thalidomide	Unknown	Unknown
Clofazimine	Unknown	Unknown
Hydroxychloroquine	TAP proteins	Blocks lymphocyte activation
CD4-D1 peptide	CD4	Blocks lymphocyte activation
CTLA-4-Ig	CD28	Blocks lymphocyte activation

ATG, antithymocyte globulin; CSP, cyclosporine; DHFR, dihydrofolate reductase; IL, interleukin; IMPD, inosine monophosphate dehydrogenase; IVIg, intravenous immunoglobulin; MHC, major histocompatibility complex; MPA, mycophenolic acid; MTX, methotrexate.

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CHAPTER 82

T-cell Depletion to Prevent Graft-versus-Host Disease

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Introduction

Acute and chronic graft-versus-host disease (GVHD) account for a significant fraction of the morbidity and mortality associated with allogeneic hematopoietic cell transplantation (HCT) [1]. GVHD reduces survival directly as a result of organ damage and indirectly as a consequence of infectious complications prompted by GVHD prophylaxis and therapy. Donor T-cell depletion (TCD), when it was initially introduced, offered the potential for prevention of GVHD without the toxicity associated with immune suppressive drugs. Numerous TCD methods have been utilized over the past three decades. These have included ex vivo negative selection approaches (e.g. monoclonal antibody, elutriation, lectin agglutination, or photodepletion techniques), ex vivo positive selection through CD34$^+$ columns, or in vivo administration of anti-T cell antibody preparations such as antithymocyte globulin (ATG) and alemtuzumab (Table 82.1). Most early trials documented that TCD could substantially limit acute and chronic GVHD. However, this reduction in GVHD did not translate into improved overall survival (OS) because of unexpected high rates of graft failure [2], Epstein–Barr virus-associated lymphoproliferative disorders (EBV-LPDs) [3], and disease recurrence [4,5] following TCD-HCT. There have been very few randomized studies evaluating TCD, but those that have been performed have not demonstrated a survival advantage to this approach [6]. Nonetheless, there still remains substantial interest in perfecting the technique, particularly to limit the long-term consequences of acute and chronic GVHD and to permit HCT across human leukocyte antigen (HLA) barriers.

Early preclinical models of T-cell depletion

In the 1950s, Barnes and Loutit noted that following irradiation of mice, infusion of isologous marrow and spleen led to long-lasting survival, whereas inoculation of allogeneic cells afforded only a temporary recovery, with most animals later succumbing to a wasting condition called "secondary phase of irradiation syndrome" [7]. They subsequently proposed that this "secondary disease" was the result of a graft-versus-host immunologic response. Uphoff demonstrated that this GVHD did not develop after infusion of fetal liver/spleen tissue, which lack mature T-lymphocytes [8]. In the 1960s, Bortin and Saltzstein extended Uphoff's findings and further demonstrated that irradiated mice given allogeneic embryonic liver and thymus cells became true chimeras that did not reject donor skin grafts [9]. These pivotal observations pointed to the critical connection between mature donor T-lymphocytes and GVHD, and suggested that selective in vitro removal of these cells from the allograft could prevent this transplant complication.

In 1968, van Dicke et al. reported on the use of differential centrifugation on a discontinuous albumin gradient to separate lymphocytes from hematopoietic precursors, and demonstrated that irradiated mice given spleen cell fractions devoid of small lymphocytes resulted in 80–100% survival without evidence of GVHD. In contrast, all mice inoculated with fractions that contained increasing numbers of small lymphocytes died from severe GVHD [10]. Subsequently, Reisner et al. demonstrated that mouse bone marrow and spleen cell suspensions, when treated with soybean and/or peanut agglutinin, could generate an agglutinated fraction that is enriched in hematopoietic activity but depleted of T cells. Treatment of irradiated mice with this fraction conferred hematopoietic recovery with a low incidence of GVHD [11].

In the 1970s, absorbed antisera with retained activity against mature lymphocytes were developed and successfully used in animal models as the method for ex vivo T-cell depletion. Mice, rats, and dogs were successfully transplanted with minimal or no GVHD, across both major and minor histocompatibility barriers [12,13]. In the late 1970s, the development of monoclonal antibody technology helped to advance efforts to eliminate T cells from the donor graft and markedly decrease the incidence of GVHD in animal models [14,15].

T-cell dose and GVHD

Bone marrow used for human transplantation contains approximately $1–2 \times 10^{10}$ nucleated cells, of which up to 10–20% ($\sim 10^9$ cells) are mature lymphocytes. An average patient transplanted with unmodified bone marrow would thus receive a T-cell dose on the order of $1–4 \times 10^7$ cells/kg recipient body weight. Initially, it was not known what degree of TCD would be necessary to prevent GVHD, as immunofluorescence and erythrocyte (E) rosette assays for quantifying T-lymphocytes were not sufficiently sensitive to evaluate marrow that had been manipulated with antibodies. Limiting dilution assays suggested that at least a 2-log depletion of T cells to $1–3 \times 10^5$ cells/kg is necessary to significantly reduce GVHD in the HLA-identical related donor marrow transplant setting without exogenous immune suppression [16,17]. The target for ex vivo TCD studies is a T-cell inoculum below $0.5–1.0 \times 10^5$ cells/kg recipient body weight for HLA-matched related grafts. The extent of TCD needed to prevent GVHD likely depends not only on T-cell dose or log reduction, but also on other factors including donor source, type of graft, degree of major and minor HLA disparity, and

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Table 82.1 Methods of T-cell depletion

Ex vivo negative selection	
Soybean lectin agglutination + E-rosette depletion	
Counterflow centrifugal elutriation	
Monoclonal antibody + complement	
Immunotoxin	
Immunomagnetic beads	
Ex vivo positive selection	
CD34$^+$ selection with immunomagnetic beads	
CD34$^+$ selection combined with negative selection	
In vivo anti-T-cell antibody infusion	
Antithymocyte globulin (ATG)	
Atgam	horse
Thymoglobulin	rabbit
ATG-Fresenius	rabbit
Alemtuzumab	humanized monoclonal antibody (anti-CD52)

polymorphisms in immune response genes present in donor and recipient pairs. For example, granulocyte colony-stimulating factor (G-CSF)-mobilized blood hematopoietic cell products contain approximately 10-fold more T cells than unstimulated bone marrow grafts. Therefore, methods removing only 2 logs of T cells would likely be insufficient for GVHD prevention. CD34$^+$ selection of peripheral blood progenitor cells (PBPCs) with a 3–4 log reduction in CD3$^+$ count below 1×10^5 cells/kg is associated with lower rates of GVHD in matched sibling transplantation [18]. The T-cell threshold for patients receiving unrelated or haplotype mismatched hematopoietic cell products is likely at least 1 log lower.

T-cell number must also be considered in the context of the hematopoietic stem-cell progenitor dose, since the CD34$^+$ count may be also an important determinant of engraftment, GVHD, and survival after PBPC HCT [19,20]. The number of CD34$^+$ cells infused may have a greater impact in patients undergoing TCD transplants than those receiving conventional procedures. A report on 141 patients receiving mobilized PBPCs demonstrated that a higher number of CD34$^+$ cells infused was associated with lower non-relapse mortality (NRM), improved OS, and improved progression-free survival (PFS) after TCD, but not T-cell-replete, transplants [21].

Specificity of TCD

TCD techniques may vary not only in the extent of T-cell removal, but also in the selectivity of depletion. Some strategies remove all lymphoid and accessory cell fractions, including natural killer (NK) cells, B cells, and dendritic cells. These other cellular elements may play an important role in immune surveillance, engraftment, and elimination of residual disease post-transplantation. Other approaches have exclusively removed T cells, while still others have focused on specific phenotypically defined T-cell subsets. Studies have attempted to target donor T-lymphocytes expressing activation antigens after in vitro exposure to recipient antigen-presenting cells in order to eliminate only those cells likely to cause GVHD.

In 2000, a retrospective analysis of alternative donor bone marrow transplantation (BMT) at the Center for International *Blood* and Marrow Transplant Research (CIBMTR) compared negative selection TCD techniques with "narrow" and "broad" spectra of reactivity [22]. Examples of "narrow" specificity techniques included antibodies targeting mature T cells only, such as anti-T-cell receptor (TCR), anti-CD6, or anti-CD5, or T cells with specific functional roles, such as anti-CD8. Broad-specificity techniques include antibodies such as alemtuzumab (anti-CD52), multiple antibody combinations, and physical separation techniques such as lectin agglutination. Patients who received T-cell-depleted marrow using narrow-specificity antibodies had a superior 5-year leukemia-free survival to those receiving broad-specificity depletion, owing to a combination of increased relapse, graft failure, and transplant-related mortality in the broad-specificity TCD cohort [22]. Unfortunately, after over 30 years of research, it still is uncertain what anatomic T-cell subset mediates GVHD in humans and if that subset might vary between donor–patient pairs as a consequence of Class I or II presentation of different minor histocompatibility antigens driving the GVHD reaction. Selective CD8$^+$ TCD had attracted particular interest because preclinical data suggested CD8$^+$ T cells preferentially mediate GVHD. Early recovery of CD8$^+$ T cells after transplantation has been associated with subsequent development of GVHD [23]. Initial trials in HLA-identical sibling BMT in the early 1990s suggested that CD8$^+$ depletion with post-transplant cyclosporine alone could reduce the incidence and severity of GVHD without compromising the graft-versus-leukemia (GVL) effect [24]. However, results of CD8$^+$ depletion of mobilized PBPCs achieving a 2-log reduction have been disappointing, particularly if unrelated donors are used [25]. Nonetheless, CD8$^+$ TCD of donor lymphocyte infusions has been used successfully after TCD-HCT as a prophylactic and therapeutic strategy for disease relapse and for falling donor T-cell chimerism [26,27]. In these uncontrolled studies, low relapse rates were noted in the absence of significant GVHD.

Ex vivo negative selection TCD

Early studies of TCD using murine monoclonal antibodies alone reported acute GVHD rates of approximately 50% in HLA-identical sibling BMT recipients [28]. These results suggested that opsonization of T-lymphocytes alone might be insufficient to prevent acute GVHD in humans, and that additional lymphocyte lysis, facilitated by addition of complement to the antibody, was necessary. In the 1980s and early 1990s, phase II trials of ex vivo negative selection TCD with monoclonal antibodies plus complement or immunotoxins, which removed 2–3 logs of T-lymphocytes, decreased the incidence of GVHD to 10–20% after matched sibling BMT even in the absence of immune suppression post-transplantation [29–31]. Addition of anti-CD52 antibody directly to the hematopoietic cell product as it is being infused (alemtuzumab "in the bag") also resulted in a low incidence of grade II–IV acute GVHD in recipients of both bone marrow and mobilized peripheral blood cells [32]. A low frequency of acute GVHD was also noted with ex vivo physical separation techniques, such as soybean lectin agglutination/elutriation and counterflow centrifugal elutriation [17,32,33].

When unrelated donor transplants came into widespread use in the 1990s, there was considerable enthusiasm for applying this technology to this setting in light of the increased incidence and severity of acute GVHD. In a report from the National Marrow Donor Program (NMDP) in 1993, the use of TCD was the most significant factor predicting freedom from severe (grade III–IV) acute GVHD [34]. A subsequent analysis at the CIBMTR involving 1868 leukemia patients receiving marrow transplants from donors other than HLA-identical siblings revealed that the grade II–IV acute GVHD incidence was 34–38% for the TCD groups compared with 57% for the non-TCD cohort ($p < 0.0001$) [22]. While TCD did appear to reduce acute GVHD in unrelated marrow recipients, rates were not as low as those observed in the matched related donor setting. It is possible that in these early studies, unrecognized HLA disparities due to less sophisticated typing techniques contributed to higher rates of GVHD in the unrelated marrow recipients.

Despite over three decades of ex vivo TCD studies, only one large prospective randomized trial has been performed. This study, reported by Wagner et al. in 2005, included 410 recipients of HLA-identical unrelated bone marrow (Table 82.2). Patients were randomized to receive either T-cell-depleted bone marrow (by the monoclonal antibody $T_{10}B_9$ or elutriation) with post-transplant cyclosporine alone versus T-replete bone marrow with post-transplant cyclosporine and methotrexate as GVHD prophylaxis [6]. Both groups received cyclophosphamide and total body irradiation (TBI) for conditioning. Patients in the TCD arm received additional treatment to promote engraftment: ATG in the elutriation group and cytarabine in the $T_{10}B_9$ antibody group. The mean T-cell dose infused for the TCD was 1 log lower than that in the non-TCD arm. There was no difference in incidence of primary graft failure in the two groups. The incidence of grade II–IV acute GVHD was significantly lower in the TCD arm than the non-TCD arm (39% vs. 63%, $p < 0.0001$), and the cumulative rates of grade III–IV acute GVHD were 18% vs. 37%, respectively ($p < 0.0001$). Regimen-related toxicity was lower in the TCD arm. There was no difference in relapse rates in patients with acute leukemia although patients with chronic myelogenous leukemia (CML) were more than twice as likely to relapse if they received TCD bone marrow. Viral infection rates, particularly from cytomegalovirus (CMV) and other herpesviruses, were higher in the TCD group. Disease-free survival (DFS) and OS at 3 years were similar in the TCD and non-TCD arms. It is uncertain whether this trial represented a true test of the potential impact of TCD as the methods utilized only removed 1–2 logs of T cells. In addition, the inclusion of additional agents to promote engraftment and the use of cyclosporine for GVHD prophylaxis in the TCD arms cloud interpretation of the results.

Most early studies of ex vivo T-cell-depleted BMT concentrated on acute GVHD. As the devastating long-term consequences of chronic GVHD became more widely recognized, attention turned towards the impact of TCD on this late effect. The 1993 retrospective analysis from the NMDP had suggested that TCD reduced the risk of chronic GVHD after unrelated BMT [34]. However, the large prospective multicenter randomized trial of unrelated donor BMT described above failed to demonstrate any difference in the incidence and time to development of chronic GVHD between patients receiving cyclosporine plus TCD versus standard cyclosporine/methotrexate [6]. It is possible that the 1-log reduction in T cells achieved by the methods used in this study was insufficient to protect against chronic GVHD. Indeed, the retrospective study at the CIBMTR in 2000 examining the effect of broad and narrow specificity TCD demonstrated no protection by narrow antibody TCD against chronic GVHD despite a reduction in acute GVHD whereas exhaustive TCD with broad-based approaches did reduce chronic GVHD [22].

Ex vivo positive selection TCD

The increasing utilization of peripheral blood HCT and its attendant increase in chronic GVHD [35] make TCD a particularly attractive modality in this setting. Around the time when the use of mobilized PBPCs eclipsed bone marrow as the more common graft source for allogeneic HCT in adults, there also occurred a shift in the techniques employed for ex vivo TCD. Regulatory and commercial hurdles curtailed the use of negative selection strategies, replaced in large part by ex vivo $CD34^+$ positive selection technology. Initial studies of HLA-identical sibling PBPC HCT using ex vivo $CD34^+$ positive selection initially reported a wide variation in acute GVHD incidence from 8% to 100% [36,37]. This variability was likely influenced by factors such as the number of $CD3^+$ cells infused, immune suppressive agents given after transplant, and the manner in which these devices were used. A study comparing three devices (CEPRATE, Isolex 300i, and CliniMACS) found no apparent differences in the allograft composition or clinical outcomes, although ease of processing did differ [38]. Initial studies using CD34 selection columns showed that a 2-log reduction in T cells from matched related donor PBPCs was insufficient to control GVHD despite the use of cyclosporine ± methotrexate as post-transplant immune suppression [36]. This was perhaps not surprising given the larger number of T cells in peripheral blood compared with bone marrow. However, subsequent studies demonstrated that when the T-cell content in the sibling PBPC graft was reduced by

Table 82.2 Impact of TCD on transplant outcome

	TCD method				
	Ex vivo negative selection TCD (Wagner et al. 2005 [6])	**In vivo TCD with ATG-Fresenius (Finke et al. 2009 [70])**	**Ex vivo positive selection TCD (Pasquini et al. 2012 [42])**	**In vivo TCD with ATG or alemtuzumab (Soiffer et al. 2011 [79])**	**In vivo TCD with ATG or alemtuzumab (Baron et al. 2012 [80])**
Patient population	Unrelated Myeloablative BMT	Unrelated Myeloablative BMT	Related Myeloablative PBPC (AML only)	Related/unrelated RIC HCT	Related/unrelated RIC HCT (AML only)
Trial design	Prospective randomized trial	Prospective randomized trial	Prospective contemporaneous trials	Retrospective registry analysis	Retrospective registry analysis
Engraftment	No impact	No impact	No impact	Delayed *ATG only*	Not reported
Acute GVHD	TCD lower grade III–IV	TCD lower grade II–IV	TCD lower grade III–IV	TCD lower grade II–IV *Alemtuzumab only*	Not reported
Extensive chronic GVHD	No impact	TCD lower	TCD lower	TCD lower ATG and alemtuzumab	TCD lower ATG and alemtuzumab
Non-relapse mortality	No impact	No impact	No impact	TCD higher *ATG only*	No impact
Relapse	TCD higher CML No impact AML	No impact	No impact	TCD higher ATG and alemtuzumab	TCD higher ATG and alemtuzumab
PFS	No impact	No impact	No impact	TCD lower ATG and alemtuzumab	TCD lower *Alemtuzumab only*
OS	No impact	No impact	No impact	TCD lower *ATG only*	TCD lower *Alemtuzumab only*

3.0–3.5 logs, grade II–IV acute GVHD was only 10% [37]. Single institution studies at the Memorial Sloan Kettering Cancer Center have demonstrated very low rates of acute and chronic GVHD in a variety of disease settings when CD34 selection was combined with sheep erythrocyte resetting to deplete residual donor T cells [39].

One of the challenges of ex vivo TCD has been reproducibility at different sites. Historically, most centers developed their own technologies, and multicenter trials were uncommon. However, in 2005, the *Blood* and Marrow Transplantation Clinical Trials Network (BMT CTN) launched a multicenter trial of $CD34^+$ selection as the sole form of GVHD prophylaxis in patients receiving matched related donor transplantation for acute myeloid leukemia (AML) after TBI-based conditioning [40]. AML was chosen as the target indication as the prospective randomized trial in unrelated BMT reported by Wagner et al. demonstrated that TCD was not associated with an increased rate of relapse in patients with AML [6]. In this BMT CTN trial, a total of 44 patients with AML were transplanted at eight centers. All patients engrafted promptly. The incidences of grade II–IV and grade III–IV acute GVHD were 22.7% and 4.8%, respectively, while the incidence of extensive chronic GVHD was 6.8% at 24 months. Differences in processing outcomes between centers were minimal. All products infused met the prespecified goal of less than 1×10^5 CD3 cells/kg recipient body weight. All patients received $>2 \times 10^7$ $CD34^+$ cells/kg and 82% achieved the goal of 5×10^7 $CD34^+$ cells/kg. $CD34^+$ cell recovery was 66.1% and was inversely associated with the starting number of $CD34^+$ cells. The median purity of the CD34-enriched fraction was 96.7% with monocytes and B cells the most common impurity [41]. These results were compared with outcomes of 84 AML patients receiving T-replete matched related donor grafts and pharmacologic immunosuppressive therapy (IST) treated in contemporaneous trials sponsored by the CTN [42]. The 100-day rates of grade II–IV acute GVHD were 23% and 39% with TCD and IST grafts, respectively ($p = 0.07$). Two-year rates of chronic GVHD were lower with TCD grafts than IST (19% vs. 50%, $p < 0.001$). There were no differences in rates of graft rejection or leukemia relapse in the TCD and IST cohorts, Treatment-related mortality and disease-free and OS rates were also similar in the two groups. At 1 year, 54% and 12% of patients were still on immune suppression in the IST and TCD cohorts, respectively. TCD led to a higher GVHD-free survival at 2 years compared with IST (41% vs. 19%, $p = 0.006$). A retrospective study from the Memorial Sloan Kettering Cancer Center and the M.D. Anderson Cancer Center examined outcomes of transplants for patients with AML in first remission at the two institutions, where TCD was utilized at one center and conventional GVHD prophylaxis at the other. The incidences of grade II–IV acute GVHD and chronic GVHD were significantly lower in the TCD graft group (5% vs. 18% and 13% vs. 53%). Three-year relapse-free survival (RFS) and OS rates were comparable in recipients of TCD and unmodified grafts [43]. These comparative analyses emphasize the need for a prospective randomized trial to assess the impact of ex vivo TCD on chronic GVHD free survival.

Haploidentical donor TCD transplantation

Overcoming major HLA barriers to permit transplantation of mismatched grafts remains a major goal of allogeneic transplantation. It has long been hoped that TCD could make that goal a reality. Although early studies of TCD in HLA-mismatched related BMTs were plagued by high incidences of graft failure and GVHD, later series have demonstrated better results. Grade II–IV GVHD incidence has ranged from 13% to 40% in recipients of HLA-mismatched BMT after TCD using monoclonal antibody and complement alone or in conjunction with steroids, cyclosporine, and ATG [44,45]. Unfortunately, the overall outcome was compromised by increases in graft failure, infection, and relapse.

Improved engraftment of T-cell-depleted haploidentical hematopoietic cells has been achieved through infusion of high ("mega") doses of mobilized peripheral blood cells depleted of T cells after $CD34^+$ selection [46,47]. Immunologic analysis of these haploidentical extensively T-cell-depleted transplants led to an appreciation of the role of NK cells and killer-cell immunoglobulin-like receptors (KIRs) in allorecognition and GVHD [46,48]. In these studies, acute leukemia patients underwent haploidentical transplantation with an intensive high-dose conditioning regimen coupled with extensive TCD using sheep red blood cell (RBC) rosetting and CD34 selection of the PBPC product. No post-transplant immune suppression was given. Among 60 patients transplanted, only four cases of GVHD were observed. Notably, patients in this group whose donor–recipient HLA disparity predicted KIR mismatch and NK-cell alloreactivity in the graft-versus-host direction experienced no graft failure, acute GVHD, or myeloid leukemia relapse. High frequencies of alloreactive donor NK cells were detected in recipient blood post-transplant that could specifically lyse leukemia targets missing KIR ligands, and not control target cells that expressed the "self" KIR ligand. Specific KIR and HLA genotypes associated with NK activation have also been demonstrated to have prognostic importance in the setting of HLA-identical T-cell-depleted transplantation for AML [49]. A European Group for *Blood* and Marrow Transplantation (EBMT) survey of 173 adults undergoing haploidentical HCT after exhaustive TCD demonstrated similar results [50]. The most frequently encountered limitation of this approach has been immune incompetence and increased risk of fatal infections. Attempts to address this stumbling block have focused infusions of conventional donor T cells either alone, after photodepletion, or in conjunction with regulatory T cells, with promising results reported in single-institution studies [51,52].

In vivo anti-T-cell antibody TCD

Whereas ex vivo methods of TCD may allow engineering of a graft with a precise T-cell dose, in vivo administration of anti-T-cell antibodies yields a level of T-cell reduction that is much less predictable, owing to the variable half-lives of these antibody preparations, the doses of antibodies used, the administration schedule, and the number of T cells contained in the donor graft. There are currently four products in widespread use in different geographic locations around the world. Three of these are polyclonal anti-T-cell antibody preparations that are considered antithymocyte globulins (ATGs). These products are all different and should be evaluated individually. Atgam is produced from horses inoculated with human thymocytes. Thymoglobulin is also directed against human thymocytes but is raised in rabbits. ATG-Fresenius, also rabbit derived, is actually not an antithymocyte preparation at all but is directed against a Jurkat T-cell leukemia line. Consequently, the antigens targeted and cells eliminated will vary depending on antibody preparation used [53]. Alemtuzumab is a monoclonal antibody directed against CD52, which is broadly expressed on T cells and also other cellular elements including B cells and dendritic cells.

Most comparative studies on the efficacy of adding in vivo TCD to conventional pharmacologic GVHD prophylaxis have been retrospective. In a matched pair analysis, Russell et al. found that at a Thymoglobulin dose of 4.5 mg/kg, acute and chronic GVHD rates were significantly lower compared with immunosuppressive therapy alone in matched related graft recipients following high-dose busulfan/fludarabine-based conditioning. Non-relapse mortality was also lower. Despite a higher relapse rate in the ATG cohort, OS was superior (66% vs. 50%, $p = 0.046$) in ATG recipients [54]. This report is the only retrospective or prospective study that has shown a survival advantage for in vivo TCD. Bredeson et al. at the CIBMTR conducted a matched-pair analysis of Thymoglobulin use in patients conditioned with high-dose busulfan plus cyclophosphamide. Again, ATG was associated with lower acute GVHD rates, but not chronic GVHD rates. Non-relapse mortality was lower but relapse was higher in the ATG cohort, resulting in no difference in disease-free survival [55]. Similar patterns have been noted in single-institution series utilizing alemtuzumab, lower GVHD rates, but higher infection and relapse rates with no difference in OS [56]. The CIBMTR compared pediatric acute lymphoblastic leukemia (ALL) patients undergoing myeloablative HCT receiving ATG preparations ($n = 191$), alemtuzumab ($n = 131$), or no in vivo TCD ($n = 392$). Doses and origin of ATG preparations were not specified. Both ATGs as a class and alemtuzumab were associated with lower rates of acute and chronic GVHD. Lower GVHD rates did not affect relapse incidence, nor was there any effect on NRM. Consequently, in vivo TCD did not improve event-free survival or OS [57]. Mohty et al. reported a retrospective experience of myeloablative HCT with or without Thymoglobulin in adult patients. ATG recipients did not have a statistically significant reduction of acute GVHD although there was a dramatic decrease in chronic GVHD [58]. Again, there was no impact on relapse, NRM, leukemia-free survival (LFS), or OS.

There is substantial evidence that the dose of in vivo T-cell antibodies influences both GVHD incidence and immune reconstitution. Retrospective French series in matched related donors examining doses of 2.5, 5.0, and 7.5–10 mg/kg Thymoglobulin demonstrated that higher doses were associated with lower rates of acute and chronic GVHD, but that did not translate into an improvement in LFS or OS [59,60]. Fewer comparative studies have been performed with other ATG preparations, but one study of ATG-Fresenius suggested that equivalent GVHD control can be achieved with lower doses than in standard use (30 vs. 60 mg/kg) with a suggestion of lower NRM [61].

ATG levels have been measured after infusion in an attempt to correlate these levels with clinical outcomes. Remberger and Sundberg studied rabbit IgG levels on days 0, 1, and 7 after unrelated donor HCT and found a correlation between high levels and protection from acute GVHD [62]. Podgorny et al. examined ATG levels on days 7 and 28 after transplant in patients who had received 4.5 mg/kg of Thymoglobulin. Low levels at those times correlated with increased acute and chronic GVHD whereas high levels were associated with the development of post-transplant lymphoproliferative disorder (PTLD). ATG levels, however, did not impact relapse or OS [63]. After umbilical cord blood transplantation, low serum levels of ATG (Thymoglobulin) on day 11 were associated with higher rates of grade III–IV acute GVHD and higher NRM, but a lower incidence of relapse [64]. Hoegh-Petersen et al. went one step further, examining day 7 serum levels of ATG antibodies specific for particular lymphoid subsets in 120 patients who had received 4.5 mg/kg of Thymoglobulin. They found that high levels of antibodies with specificities for conventional T and B cells were associated with lower acute GVHD and higher viral infection rates, without relation to relapse. Of interest, high specificities to both regulatory T (Treg) cells and invariant natural killer T (NK-T) cells were linked to lower relapse rates [65].

There may be other factors in addition to the actual ATG dose and number of T cells in the graft that affect ATG dose levels. Studies of solid organ transplant recipients have identified that the presence of antibodies to rabbit-derived globulin in the serum accelerates clearance and results in lower serum level of ATG. One group reported that 20 of 72 pediatric patients who had received ATG developed anti-ATG antibodies. In those who developed IgG (as opposed to IgM) anti-ATG antibodies before day 22, rapid clearance of ATG was observed, as was faster T-cell recovery and a higher rate of acute GVHD [66].

Unfortunately, there are only a limited number of prospective randomized controlled trials available to assess intravenous TCD. Kumar et al. reviewed seven such trials enrolling 733 patients randomized to receive ATG. Pooled results showed no difference in NRM or OS with use of ATG. There was a significant benefit for prevention of grade III–IV acute GVHD. Insufficient data were available to assess chronic GVHD in this review [67]. In two of these randomized trials, Bacigalupo et al. compared cyclosporine/methotrexate with cyclosporine plus Thymoglobulin (15 mg/kg in one study, 7.5 mg/kg in the other) in recipients of matched unrelated donor myeloablative BMT. Only the higher dose of ATG was associated with a significant reduction in grade III–IV acute GVHD. However, this reduction in severe acute GVHD did not improve outcomes because 30% of the patients in the high-dose ATG arm died of severe infections. OS was not affected by the use of either dose of ATG [68]. Data from these randomized ATG trials suggested a reduction in chronic GVHD for those receiving ATG with a reduction in long-term pulmonary complications and late deaths [69].

The largest prospective randomized trial of in vivo TCD was conducted in Europe and compared patients randomized to conventional pharmacologic GVHD prophylaxis alone with those randomized to immune suppression plus ATG-Fresenius at a dose of 20 mg/kg administered on days –3, –2, –1 (60 mg/kg total). A total of 250 patients were registered. Finke et al. reported lower grade II–IV acute GVHD in the ATG-Fresenius group (33% vs. 51%, $p = 0.011$) and lower grade III–IV rates (11.7% vs. 24.5%, $p = 0.054$). The most striking difference was in extensive chronic GVHD incidence (12.2% vs. 42.6%, $p < 0.0001$). Relapse rates, NRM, DFS, and OS were not significantly different in the two groups [70]. Socié et al. reported the extended follow-up of these patients and found that immune suppression-free survival was markedly superior for the ATG-Fresenius group [71]. As of 2013, two additional large prospective randomized trials are under way, one assessing Thymoglobulin in Canada and a blinded trial testing ATG-Fresenius in myeloablative transplantation in the United States. Results of these trials will be extremely valuable in establishing the role of these agents in recipients of full-intensity conditioning. If these studies demonstrate lower GVHD rates despite no OS advantage, they still might represent an important advance, as Yu et al. demonstrated in a comparative series that ATG administration was associated not only with lower GVHD rates, but also improved quality of life parameters [72].

In vivo TCD and reduced-intensity conditioning HCT

Over the past decade, there has been a dramatic increase in the use of reduced-intensity conditioning (RIC) allogeneic HCT. The primary antitumor activity in RIC procedures stems not from the cytotoxic effects of low-dose chemotherapy, but from the alloimmune effect of the donor cells. Hence there might appear to be little rationale for TCD in RIC HCT if TCD substantially blunted the GVL effect, thereby defeating the purpose of the transplant itself. Furthermore, RIC preparative regimens might not be sufficiently immune suppressive to support and sustain the engraftment of T-cell-depleted hematopoietic cells or marrow. Nonetheless, both ATG preparations and alemtuzumab have been used to achieve simultaneous depletion of donor and host T cells to ensure engraftment and reduce in GVHD in RIC HCT. As with high-dose transplantation, these antibodies lead to delayed immune reconstitution and a higher incidence of disease relapse and virus infections, especially from CMV and adenovirus [73–75].

There is no consensus on which antibody preparation yields the best results. In a comparative analysis of two prospective studies using either alemtuzumab/cyclosporine or cyclosporine/methotrexate as GVHD prophylaxis after RIC, there was a significantly lower incidence of acute and chronic GVHD in the alemtuzumab patients, but a higher incidence of CMV reactivation and disease relapse/progression [73]. However, long-term disease and OS status were similar in the two cohorts when responses were considered after donor lymphocyte infusion (DLI) in the patients in the alemtuzumab arm. These data and others suggest that TCD can be used to induce a state of mixed chimerism with minimal GVHD, and provide a platform for additional adoptive immunotherapy interventions such as DLI or vaccinations to bolster immune function and GVL [27,76,77]. Ex vivo TCD has also been studied in RIC HCT. $CD34^+$ selection of donor PBPCs after RIC led to a low rate of GVHD [78]. However, both engraftment and disease relapse have been problems in the absence of additional DLI.

Unfortunately, there has yet to be a prospective randomized trial evaluating the consequence of in vivo anti-T-cell antibody administration in RIC HCT. It is critical to determine whether immune modulation with anti-T-cell antibody infusion abrogates the therapeutic benefits of transplantation. The CIBMTR examined 1676 adults undergoing RIC-related ($n = 792$) and -unrelated ($n = 884$) HCT for hematologic malignancies [79]. Outcomes after in vivo TCD ($n = 584$ ATG, $n = 213$ alemtuzumab) were compared with T-cell-replete ($n = 879$) transplantation. Engraftment was not aided by in vivo TCD; in fact, it was slightly inferior. Grade II–IV acute GVHD was lower with alemtuzumab than ATG or T-cell-replete regimens (19% vs. 38% vs. 40%, $p < 0.0001$) and chronic GVHD, and lower with alemtuzumab and ATG regimens than T-cell-replete approaches (24% vs. 40% vs. 52%, $p < 0.0001$). However, relapse was more frequent with alemtuzumab and ATG than T-cell-replete regimens (49%, 51%, and 38%, respectively, $p < 0.001$). DFS was lower with both alemtuzumab and ATG than T-cell-replete regimens (30%, 25%, and 39%, respectively, $p < 0.001$). Corresponding probabilities of OS were 50%, 38%, and 46% ($p = 0.008$). The EBMT conducted a similar analysis in 1859 AML patients undergoing RIC HCT [80]. In vivo TCD with ATG or alemtuzumab was successful at preventing extensive chronic GVHD ($p < 0.001$), but without improving OS for ATG and even with worsening OS for alemtuzumab (hazard ratio 0.65, $p = 0.001$). These collective data suggest adopting a cautious approach to in vivo TCD with RIC regimens and emphasize the need for randomized controlled trials in the RIC setting.

Organ Dysfunction and TCD

In single-institution TCD series, low rates of hepatic, renal, and pulmonary complications have been reported [81–83]. In an analysis of 199 allogeneic transplants, the incidence of severe pulmonary complications was 8% among those who received CD6 TCD as the sole form of GVHD prophylaxis, but 33% among those who received cyclosporine and methotrexate [83]. The protective effect of TCD against pulmonary complications was independent of acute GVHD. The incidence of hepatic sinusoidal obstructive syndrome (SOS), has been reported to be fairly low after TCD BMT [81,82]. This reduction in hepatotoxicity may be due to the fact that the TCD patients are spared from injurious effects of methotrexate and/or cyclosporine/tacrolimus. Alternatively, decreases in alloreactivity may lead to lower levels of circulating cytokines that could damage hepatic endothelium. In the 410 patient prospective randomized study reported by Wagner et al., recipients of T-cell-depleted marrow experienced less regimen-related toxicity within the first 28 days of transplant, including stomatitis ($p < 0.0001$), hepatic ($p = 0.0003$), pulmonary ($p = 0.012$), and central nervous system complications ($p = 0.024$) [6].

Engraftment and TCD

Graft failure or rejection has been reported to occur more frequently in patients receiving TCD bone marrow than in recipients of T-cell-replete grafts. Whereas graft failure usually occurs in 1% or less of patients with leukemia receiving HLA-identical products, studies from the 1980s and early 1990s reported graft failure rates of 3–40% depending on the methodology used. Registry analysis from the CIBMTR in the era identified a significantly increased risk for graft failure compared with unmanipulated BMT (relative risk 9.29, $p < 0.0001$) [34].

Graft failure after TCD HCT may occur in two patterns: (1) failure of initial engraftment or graft rejection within 2 weeks of HCT, and (2) delayed graft failure which can occur months following transplant. Early graft failure after TCD transplantation likely represents primary immunologic rejection of donor hematopoietic elements by host lymphoid cells that have survived the conditioning regimen. Host T-lymphocytes with donor-specific activity have been isolated from the blood of patients at the time of graft rejection [84,85]. It is likely that TCD removes cells that are important for promoting engraftment. However, it remains uncertain what roles specific donor cells play in establishing and maintaining a graft in human transplantation. Graft failure has been much less commonly observed in recent studies of TCD-mobilized peripheral blood HCT. In a 2007 report, all 52 patients transplanted with $CD34^+$-selected TCD-matched sibling-mobilized PBPCs engrafted after conditioning with fractionated TBI, *N*-triethylenethiophosphoramide (thioTEPA), and fludarabine without ATG, despite only receiving a median of 0.99×10^3 cells/kg [86]. Other series from the past decade have likewise reported low graft failure rates [39,40].

The mechanism behind late graft failure remains unknown. Unlike primary rejection, donor-specific host T-lymphocytes have not been isolated from the blood of recipients at the time of late graft failure. Since mixed hematopoietic and lymphoid chimerism is common after TCD transplantation, late graft rejection may result when this state of immune tolerance is broken by some event. Even in patients in whom hematopoietic recovery is observed, there is an increased frequency of mixed hematopoietic chimerism [87]. This has been particularly noted in the T-cell compartment. Viral infections with CMV or human herpesvirus 6 (HHV6) may also contribute to late graft failure after HCT [88,89].

Attempts to thwart immunologic rejection after HCT have included increasing host immune suppression, intensification of the conditioning regimen to create space or additional immune suppressive agents after transplantation [32,44,90]. It is not clear how intensive conditioning must be to achieve hematopoietic recovery after TCD transplantation. Successful engraftment of haploidentical family donor PBPCs using an RIC regimen plus alemtuzumab has been reported with a 16% incidence of grade II–IV acute GVHD [91]. Other approaches have included narrowing the spectrum of TCD, infusion of increasing doses of CD34$^+$ cells in the graft, and administration of donor lymphocytes after transplantation. There are no randomized data available to support the use of any of these interventions.

Immune reconstitution and infectious complications after TCD HCT

Immune reconstitution is delayed after ex vivo TCD [92–95]. In general, total lymphocyte counts are lower in recipients of TCD grafts. Prolonged inversion of CD4$^+$/CD8$^+$ ratios are observed with delays in CD4$^+$ T-cell recovery. The number of T cells with memory phenotypes is decreased. Delays in functional recovery of T cells after TCD HCT have also been described. Proliferative responses of PBMCs to mitogenic stimuli are impaired far longer after TCD transplants than after conventional procedures [92]. T-lymphocytes from recipients of TCD BMT have restricted variability in their T-cell receptor repertoires, limiting the breadth of potential cellular responses [93]. The capacity to generate new T cells is impaired after TCD, as noted by decreased numbers of thymic emigrants measured by T-cell receptor excision circle (TREC) analysis [94,95]. Deep sequencing techniques have demonstrated that recovery of T-cell receptor diversity is particularly delayed in CD8$^+$ cells after TCD HCT, even more so than in T cells following umbilical cord blood HCT [96]. Comparative studies on immune reconstitution after in vivo TCD with ATG administration have demonstrated high levels associated with slower T-cell but more rapid B- and NK-cell recovery [97].

Delayed immune reconstitution and loss of TCR repertoire diversity may place TCD HCT recipients at increased risk for opportunistic infections. CMV reactivation and adenovirus infections occur more frequently after TCD transplants and in recipients of in vivo anti-T-cell antibodies, particularly alemtuzumab [73–75,98]. Partial TCD may not abrogate transfer of anti-CMV T-cell immunity although protection against CMV appears lost with more extensive depletion [99,100]. In a large randomized study of ex vivo TCD in unrelated BMT, the overall rate of fatal infections did not differ between the TCD and non-TCD arms. Bacterial infections were not increased. However, higher incidences of severe CMV (28% vs. 17%, $p = 0.02$) and life-threatening *Aspergillus* (16% vs. 7%, $p = 0.01$) infections were observed in the patients receiving TCD [6,101].

A number of studies have demonstrated a higher probability of Epstein–Barr virus (EBV) reactivation in recipients of TCD grafts [3]. Recipients of TCD transplants using HLA-mismatched or unrelated donor marrow appear to be at particularly high risk, as are patients with severe GVHD and those treated with specific anti-T-cell monoclonal antibodies. Recipients of cord blood transplantation are also at high risk for EBV–PTLD, especially if they are conditioned with a reduced-intensity regimen that includes ATG [102]. Rituximab has been utilized as therapy for PTLD [103] and in a preemptive mode in the setting of rising EBV viral loads in the absence of documented disease [104]. Donor lymphocyte infusions have been successful in treating PTLD but may induce GVHD [105]. EBV-specific cytotoxic T cells have been demonstrated to lead to regression of PTLD with only limited GVHD [106]. The production of virus-specific T-lymphocytes with the capacity to recognize CMV, EBV, and adenovirus but without alloreactive potential is being studied in both therapeutic and prophylactic settings [107,108]. Other strategies to accelerate recovery of T-cell immunity after TCD HCT include attempts to improve thymic function through administration of interleukin (IL)-7 and luteinizing hormone-releasing hormone (LHRH) antagonists [109,110].

Disease relapse and TCD

The increased risk of disease relapse after TCD BMT was initially recognized in patients with CML. In patients receiving matched sibling BMT for first chronic-phase CML, the risk of relapse with TCD has been estimated to be 5–6-fold higher than in conventional transplantation [4,5]. The incremental risk of relapse is less apparent after TCD transplantation using matched unrelated marrow [111]. A study at the EBMT demonstrated that in patients with CML who received unrelated donor BMT, TCD was not associated with a higher incidence of relapse in the multivariate analysis [112]. However, in the large multi-center randomized trial of TCD reported by Wagner et al., the 3-year relapse incidence was higher in the TCD patients transplanted for CML (20% vs. 7%, $p = 0.017$) [6].

The increased incidence of CML relapse after TCD HCT has been linked, at least in part, to the reduction in GVHD. It is well established that allogeneic HCT is superior to autologous or syngeneic transplants in CML because donor T cells can mediate alloimmune responses, and patients who develop clinically significant GVHD have a better LFS after HCT [113]. Direct evidence of the importance of donor T cells in the GVL effect have been derived from DLI studies for patients with CML who have relapsed after allogeneic BMT, where complete remission rates of 70–80% were achieved [114,115]. The diseases for which DLI appears efficacious are the same as those in which TCD has been linked to increased relapse rates. These include, in addition to CML, both multiple myeloma and chronic lymphocytic leukemia. The risk of leukemia relapse may also vary depending on the extent and specificity of TCD employed. “Narrow-specificity” antibodies appear to be associated with a lower relapse rate than “broad-specificity” TCD methods. The 5-year probability of leukemia relapse was 28% for recipients of narrow-specificity TCD BMT versus 51% for recipients T-depleted by other techniques ($p < 0.001$) [22]. The 5-year relapse rate for recipients of “narrow-specificity” TCD was similar to that observed in patients who received unmanipulated BMT. Risks factors for relapse after TCD HCT may be linked to genetic polymorphisms in the donor CTLA4 genotype and the capacity to generate and sustain a GVL response [116].

In contrast to CML, TCD appears to have a less significant effect on the relapse rates of patients transplanted for acute leukemia [117,118]. In two separate randomized trials comparing TCD with methotrexate and cyclosporine as GVHD prophylaxis for patients undergoing HLA-matched related or unrelated BMT, a higher relapse rate was observed after TCD BMT only in patients with CML, not in patients with acute leukemia [6,119]. In addition, in the comparative study of CD34$^+$ TCD reported by the BMT CTN in AML patients and in the randomized ATG-Fresenius trial including AML, myelodysplastic syndrome, and ALL, both after myeloablative conditioning, there was no increase in relapse rate with either ex vivo or in vivo TCD [42,70,71]. These trials excluded patients with advanced disease, hence this observation should not be extrapolated to all patients. Nor should it be extended to patients receiving RIC conditioning, as the retrospective analyses of the CIBMTR and EBMT clearly show a higher relapse rate for both ATG and alemtuzumab in this setting [79,80].

Efforts aimed at reducing leukemia relapse after TCD have focused primarily on immune-based strategies to enhance GVL activity without compromising GVHD prevention. These approaches have included selective purging of T-lymphocyte subsets (e.g. $CD8^+$ T cells), prophylactic donor lymphocyte infusion, and induction of NK cell-mediated antitumor activity. Donor lymphocyte infusion or T-cell add-back strategies have been used in conjunction with TCD to restore GVL activity and prevent disease relapse. Prophylactic DLI strategies may be useful after TCD HCT using CD34 selection, ATG, or alemtuzumab, where the extensive depletion leads to profound and long-lasting impairment of immunity [69,75–78,120]. Unfortunately, GVHD is common after these infusions. Although the incidence of GVHD after prophylactic DLI is similar to that with conventional transplantation, a report suggests that chronic GVHD after T-cell add-back is associated with less mortality, but retains its protective effect on relapse [120]. Retrospective studies in patients with CML and multiple myeloma undergoing high-dose transplantation have suggested that TCD plus DLI is a reasonable alternative to conventional GVHD prophylaxis, and may in some circumstances be associated with superior outcome [121–123]. An update of the Spanish and British RIC experience in Hodgkin lymphoma suggested that with the addition of DLI, current DFS was superior for patients treated with cyclosporine/alemtuzumab than those receiving cyclosporine/methotrexate for GVHD prophylaxis, although again randomized data are lacking [124].

Novel approaches to graft manipulation

Novel strategies to limit the risk of GVHD associated with either hematopoietic cell or donor lymphocyte infusions are under investigation and include photoinactivation, anergy induction, and elimination of cells expressing activation antigens (e.g., CD25, CD69). These approaches usually involve a brief coincubation of donor cells with recipient-derived antigen-presenting cells. Theoretically, this approach would preferentially purge alloreactive lymphocytes responsible for GVHD, but retain "non-alloreactive" T cells that might improve post-transplant immune reconstitution against infectious pathogens [125]. If minor histocompatibility antigens responsible for GVH, but not GVL, can be routinely identified, then targeted depletion of those cells recognized those minor antigens could represent a major advance [126].

Herpes simplex thymidine kinase (HSV-TK) gene insertion into donor T cells represents another approach for controlling GVHD after allogeneic transplantation or DLI. These cells have been rendered susceptible to destruction after exposure to ganciclovir. Infusion of $HSV\text{-}TK^+$ T-lymphocytes with TCD marrow graft did not hamper engraftment, and resulted in stable establishment of circulating TK^+ cells in the recipient post-transplant [127]. HSV-TK gene insertion has also been studied in the DLI setting without compromise of antitumor activity [128]. In one study, a direct correlation between antitumor response with peak levels of TK^+ cells in circulation was seen. Moreover, circulating TK^+ cells could be disrupted by the administration of ganciclovir.

More recently, the recognition that certain T cells have regulatory function that can suppress graft-versus-host reactions may play a role in the design of future TCD strategies that spare these negative regulators [129,130]. $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells have been identified as important mediators of self-tolerance, auto/alloimmunity, and immune reconstitution after HCT. Murine transplant models have demonstrated that Treg cells are potent suppressors of alloimmune responses, and can protect against GVHD [129,131]. Lower levels of Treg cells have been associated with development of acute and chronic GVHD [132,133]. Infusions of cultured Treg cells have been used to try to prevent GVHD after umbilical cord blood transplantation [134]. Early uncontrolled data suggest that infusion of freshly isolated Treg cells can prevent induction of GVHD by conventional T cells when administered around the time of transplantation [52]. In vivo expansion of Treg cells with low doses of recombinant IL-2 are being investigated in patients with established chronic GVHD based upon the observations from clinical trials that this cytokine preferentially expands Treg cells in cancer and transplant patients [135]. Low dose IL-2 has been used as a means to treat correct Treg cell deficiency in patients with established refractory chronic GVHD with promising results reported [136]. Results from trials combining adoptive Treg cell immunotherapy with IL-2 administration are under way.££

Conclusion

Although it is clear that TCD reduces the incidence of acute and chronic GVHD, there is as yet no definitive evidence that TCD improves OS after HCT. Nonetheless, many investigators remain optimistic that graft engineering and targeted T-cell antibodies will one day fulfill the promise of separating GVH and GVL. Current evidence suggests that in patients with acute leukemia undergoing myeloablative transplantation, in vivo and ex vivo TCD can do just that. However, that may not be true after RIC HCT. Further delineation of the cellular subsets that promote engraftment and control alloimmune, antitumor, and antiviral responses is needed to optimize graft engineering. It is critical that as promising ex vivo or in vivo TCD strategies are developed, they be definitively tested in prospective randomized trials.

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CHAPTER 83

Manifestations and Treatment of Acute Graft-versus-Host Disease

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Background, definition, and incidence of acute graft-versus-host disease

The first description of graft-versus-host disease (GVHD) is ascribed to Barnes and Loutit in 1955, who reported on "secondary disease" in animals undergoing transplantation [1]. Since then, much of the pathophysiology of acute GVHD has been deciphered (reviewed in Chapter 13); however, much remains to be learned. A review of methods to prevent acute GVHD through pharmacologic and graft manipulation can be found in Chapters 81 and 82, and this chapter discusses the diagnosis and treatment of established acute GVHD.

The term acute GVHD traditionally described a distinctive syndrome of cutaneous, hepatic, and gastrointestinal (GI) inflammation developing within 100 days of allogeneic hematopoietic cell transplantation (HCT). The term chronic GVHD described a more pleiotropic syndrome that developed after day 100 (see Chapter 84). However, it is now recognized that acute GVHD can occur after day 100 and chronic GVHD may occur before then, and the syndromes are currently defined by their clinical features, rather than their timing in relation to transplantation. Late-onset acute GVHD predominantly occurs after reduced-intensity conditioning (RIC) regimen-based allogeneic transplantation [2] or in the context of rapid immunosuppressant tapering.

Incidence and staging

Table 83.1 presents the commonly used Consensus criteria for the staging and grading of acute GVHD [3,4]. A modification of grade II disease includes symptoms of nausea, anorexia, food intolerance, or vomiting confirmed to be enteric GVHD on upper intestinal biopsy [4,5]. The staging system proposed by the International Bone Marrow Transplant Registry (IBMTR) uses the same individual organ staging but modifies the overall grading scheme slightly (Table 83.2) [6]. Although both systems predict overall survival equally well, the Consensus system predicted earlier survival better in a comparison of 607 patients. The IBMTR index, however, was easier to use and was associated with less physician bias or error in stage assignment [7].

Depending on the prophylactic regimen used, clinically relevant grade II–IV acute GVHD occurs in 20–40% of patients transplanted from a matched sibling donor [8] and in 30 to >50% of recipients of unrelated donor grafts [9,10]. Severe acute GVHD (grade III–IV) occurs in up to 20% of recipients of related donors [8] and up to 35% of unrelated donors [9,10]. Reliable incidence figures for acute GVHD are limited by interobserver and intercenter variation. This variability largely derives from uncertainty in establishing grade II GVHD [11,12]. The median time to the diagnosis of acute GVHD varies with conditioning, with recipients of myeloablative conditioning being diagnosed at a median of 17 days from transplantation [13], in comparison with recipients of RIC transplantation being diagnosed at a median of 3 months [2], and is more commonly associated with the tapering of immunosuppressive agents.

Risk factors for acute GVHD

Human leukocyte antigen disparity

Human leukocyte antigen (HLA) disparity between the hematopoietic cell donor and recipient is the most powerful factor governing the severity and kinetics of acute GVHD. The incidence of acute GVHD is much increased with HLA-non-identical donors compared with HLA-identical donors [14].

Although traditionally the HLA class I antigens HLA-A and HLA-B and the class II antigen HLA-DRβ1 have been used for matching purposes, other HLA antigens, namely HLA-C, HLA-DQ, and HLA-DP, are also relevant in acute GVHD and transplantation outcomes [15–19]. In a large, retrospective National Marrow Donor Program (NMDP) study, Lee et al. demonstrated that both allelic and antigenic mismatches at any single locus, when compared with fully matched unrelated donor transplantation, were associated with excess acute GVHD; however, no differences between antigenic and allelic mismatches were noted overall [20]. When examined at individual HLA loci, mismatches at any given locus were associated with a relative risk increase of acute GVHD of approximately 1.6-fold, although not all comparisons were statistically significant owing to small sample sizes.

Although some studies have identified individual HLA antigens that are associated with increased rates of GVHD [21], these correlations have not been observed consistently [22,23]. It is likely that as further refinements to HLA-typing techniques are made, and an improved understanding of how individual disparities in HLA affect antigen presentation, recognition, and signal transduction is developed, the role of individual HLA disparities will become more evident.

Hematopoietic stem-cell source and graft composition

The source of allogeneic hematopoietic cells may also influence the development of GVHD. For more than two decades, experience was derived from bone marrow transplant (BMT) recipient cohorts

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Table 83.1 Modified Glucksberg grading of acute GVHD [4]

	Skin[a]	Liver	Gut
Organ stage			
1	Rash <25%	Bilirubin 2–2.9 mg/dL	Diarrhea 500–1000 m^3/day or biopsy-proven upper GI involvement
2	Rash 25–50%	Bilirubin 3–6 mg/dL	Diarrhea 1000–1500 cm^3/day
3	Rash >50%	Bilirubin 6.1–15 mg/dL	Diarrhea 1500–2000 cm^3/day
4	Generalized erythroderma with bullae	Bilirubin >15 mg/dL	Diarrhea >2000 cm^3/day or severe abdominal pain with or without ileus
Overall grade			
I	Stage 1–2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	–	Stage 2–3 or	Stage 2–4
IV	Stage 4 or	Stage 4	–

[a] Use "rule of nines" to determine body surface area.

Table 83.2 IBMTR grading of acute GVHD [6]

Overall grade	Skin*	Liver	Gut
A	Stage 1	None	None
B	Stage 2	Stage 1 or 2	Stage 1 or 2
C	Stage 3	Stage 3	Stage 3
D	Stage 4	Stage 4	Stage 4

describing rates and risk factors for GVHD [21–23]. Recently, peripheral blood progenitor cells (PBPCs) have become the most utilized source of hematopoietic cells for both related and unrelated donor transplantation [24]. Initial phase 2 studies of matched, related donor PBPC transplantation demonstrated an increased incidence of acute GVHD [25–28], and multiple randomized, controlled trials have compared GVHD outcomes between matched sibling BMT and PBPC transplantation [29–36]. In the largest randomized trial involving 350 patients enrolled between 1995 and 1999, grade II–IV and grade III–IV acute GVHD were significantly higher in the PBPC group (52% vs. 39%, p = 0.014, and 28% vs. 16%, p = 0.01, respectively). In this trial, however, an abbreviated three-dose course of methotrexate was used in both arms as GVHD prophylaxis [35]. A randomized trial involving the Seattle, City of Hope, and Stanford groups demonstrated a non-significant increase in the hazard ratio (HR) for both acute GVHD [HR 1.21, 95% confidence interval (CI) 0.81–1.81] and severe acute GVHD (HR 1.27, 95% CI 0.55–2.89) in the PBPC arm of the trial [29]. Other trials have reported non-significant increases [32,36] or similar risks in the PBPC and BMT groups [30,31,34], with only one small trial demonstrating a statistically significant increase in acute GVHD in the BMT arm [33]. Two meta-analyses found that the relative risk for acute GVHD after PBPC transplantation was 1.16-fold greater (p = 0.006) [37] or 1.14-fold greater (p = 0.2) [38] compared with BMT. The latter meta-analysis demonstrated a statistically significant increase in severe acute GVHD after PBPC transplantation [odds ratio (OR) 1.13, p = 0.03] [38].

In unrelated donor transplantation, initial small phase II studies of PBPC transplantation with retrospective comparisons with BMT did not demonstrate differences in the rates of acute GVHD [39,40]; however, a large retrospective review of over 1300 patients from the Center for International Blood and Marrow Transplant Research (CIBMTR) database demonstrated that acute GVHD may be more prevalent (56% vs. 46%, p < 0.001) after PBPC transplantation than BMT for leukemia and myelodysplasia [41]. In the largest prospective comparison of BMT and PBPC transplantation in unrelated donors, 551 transplant recipients were randomized to receive either PBPC transplantation or BMT. In this study, the cumulative incidence of acute GVHD (approximately 50%) and severe acute GVHD was no different between treatment groups [42].

Umbilical cord blood (UCB) transplantation is associated with the lowest relative rates of acute GVHD given the multiple HLA mismatches commonly encountered. This difference in acute GVHD incidence is likely attributable to the paucity and skewing of T-cell subsets found in UCB units. Since T cells are found in much higher concentrations in PBPC grafts, it is commonly assumed that this accounts for the differences in GVHD outcome between bone marrow (BM) and PBPC grafts [43–46]. The fact that either in vivo or ex vivo T-cell depletion is associated with reduced rates of acute GVHD is the strongest evidence correlating this cellular subset and the pathogenesis of acute GVHD [47,48]. The role of specific T-cell subsets remains controversial, with some investigators demonstrating important correlations between $CD8^+$ T-cell content [46,49,50] and $CD4^+CD25^+$ regulatory T-cell content [51–55] to be important predictors of the occurrence of acute GVHD.

The number of $CD34^+$ progenitors in a granulocyte colony-stimulating factor (G-CSF)-mobilized PBPC graft is generally higher than in a BM harvest [56]. The dose of $CD34^+$ progenitors has been shown to affect the development of chronic GVHD [45,57], but this association is less clear for acute GVHD [43,45,58–60]. Similarly, the contribution of other cellular subsets contained within a graft to the incidence of acute GVHD remains unknown.

Donor–recipient clinical factors

Both donor and recipient age can influence the occurrence of acute GVHD. In a large analysis of NMDP donor–recipient pairs, increasing donor age was associated with an increased incidence of severe acute GVHD [61]; however, a CIBMTR analysis did not associate donor age with GVHD occurrence [62]. The association of increasing donor age and acute GVHD is also noted among pediatric transplant recipients, where rates of acute GVHD are typically lower [63], possibly related to the presence of a functional thymus in young people. Increasing recipient age is also a known risk for GVHD, but since sibling ages are often closely linked, it has been difficult to demonstrate the independent effect of donor age on GVHD incidence in matched, related donor transplantation. For older recipients, however, it appears that older matched sibling donors remain preferable to younger matched unrelated donors [64].

Donor–recipient gender mismatching and donor parity are associated with an increased risk of acute GVHD, with male recipients of female donor grafts having the highest rates of GVHD [21–23,63,65]. The pathogenesis of this phenomenon is due to the recognition of Y chromosome-encoded antigens by female donor T cells. Parity, by virtue of maternal alloimmunization to fetal minor antigens, would be expected to be associated with vigorous anamnestic-type GVHD responses after transplantation. More recent analyses in matched related donor transplantation and in RIC transplantation, however, failed to note an impact of donor gender [66,67] or age on acute GVHD [67].

The influence of ABO blood type on acute GVHD remains controversial. ABO glycotransferase peptides may act as minor histocompatibility antigens and stimulate allogeneic T-cell responses [68]. A large CIBMTR analysis of 3103 donor–recipient pairs demonstrated an increased risk of severe acute GVHD among bidirectional ABO-mismatched pairs (HR 1.869, $p = 0.006$) [69]; however the recent NMDP analysis associated ABO mismatch only with overall mortality, and not acute GVHD [61], and several smaller studies were not able to replicate this finding [53–55]. Since ABO incompatibility has other consequences on graft processing and transfusion requirements after transplantation, it is often considered in the search strategy in unrelated donor transplantation.

Donor and recipient cytomegalovirus (CMV) serostatus may also influence the rates of acute GVHD. For CMV-seronegative BMT recipients, matching with seronegative donors also appears to reduce the risk of both CMV infection and GVHD [21].

Non-HLA genetic factors

A higher incidence of acute GVHD has also been observed with HLA-matched unrelated donors compared with matched, related donors, necessarily implicating minor or non-HLA antigens in the pathogenesis of GVHD [70]. Minor HLA (mHA) antigens, short peptides presented in the context of HLA, have been identified and sequenced, and disparities among mHAs appear to correlate with the risk of GVHD in adults given HLA-identical donor grafts [71–74]. The importance of mHAs is further supported by the observed lower rate of GVHD in populations that feature genetic homogeneity [75]. In more genetically heterogeneous racial groups, data are less consistent concerning increased rates of GVHD [76–78]. Disparities in antigens with presentation restricted to certain class I and II alleles have been associated with GVHD, demonstrating that altered peptide–ligand binding may have profound effects on alloreactivity [79,80]. Even within the HLA gene complex on chromosome 6, there is evidence that there are relevant transplant mHA genes present. HLA haplotype matching has demonstrated that mismatching of HLA haplotypes is associated with an increase in acute GVHD [81].

Polymorphisms in genes [such as interleukin (IL)-2, IL-6, IL-10, the tumor necrosis factor (TNF) superfamily, and others] that lead to altered expression and levels of cytokines have been linked to differences in acute GVHD incidence (reviewed by Chien et al. [82]), presumably by modulating inflammation in the context of alloimmunity. An additional group of distinct polymorphisms that occur in the natural killer (NK) cell receptor and ligand group, the KIR family, have been linked to differences in both GVHD and relapse rates after transplantation [83,84]. The genes involved in the metabolism of methotrexate have been linked to toxicity and GVHD after allogeneic transplantation [85–87]. Finally, genes with only loose associations with the immune system can also influence rates of GVHD [88–92]. Currently, the presence of cytokine polymorphisms is not ascertained prior to allogeneic transplantation; however, several investigators have suggested that search strategies that include donor genotype of cytokines and other relevant genes should be incorporated into search strategies [93–95].

Tolerance

Chapter 12 reviews the mechanisms of graft–host tolerance after allogeneic HCT. Tolerance can be achieved by elimination (clonal deletion) in the thymus of host reactive cells; accordingly, thymic damage could abrogate self-tolerance [96,97]. Alternatively, functional suppressor $CD4^{+}CD25^{+}FoxP3^{+}$ regulatory T cells may be important in transplantation tolerance [98], and strategies to expand or transfer these T cells selectively to abrogate experimental acute GVHD have been developed [99]. Tolerance can also be attained via the transplacental bidirectional passage of maternal and fetal antigens during pregnancy. In this fashion, tolerance to non-inherited maternal antigens (NIMAs) in utero develops [100]. In experimental and human models of haploidentical transplantation, transfer of stem cells from an offspring to a mother or vice versa results in less acute GVHD than transplantation to or from the father, suggesting that NIMA-induced tolerance exists. This tolerance may be dependent on T cells [101]. Consistent with this finding is that among haploidentical sibling transplants, mismatch for non-inherited paternal antigens (NIPAs) appear associated with higher rates of GVHD, suggesting that exposure to maternal HLA antigens in utero could have a lifelong effect on subsequent immune responses [102,103]. Similarly, in UCB transplantation, when maternal HLA-typing is known, the selection of a NIMA-matched UCB unit is associated with improved outcome [104,105].

Conditioning regimen intensity and immunomodulation

The pathophysiology of acute GVHD, reviewed in Chapter 13, describes an afferent activation loop of conditioning-related tissue injury leading to acute GVHD. As such, it has been postulated that lower intensity preparative regimens, by virtue of causing less tissue injury and lower concentrations of elucidated cytokines, would be associated with a reduced incidence of acute GVHD. In a retrospective single-center review of 238 patients who underwent reduced-intensity compared with myeloablative transplantation, the hazard ratio for grade II–IV acute GVHD was 1.73 ($p = 0.015$) despite similar GVHD prophylaxis in both arms [106]. Similarly, the EBMT noted a 40% reduction in the incidence of acute GVHD among 315 reduced-intensity conditioning recipients when compared with over 400 myeloablative conditioning recipients ($p = 0.01$) [107]. A more recent CIBMTR analysis analyzed GVHD risk by grouping conditioning intensity, stem-cell source, and the use of total body irradiation (TBI), and was able to demonstrate a lower incidence of acute GVHD among unrelated donor recipients who received RIC conditioning when compared with myeloablative, TBI-based PBPC grafts [62]. Even among patients undergoing high-dose conditioning, the intensity of the regimen may influence acute GVHD outcomes. Among HLA-identical transplant recipients who received GVHD prophylaxis with methotrexate and cyclosporine, acute GVHD developed in 48% of patients given 1575 cGy TBI, compared with 21% of patients given 1200 cGy TBI ($p = 0.02$) [108]. An alternative hypothesis linking conditioning intensity and acute GVHD is that more intensive conditioning more effectively eliminates host cells and prevents the development of mixed donor–host chimerism, which has been associated with a decreased probability of GVHD [109,110].

Microbial environment

The microbial milieu of the host may influence the development of GVHD. Compared with conventionally housed, irradiated mice, enteric GVHD is significantly reduced in germ-free mice given incompatible marrow [111]. Human studies confirmed this gnotobiotic effect [112,113]; however, decontamination of the human GI tract using broad-spectrum antibiotics may be associated with an increased susceptibility to fungal overgrowth of the GI tract [114]. Microorganisms could act as triggers of GVHD, by sharing antigenic epitopes with gut epithelial cells, by activating latent virus-inducing antigens on cell surfaces to become targets of alloreactivity [115], or by direct activation of Toll-like receptor (TLR) or non-obese diabetic (NOD)-like receptors on epithelial cells [116]. The effect of GVHD on the intestinal microbiome has also been studied, with marked shifts noted in intestinal microflora occurring in patients with active GVHD [117]. One postulated mechanism for these changes is the targeted destruction of Paneth cells in the intestinal mucosa, leading to a reduction in locally produced antimicrobial α-defensins [118]. Changes in gut microflora are associated with inflammatory changes in the intestinal mucosa, which in turn leads to translocation of bacteria across the mucosal barrier into the bloodstream, elaboration of lipopolysaccharide (LPS) in the circulation, and an increase in cytokine effectors of GVHD [119,120].

Laminar airflow protective isolation with gut decontamination has been shown to decrease the incidence of acute GVHD and improve survival in patients with aplastic anemia [112], but improved outcomes have not been generalizable to all transplant scenarios [121,122]. Randomized clinical trials of antimicrobial prophylaxis demonstrate a reduction in GI GVHD after administration of either antifungal prophylaxis with fluconazole compared with placebo [123], or anaerobic intestinal decontamination with metronidazole plus ciprofloxacin compared with ciprofloxacin alone [113]. Keratinocyte growth factor (KGF) has specificity for gut epithelial cells and can prevent epithelial injury from chemoradiotherapy, reduce levels of LPS and TNF, and decrease enteric GVHD in animal models [124]. However, the use of KGF after transplantation has not been shown to reduce acute GVHD in a randomized study [125].

Clinical features of acute GVHD

Cutaneous acute GVHD

The skin is the most commonly involved organ in acute GVHD, affecting over 75% of acute GVHD patients, and is the sole manifestation in 44% of acute GVHD patients [126]. Acute GVHD presents as a maculopapular exanthem, and may be asymptomatic, pruritic, or painful [127]. It may involve the palms and soles, which helps differentiate the rash of acute GVHD from a drug eruption, which generally spares these areas. The characteristic predilection for these sites appears related to concentration of epidermal stem cells in the rete ridges [128]. As the rash intensifies, confluent involvement ensues. In its most severe form, bullae formation ensues and epidermal necrosis with desquamation occurs, mimicking toxic epidermal necrolysis. The bullae contain sterile serous fluid, and unless the acute alloreactive inflammatory reaction has abated, the regenerating skin exposed by the bullae also may be erythematous.

Gastrointestinal acute GVHD

The GI tract is the second most commonly involved organ in acute GVHD, with up to half of individuals being affected [126]. In general, signs of enteric GVHD develop as the chemoradiotherapy effects resolve following the first several weeks after transplantation; however, these syndromes may overlap, making an early diagnosis difficult. Symptoms of acute GVHD of the distal small bowel and colon include profuse watery diarrhea, intestinal bleeding, crampy abdominal pain, and, in its severest form, ileus. The diarrhea is often green, mucoid, watery, and mixed with exfoliated cells, forming fecal casts. Upper GI tract GVHD is associated with anorexia, nausea, and dyspepsia; patients with upper GI disease may not manifest lower GI tract involvement. In the initial description of upper GI GVHD, 13% of related donor recipients and 31% of unrelated donor recipients were noted to be affected [5]. Importantly, the occurrence of isolated upper GI GVHD did not influence survival outcomes, but upper GI GVHD is now included in the modified Glucksberg acute GVHD grading system.

Imaging of the GI tract using computed tomography often reveals diffuse edema, but this finding is non-specific and should be evaluated with endoscopy and biopsy. Endoscopic findings of enteric GVHD range from normal to extensive edema and mucosal sloughing. Lesions may be most prominent in the cecum, ileum, and ascending colon, but may also involve the stomach, duodenum, and rectum. Gastrointestinal endoscopy and biopsy are mandatory for the diagnosis of upper GI tract disease [5,129], since anorexia and nausea are common symptoms of conditioning-related injury. Endoscopy and biopsy are recommended for the diagnosis of lower GI tract disease in order to exclude other diagnoses, such as viral enteritis.

Hepatic acute GVHD

The liver is the least commonly affected organ in acute GVHD, with less than 20% of acute GVHD patients having hepatic involvement [126]. Cholestatic jaundice is the most common manifestation; however, a concomitant or isolated transaminitis is not uncommon [130–134]. When severe, hepatic failure with encephalopathy may occur. Percutaneous or transjugular liver biopsy is recommended for the diagnosis of hepatic GVHD, when feasible, as there are no imaging studies that can differentiate reliably hepatic GVHD from other causes of liver dysfunction.

Other findings

Ocular symptoms have been described in patients with acute GVHD [135–138]. Manifestations may include photophobia, hemorrhagic conjunctivitis, pseudomembrane formation, corneal ulceration, and lagophthalmos. Involvement of the central nervous system with acute GVHD has been postulated to occur [139,140], although in most cases the definite etiology of the central nervous system pathology cannot reliably be ascribed to an alloimmune effect, with infections and calcineurin inhibitor-induced leukoencephalopathy as the primary diagnostic alternatives. The syndromes of diffuse alveolar hemorrhage and idiopathic pneumonia syndrome are more common among individuals with acute GVHD [141], but whether this in fact represents a true pulmonary manifestation of acute GVHD is unclear. The kidney appears to be susceptible to alloimmune attack in the context of chronic GVHD; however, acute GVHD involving the kidney has not been reported, although there is an association between transplant-associated thrombotic microangiopathy and acute GVHD [142,143]. Thrombocytopenia and anemia have been reported in patients with GVHD and failure of platelet recovery was shown to be 3.5-fold more common in patients with acute GVHD [144,145], with persistent thrombocytopenia having an adverse prognosis [146].

Diagnosis and differential diagnosis

Acute GVHD is a clinicopathologic syndrome with clinical features that may be difficult to distinguish from other early post-transplantation events. For example, the skin rash of acute GVHD is often confused with eruptions related to the effects of chemoradiotherapeutic conditioning, allergic reactions to medications, or viral exanthems. Similarly, the pattern of hepatic enzyme perturbation may be difficult to distinguish from venocclusive disease (VOD) of the liver or viral hepatitis. Complicating the diagnosis is that many of these complications may coexist. The nausea and anorexia of upper GI GVHD and diarrhea of lower GI GVHD are often indistinguishable from conditioning-related injury and intestinal infection with agents such as *Clostridium difficile*, *Candida*, and CMV. Occurring around the time of neutrophil engraftment, an engraftment syndrome, consisting of fever in the absence of infection, diffuse erythroderma, and non-cardiogenic pulmonary edema may also be difficult to distinguish clinically from acute GVHD [147].

Biopsy of the target organs of GVHD (reviewed in Chapter 26) is often obtained for diagnostic confirmation; however, the diagnosis remains largely a clinical one, and therapy often must be initiated before the results of biopsies are known. The utility of skin biopsy in the diagnosis of acute GVHD is questionable in retrospective reviews [148–150]. Similarly, biopsy of the lower and upper GI tract may be difficult to interpret early after transplantation, since the apoptotic changes associated with acute GVHD can often be confused with conditioning-related injury. However, viral or other infectious etiologies of diarrhea after transplantation are more readily distinguished. Biopsy of the liver is often indicated, as the histologic findings of acute hepatic GVHD, including portal triad inflammation, are often easily distinguishable from the findings of VOD of the liver, viral hepatitis, or the cytotoxic effects of medications, unless massive necrosis obscures the diagnostic picture.

Prediction and prognostication of acute GVHD

The role of *in vitro* functional assays to predict the occurrence of acute GVHD is largely historical, with mixed lymphocyte reaction testing no longer being routinely performed. More relevant now are biologic assays that can predict the occurrence of GVHD in advance of clinical features. Several biological markers (biomarkers) of acute GVHD have been postulated to predict the occurrence of acute GVHD better than clinical acumen. Assays for single inflammatory molecules such as TNF-α [151], IL-13 [152], or the soluble IL-2 receptor α chain (sIL-2Rα) [153], or markers of GVHD-target tissue injury (Elafin, REG3α) [154], most often discovered in proteomic assays, may, in the future, be the best markers of acute GVHD, but at present are experimental in nature.

In addition to their potential usefulness to predict GVHD occurrence, some of these biomarker panels have now been used to follow patient progress and to predict outcomes. In a prospective validation study, a six biomarker panel (sIL-2Rα, TNF-α, HGF, IL-8, Elafin, and REG3α) predicted day 28 therapy unresponsiveness and day 180 mortality [155]. However, simple clinical factors may also be useful. Response to steroids and outcome of acute GVHD are correlated with the initial GVHD grade at presentation [3]. Long-term survival for patients with grade 0–I disease approaches 50%, whereas for those with grade IV disease, the long-term survival rate has been reported to be as low as 11% [156]. Response to treatment is also a key predictor of outcome, as mortality in patients with grade II–IV acute GVHD is lowest in those who achieve a complete response to initial treatment (Figure 83.1) [13,156,157]. At least two large analyses have suggested that day 28 response to therapy correlated best with non-relapse mortality and survival [158,159], suggesting that this should be the end-point of acute GVHD clinical trials. A very early response, as evidenced by the ability to begin a steroid taper on day 5 of therapy, is associated with a favorable prognosis [160]. It is likely that early responders do better at least in part because the steroid taper begins sooner and reduces the risk of complicating opportunistic infections, a common cause of death even among responders. In general, skin disease responds promptly, but lower GI tract involvement responds poorly to therapy [157]. This may reflect the fact that the regeneration of a denuded intestinal mucosa requires days to weeks to occur and the use of escalating immunosuppression during this period can result in fatal infections. Other risks for failure of initial therapy and adverse outcome include early onset of GVHD, HLA disparity, and age [13,157], and also protean factors such as caloric intake and performance status [161].

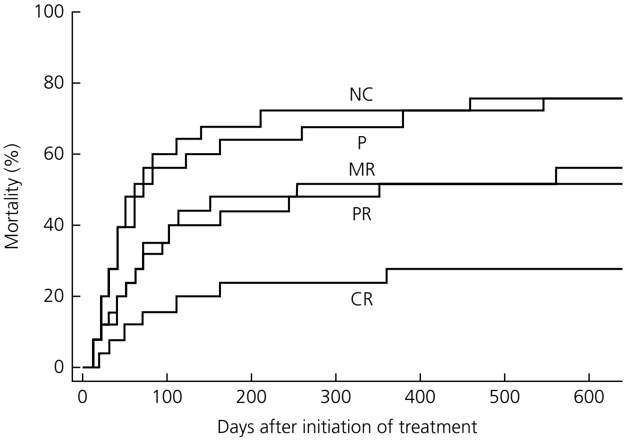

Figure 83.1 Non-relapse mortality following primary therapy for acute GVHD [13]. CR, complete response; PR, partial response; MR, mixed response; P. progression; NC. no change.

Therapy of acute GVHD

Primary therapy

The mainstay of therapy for acute GVHD is corticosteroids, with the route of administration (topical, oral, or intravenous) determined by the severity of acute GVHD and the degree of organ involvement. Corticosteroids are lympholytic and decrease the inflammatory cytokine cascades that propagate acute GVHD. Other agents have been utilized as front-line therapy, but none is superior to corticosteroids [13,162].

There is substantial center and investigator variability regarding the stage of acute GVHD that should be treated and the dose of corticosteroids to be used, particularly in early-stage GVHD. Cutaneous involvement with less than 50% body surface area involvement in the absence of hepatic or GI involvement (stage 1 or 2 skin disease, overall grade I acute GVHD) can often be treated with the topical administration of medium- or high-potency glucocorticoids alone; however, individuals with multiorgan involvement, and also some individuals with near 50% body surface cutaneous involvement, require systemic corticosteroid administration. The recommended initial dose of corticosteroids for grade II–IV acute GVHD is 2 mg/kg/day of methylprednisolone, or an equivalent steroid [163,164]. Initial steroid doses lower than 2 mg/kg/day have been analyzed retrospectively. In an analysis of 733 patients, no difference in adjusted hazard ratios for non-relapse mortality, recurrent or progressive malignancy, overall mortality, and extensive chronic GVHD were noted when doses of 1 and 2 mg/kg/day were compared; however, the former group was comprised predominantly of patients with grade I–II acute GVHD [165]. Initial doses of corticosteroids higher than 2 mg/kg/day were not been associated with improvement in response rates. In a prospective trial comparing 2 with 10 mg/kg/day of methylprednisolone in 94 patients with grade II–IV acute GVHD, response rates, progression to grade III–IV disease, non-relapse mortality, and overall survival were similar in both treatment groups [160].

For steroid-responsive acute GVHD, treatment is generally continued until complete resolution of signs and symptoms of acute GVHD and for at least 1–2 weeks thereafter. A gradual taper of the corticosteroid dose is then initiated, generally at no greater than a 10% reduction per week, but modified by factors such as the durability of the response to steroids and the toxicity of high-dose steroids. In a prospective trial, patients with acute GVHD who were responding by day 14 of corticosteroid treatment were randomized to a fast taper of corticosteroids over 86 days ($n = 14$) or a slow taper over 147 days ($n = 16$). The median time to acute GVHD resolution was 42 days (range 12–74 days) in the short treatment group, compared with 30 days (range 6–30 days) for the long taper schedule ($p = 0.01$), but without significant differences in the number of patients with acute GVHD flares, chronic GVHD, median dose of corticosteroid administered, infection, or death, suggesting that lower total cumulative doses of steroids appear equally effective and might minimize steroid-related complications [166].

The response rate to corticosteroid therapy in large retrospective reviews was approximately 50% [13,157], although it was higher in smaller clinical trials [167]. Durable responses are even less common. The complete response rate to corticosteroids is low enough to have prompted investigators to examine the role of additional immunosuppressive agents in the initial therapy of acute GVHD. The most widely studied agent in this setting is antithymocyte globulin (ATG). Phase II data demonstrated the response rate to steroids and ATG to be 67–80% [168,169]. In a randomized trial of prednisone with or without ATG ($n = 100$), no difference in response rates (76% in both arms) or survival at 1 and 2 years was noted, but infectious morbidity was higher in the combined immunosuppression group [167]. Lee et al. randomized 102 patients to receive the monoclonal anti-CD25 antibody daclizumab (1 mg/kg) on days 1 and 4 and weekly thereafter or placebo in conjunction with corticosteroids. Response rates were similar in both groups (53 vs. 51%), but survival at 100 days and 1 year was inferior in the daclizumab group [170]. Randomized trials have similarly demonstrated no benefit to the addition of the anti-IL-2 receptor antibody inolimomab [171], an anti-T-cell ricin-A-chain immunotoxin [172], or the monoclonal TNF-α antibody infliximab [173] when added to corticosteroids. In order to minimize the systemic effects of long-term high-dose steroids, corticosteroids with or without oral beclomethasone were tested in a small randomized trial and demonstrated benefit [174], but these advantages were not noted in a larger randomized trial, which was halted after an interim analysis suggested futility.

The Blood and Marrow Transplant Clinical Trials Network (BMT CTN) performed a randomized phase II trial of corticosteroids in combination with one of four promising agents [denileukin diftitox, etanercept, mycophenolate mofetil (MMF), and pentostatin]. The highest response rates were in patients treated with MMF [175], leading to a randomized phase III study of corticosteroids alone or with MMF. This trial was halted with 236 enrolled patients when the experimental and control arms demonstrated similar rates of GVHD-free survival 56 days from randomization (60.5% vs. 52.2%, $p = 0.78$) without differences in chronic GVHD incidence, severe infections, or overall survival in an interim analysis [176]. Hence there is no evidence at present that strategies using additional immunosuppressive agents will improve outcomes beyond single-agent prednisone.

Secondary therapy

Patients in whom initial therapy fails (commonly defined as progression of acute GVHD after 3 days, no change after 7 days, or incomplete response after 14 days of methylprednisolone treatment) have been given a variety of salvage regimens. There is no accepted standard for salvage treatment, and therapy of steroid-resistant acute GVHD remains unrewarding despite seemingly high response rates due to the risks of infection during intense immunosuppression.

The agent used most commonly in the setting of corticosteroid failure is ATG [177,178]. ATG use has been associated with response rates between 30 and 54%, but provides long-term survivorship in only 5–20% of treated patients [179–182], with earlier administration associated with improved survival [182]. Other pan-T-cell depleting strategies, including the monoclonal murine anti-CD3 antibody OKT3 and the non-mitogenic non-Fc-binding anti-CD3 antibody visilizumab, have been tested, but both of these were associated with excess rates of Epstein–Barr virus (EBV) reactivation and post-transplant lymphoproliferative disorder (PTLD) [183–186]. The anti-CD52 monoclonal antibody alemtuzumab, which targets all T cells and also antigen-presenting B cells and dendritic cells, has also been reported to be effective as therapy of steroid-refractory acute GVHD [187–189].

Targeting only activated T cells, antibody therapy directed against the α-subunit of the IL-2 receptor (CD25) has been attempted for more than 30 years, using the original anti-Tac antibodies [190–192], a murine antibody (BT563, inolimomab) [193–195], a chimeric murine/human monoclonal antibody (basiliximab) [196–198], and a humanized antibody (daclizumab) [199–203]. The utility of most of these agents is largely historical, since only basiliximab is currently available for use in the United States. In the original dose escalation trial, there was a response rate of 40%, with a predilection for improvement in cutaneous and GI disease [199]. Subsequently, a response rate of 50% was shown in a small cohort of patients with grade III–IV acute GVHD [203], and the largest series of patients reported demonstrated a complete response rate of 29% for patients treated with daclizumab weekly for five consecutive weeks, and a complete response rate of 47% for patients treated on a more accelerated schedule (1 mg/kg on days 1, 4, 8, 15, and 22) [201]. More recent retrospective analyses confirmed the high response rates, but poor long-term survival [200]. A large retrospective review spanning 10 years of experience noted a response rate of 42% by day 30 for inolimomab, with lymphopenia, early use, and grade IV disease as predictors of adverse outcome [195]. In phase II studies using different doses and schedules of basiliximab, the overall response rate was uniformly high (71–82.5%), with the complete response rate varying between 17.5% and 53% [196–198]. Also targeting the IL-2 receptor, denileukin diftitox is a recombinant fusion protein comprised of amino acids 1–133 of IL-2 fused to the membrane translocation and catalytic domains of diphtheria toxin. In a phase I study in steroid-refractory acute GVHD, 71% of patients responded to therapy, with 46% attaining a complete response at the MTD (9 μg/kg, days 1, 3, 5, 15, 17, and 19) [204]. A similar dose escalation trial that administered 4.5 μg/kg daily for 5 days followed by weekly therapy for 1 month showed a complete response rate of 41% at 36 days [205]. Other potential activated T-cell surface targets include CD147 (targeted by the antibody ABX-CBL), a member of the immunoglobulin superfamily, which is also found on B cells, monocytes, and dendritic cells [206]. Although active, this agent demonstrated inferior survival when compared with ATG in a multicenter, randomized phase III study [207].

Targeting the inflammatory cytokines that lead to T-cell activation and proliferation has been attempted in secondary GVHD treatment. Busca et al. treated 13 patients with steroid-refractory acute GVHD with etanercept, a genetically engineered molecule comprising two p75 extracellular domains of the human TNFα receptor fused with human IgG1, administered subcutaneously twice weekly for 4 weeks. Six of 13 responded (46%), with five attaining a complete response [208]. The chimeric antibody infliximab neutralizes TNFα and lyses cells that produce it, and has been used more extensively than etanercept. Initial case series and dose escalation trials documented the potential effectiveness of this agent [209–211], and larger series demonstrated response rates of 59–67%, with a predilection for improvement in GI GVHD [212,213], but with a high incidence of fungal infection [213,214]. Combination therapy with anti-T-cell therapy and cytokine-directed therapy has also been reported [215–219]. Other cytokines, including IL-5, IL-6, and IL-12/IL-23, are potential targets for GVHD therapy.

Agents that inhibit the proliferation of T cells are useful in the prevention and treatment of GVHD. In a phase I dose escalation study of pentostatin, an adenosine deaminase inhibitor, for steroid-refractory acute GVHD, the maximum tolerated dose (MTD) was 1.5 mg/m^2, and the overall response rate was 77% [220]. Less promising long-term results were reported recently by others [221,222]. MMF similarly suppresses T-cell proliferation by reversibly inhibiting purine synthesis; however, the use of this agent was associated with poor long-term survival in one series [223]. Sirolimus, an inhibitor of the mammalian target of rapamycin, is a T-cell immunosuppressant that inhibits DNA transcription, DNA translation, protein synthesis, and cell cycling [224]. Sirolimus may also be immunosuppressive through multiple alternative pathways. Benito et al. reported that 12 of 21 steroid-refractory patients (57%) had an objective response to sirolimus (five complete response, seven partial response); however, toxicity was limiting in this series [225]. Using more stringent serum levels, Hoda et al. demonstrated a 76% response rate (44% complete response rate) with acceptable toxicity and 44% survival at 1 year [226].

Extracorporeal photopheresis (ECP) has been used in the management of refractory acute GVHD. Postulated mechanisms of action of ECP, in addition of apoptosis of treated CD8$^+$ T effector lymphocytes, include a decrease in the production of proinflammatory cytokines and an increase in production of anti-inflammatory cytokines, both of which lead to a lower ability to stimulate T-cell responses, mediated in part by an increase in regulatory T cells [227,228]. Greinix et al. reported a 60% complete response rate to ECP, although there were no responders with GI GVHD [229], and Perfetti et al. reported response rates of 70, 42, and 0% for patients with refractory grade II, III, and IV GVHD respectively [230]. A similar approach used ultraviolet A light with a psoralen sensitizer to control refractory cutaneous GVHD, where high response rates were noted [231–233].

The use of cellular therapy to treat resistant acute GVHD has been explored. Mesenchymal stem cells (MSCs) are immunosuppressive, and transplantation of these cells from haploidentical or third-party donors improved GVHD in experimental models [234]. The first report of haploidentical MSC infusion for therapy of resistant acute GVHD was by LeBlanc et al., who obtained a single favorable outcome [235]. Since then, other case series reporting the successful use of MSCs in resistant visceral acute GVHD have been reported, with a notion that these cells may help repair the damaged organ, thus speeding up recovery from GVHD even further. Unfortunately, a large randomized trial failed to demonstrate an advantage to MSCs when added to conventional therapy in the treatment of steroid-refractory acute GVHD [236].

There are other experimental approaches to the management of refractory acute GVHD, including intra-arterial steroid [237–239] or methotrexate [240] infusion for GI and hepatic GVHD, and modulation of T-cell responses using alefacept [241] and IL-1 receptor-blocking antibodies [242]; however, all of these approaches remain experimental.

Supportive care

In addition to immunosuppressive therapy, supportive care is critical for the favorable outcome of acute GVHD. Organ-specific supportive measures for the skin include the use of topical emollient therapy and meticulous wound care, including care in a burn unit when indicated. For the GI tract, gut rest and parenteral hyperalimentation are required. Antimotility agents such as loperamide are often employed. Octreotide or other somatostatin analogs may be of benefit in controlling the secretory component of GI GVHD [243,244]. The use of ursodeoxycholic acid has been advocated as a supportive measure in hepatic acute GVHD, and hepatotoxic agents should be avoided.

Systemic supportive measures include the judicious use of antibiotics for proven or suspected infections, the routine use of antiviral prophylaxis against herpesviruses, and routine monitoring for CMV reactivation. Finally, routine antifungal prophylaxis with extended spectrum azoles or echinocandins with activity against *Aspergillus* sp. and other molds is recommended [245], as infectious morbidity during immunosuppressive therapy for GVHD therapy is a major cause of morbidity and mortality.

Special scenarios

Hyperacute GVHD

Hyperacute GVHD is a very rare complication of allogeneic HCT. Clinically similar to traditional acute GVHD, hyperacute GVHD occurs earlier, and often several days prior to engraftment, although no standard definition exists. The pathophysiology of hyperacute GVHD remains unclear. It has been postulated to be caused by mature, immunocompetent cells transferred with the stem-cell product, including natural killer T (NK-T) cells [246]. Risk factors for hyperacute GVHD include mismatched or unrelated donor transplantation, high-dose conditioning, donor–recipient gender mismatching, second transplantation, and the lack of prophylactic immunosuppression [247–250]. Therapy for hyperacute GVHD is similar to that for acute GVHD, and although it is often less successful, the long-term outlook for hyperacute GVHD appears no different than for patients with traditional acute GVHD [247,248].

Autologous GVHD

Histocompatibility differences between donor and recipient may not always be needed to produce GVHD; instead, inappropriate recognition of self-antigens may produce a GVHD-like syndrome after autologous HCT [251,252]. In a thymus damaged by conditioning therapy after autologous HCT, HLA class II-bearing cells may be absent and clonal deletion of reactive anti-self cells may not occur. In a series of patients conditioned with alemtuzumab prior to transplantation, a high incidence of autologous GVHD was reported, suggesting that dysregulation of the immune system is involved in the pathophysiology of autologous GVHD [253]. Administration of cyclosporine in the context of thymic damage may further prevent the development of regulatory immune cells and permit the development of autoreactive $CD8^+$ and $CD4^+$ T cells that recognize class II determinants [254]. Cyclosporine administration after autologous transplantation has been given in an attempt purposefully to invoke graft-versus-malignancy effects [255–257].

Clinical involvement of the skin, liver, and GI tract have all been reported in autologous GVHD [258–260], although the incidence of each of these complications is low. Therapy for autologous GVHD is similar to that for classic acute GVHD, with very favorable results with corticosteroids alone. Some have postulated that autologous GVHD may simply be a version of an engraftment syndrome.

Transfusion-associated GVHD

Transfusion-associated GVHD (TA-GVHD) is a rare complication of blood-component transfusion therapy [261]. TA-GVHD has been associated with cellular blood components, thus excluding cryoprecipitate or fresh frozen plasma [261–264]. The incidence of TA-GVHD overall remains elusive, as the disorder is often not recognized and is under-reported, but the pathobiology appears similar to that of GVHD following allogeneic hematopoietic stem-cell transplantation [265,266].

Although immunologically normal recipients may be affected by TA-GVHD, several groups are at a distinctly higher risk. Individuals with congenital immune deficiency disorders, cancer patients receiving immunosuppressive chemotherapy, and surgical patients undergoing major surgical procedures or suffering from severe trauma represent the most commonly affected subjects [263]. Among normal individuals, genetic factors appear to be the most important risk factors [267,268]. In populations with common HLA haplotypes, homozygous extended HLA haplotype donors represent the greatest risk. In this setting, donor cells would not be rejected as foreign by the recipient and could precipitate TA-GVHD [269,270]. In Japan, the incidence is estimated to be approximately 1 in 500 after major cardiothoracic procedures [267].

The clinical features of TA-GVHD are very similar to those of traditional GVHD, although the onset of TA-GVHD is generally earlier than that of traditional GVHD, often occurring 7–10 days after transfusion therapy. The diagnosis can be made by demonstrating circulating T cells of donor origin using chimerism analysis. The one distinguishing clinical feature of TA-GVHD is the development of pancytopenia, which indicates that host marrow and hematopoietic progenitors are an immunologic target of TA-GVHD. Pancytopenia and resultant infectious complications of TA-GVHD are the most common cause of death in TA-GVHD, which often occurs 3–4 weeks after the diagnosis.

Prevention of TA-GVHD is critical. The efficacy of blood-product irradiation has long been recognized, and initial BMT practice routinely employed irradiation of blood products with 1500 cGy [271]. This dose of gamma irradiation from ^{60}Co or ^{137}Cs sources effectively prevents lymphocyte proliferation without adverse effects on blood cell morphology or function [272]. Although there has been variation across individual centers, 97% of institutions surveyed by the American Association of Blood Banks employ doses of 1500–3500 cGy [273]. This practice appeared to be highly effective and only a single case of TA-GVHD has been reported in a patient given blood components irradiated with 2000 cGy from a ^{137}Cs source [274]. Based upon more sensitive limiting dilution assays of T-cell inactivation, more recent studies have called for higher doses of blood-product irradiation and since July 1993, the US Food and Drug Administration (FDA) has required a dose of 2500 cGy to the internal midplane of the canister with no area receiving less than 1500 cGy, with well-defined procedures for quality assurance, dose mapping, and storage times [275–278].

Newer methods to prevent TA-GVHD are under development. Most promising are photosensitizing psoralens and ultraviolet A (PUVA) light designed to inactivate infectious organisms. This technology also cross-links T-cell DNA and in addition prevents their proliferation [279,280]. Lymphocyte depletion of transfused blood products has been attempted; however, the minimum threshold of competent T cells to induce TA-GVHD is still unknown, and there are case reports of individuals developing TA-GVHD despite lymphocyte depletion [281,282].

Therapy of TA-GVHD using agents used to prevent or treat traditional GVHD has rarely been successful, since the pancytopenia is often irreversible, and there is therefore an almost 90% fatality rate associated with TA-GVHD. Despite this, an attempt at immunosuppression using corticosteroids and other immunosuppressive agents is often attempted, sometimes with favorable outcomes [283,284]. Allogeneic HCT is often required to restore hematopoiesis.

Conclusion

Despite a seemingly improved understanding of the biologic mechanisms of acute GVHD, this syndrome is often the first hurdle to challenge the transplant recipient. The diagnosis of acute GVHD remains largely a clinical one, with biopsy of affected organs an important adjunct. After decades of research, the standard therapy for this problem remains simple corticosteroids, although novel approaches to the initial management of acute GVHD are being developed. In addition, there remains no standard for secondary therapy of acute GVHD, as numerous agents have only modest activity in this setting. Unfortunately, most of these approaches are associated with high mortality related to infectious morbidity. Future endeavors in acute GVHD include novel assays for the diagnosis of acute GVHD, preemptive therapy for incipient acute GVHD, and the standardization of therapeutic measures when primary therapy fails.

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CHAPTER 84

Chronic Graft-versus-Host Disease – Clinical Manifestations and Therapy

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Introduction

Chronic graft-versus-host disease (cGVHD) is a pleiotropic syndrome, characterized in 1980 [1], and clinically distinguished from the typical dermatitis, hepatitis, and enteritis of acute GVHD by the reclassification of the 2005 National Institutes of Health (NIH) Consensus Development Project on cGVHD [2]. Thus, the NIH diagnosis of cGVHD requires no reference to timing after transplantation but rather requires the presence of characteristic clinical manifestations resembling collagen vascular diseases that may affect one or multiple sites, including skin, liver, eyes, mouth, gastrointestinal (GI) tract, lungs, serosae, and skeletal muscles. Manifestations that are diagnostic of cGVHD are categorized as "*classic chronic*" regardless of when they occur, those consistent only with acute GVHD but which occur at or after day 100 are defined as "*late acute*" and the concurrent presentation of chronic and acute manifestations defines the "*overlap subtype*" of cGVHD (Figure 84.1). Major causes of cGVHD morbidity include ocular and oral sicca syndromes, contractures, and ulcerations associated with sclerotic cGVHD, progressive airflow obstruction, dysphagia, wasting syndrome, and others related to chronic illness. Infection represents the major cause of mortality and is associated with immune dysregulation, which also includes eosinophilia, circulating autoantibodies, and perturbations in the serum levels of gammaglobulin.

Half of all patients who develop cGVHD are diagnosed within 6 months from transplantation and the remainder develop it within 2 years. cGVHD is the major late determinant of morbidity and non-relapse mortality (NRM) after allogeneic transplantation and is associated with decreased quality of life, impaired functional status, and prolonged duration of immunosuppressive therapy. Nonetheless, its potential benefit is the eradication of malignant cells via a graft-versus-tumor effect that remains poorly understood but an active focus of ongoing research.

After 25 years of limited progress in cGVHD due to deficiencies in our understanding of its biology, diagnosis, staging, supportive care, and response categorization, the 2005 NIH consensus conference energized the field. The fruits of these labors are beginning to emerge, for example, with the early validation results of revised classification schemes, severity scales, and response assessment tools [3–13]. Important 2014 updates to the 2005 NIH consensus reports were published after this chapter was submitted to the publisher and are available in the article by Jagasia et al. [13] and at http://www.asbmt.org/?page=GuidelineStatements.

Risk factors for onset of chronic GVHD

Factors that have consistently been associated with a higher risk for developing traditionally defined cGVHD include preceding acute GVHD, older patient age, the use of female donors for male recipients, donor lymphocyte infusions (DLIs), unrelated or HLA-mismatched donors, and, more recently, the use of granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood cells as opposed to marrow as a source of stem cells [14]. Among them, a prior history of acute GVHD seems to be the most powerful predictor, which is not surprising given the overlap of some, but not all, of these risk factors. A comparative analysis between acute GVHD and cGVHD diagnosed by NIH criteria has shown that total body irradiation is a risk factor for acute GVHD but not for cGVHD [3]. In contrast, mobilized peripheral blood grafts and older patient age increased the risk for cGVHD but not acute GVHD and the impact of female donor into male recipient was greater on cGVHD (Figure 84.2); these associations held after adjustment for prior acute GVHD (data not shown). This suggests that cGVHD is not simply a continuation of acute GVHD and that some differences in the mechanisms of pathogenesis may exist between these syndromes. Among patients with cGVHD, total body irradiation has been identified as a significant risk factor for skin sclerosis, one of the most disabling forms of cGVHD [15].

The incidence of cGVHD varies from 20 to 85%, depending on the presence of aforementioned risk factors and the diagnostic criteria used. After matched sibling transplantation, the incidence of traditionally defined extensive cGVHD at 3 years is 47% after mobilized blood versus 31% for marrow ($p < 0.000001$) [16], frequencies that are similar to those seen in the unrelated donor setting based on the 2-year rates of extensive cGVHD in the randomized Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0201 study (48% versus 32%, $p < 0.001$] [17]. It is worth noting that rates of NIH-defined cGVHD are lower than for historically defined extensive cGVHD because the latter also includes late acute GVHD (Figure 84.1).

The reason for the increased incidence of cGVHD after mobilized blood allografts is not fully understood. Although mobilized blood contains more than 10 times the number of T cells than marrow, one study showed that the number of infused CD3 positive cells may not correlate with the development of

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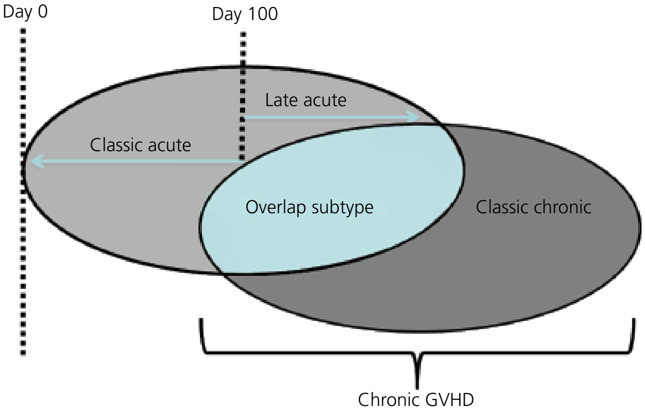

Figure 84.1 Acute GVHD and cGVHD are subcategorized into *classic* acute GVHD (maculopapular erythematous rash, GI symptoms, or cholestatic hepatitis) occurring arbitrarily within 100 days after HCT or DLI, or *persistent, recurrent* or *late* acute GVHD beyond 100 days and typically seen after withdrawal of immunosuppression. Both acute GVHD entities occur without the presence of diagnostic or distinctive cGVHD manifestations. cGVHD subcategories include classic cGVHD consisting only of manifestations attributable to cGVHD (Table 84.1), and acute GVHD and cGVHD overlap syndrome, in which features of acute GVHD and cGVHD coexist.

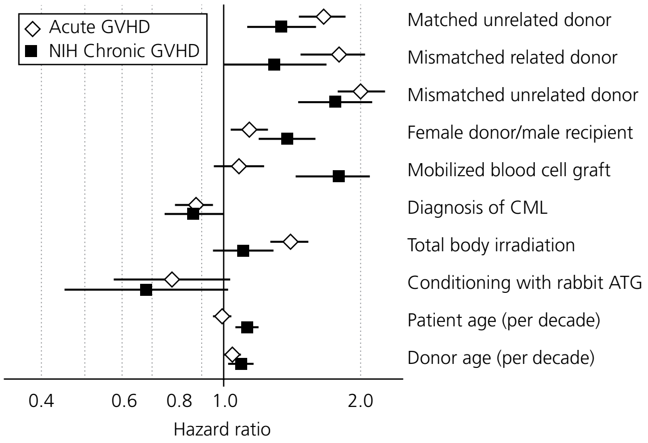

Figure 84.2 Risk profiles for acute GVHD and cGVHD. Factors associated with an increased risk (median HR > 1) or decreased risk (median HR < 1) for development of acute GVHD or NIH cGVHD are shown together with 95% confidence intervals (CIs) for each factor. There were 2355 acute GVHD events and 1022 NIH cGVHD events. Source: Flowers et al 2011 [3]. Reproduced with permission of the American Society of Hematology.

cGVHD [18]. Donor exposure to filgrastim does not appear to be a strong risk factor since rates of cGVHD are lower among recipients of filgrastim-primed allogeneic marrow versus filgrastim-mobilized blood [19]. Although ex vivo T-cell depletion is clearly associated with lower rates of acute GVHD, its effects on cGVHD rates are less clear and these effects may depend on the source of stem cells [20,21]. Early complete donor chimerism seems to be important for developing cGVHD and is frequently delayed after reduced-intensity conditioning (RIC) and may affect the incidence and severity of cGVHD [22]. In summary, although our ability to predict cGVHD has improved, additional improvements in prognostic criteria are still needed and might include biological markers [23].

Risk factors associated with increased non-relapse mortality and inferior survival

Across studies, thrombocytopenia (<100 × 10^9/L) and progressive onset of cGVHD from prior acute GVHD are the two factors most consistently associated with increased risk for late NRM among patients with cGVHD. Other factors at the time of cGVHD diagnosis that have been commonly reported to associate with increased NRM include prednisone dose >0.5 mg/kg/day, hyperbilirubinemia (>2 mg/dL), extensive (>50%) skin involvement, poor Karnofsky performance status, weight loss, intestinal involvement, history of prior acute GVHD grade II–IV, HLA mismatching, and older patient or donor age, among others [24–27]. Several retrospective studies have reported inconsistent results regarding the associations of GVHD subtypes with NRM or overall survival. Three studies reported that NRM was higher, and survival lower, for late acute compared with cGVHD classic or overlap subtypes [28–30], while three other studies reported no differences in outcomes between GVHD subtypes [6,9,31]. Two of these reports did note an independent association of NIH global severity (mild, moderate, severe) with overall survival and NRM [9,31]. A recent prospective longitudinal study of 298 patients by the North American Chronic GVHD Consortium showed that global severity scores at enrollment were associated with NRM ($p < 0.0001$) and survival ($p < 0.0001$) (Figure 84.3), and 2-year overall survival was 62% (severe), 86% (moderate), and 97% (mild) [8].

An earlier registry analysis of over 5000 adults and children with cGVHD was used to develop a 10-variable risk score [age, prior acute GVHD, time from hematopoietic cell transplantation (HCT) to cGVHD, donor type, disease status at HCT, GVHD prophylaxis, gender mismatch, serum bilirubin, Karnofsky score, and platelet count]. Risk groups were identified that predicted 5-year outcomes that stepwise ranged from 5% through to 72% for NRM and 91% down to 4% for overall survival. These results were recently validated in a contemporary population and can be broadly applied [32]. This Center for International Blood and Marrow Transplantation Research (CIBMTR) risk score may not be equally applicable to NIH-defined cGVHD.

These results suggest that intervention studies in cGVHD should consider assigning therapy according to risk group. For instance, patients at low risk for NRM might be preferentially enrolled in studies of new topical or organ-specific therapies, whereas high-risk patients might receive more aggressive systemic therapy.

Risk factors associated with duration of therapy

The results of at least one randomized study indicated that a longer duration of prednisone therapy was needed to treat cGVHD that occurred after transplantation of mobilized blood compared with bone marrow [33]. Results of a multivariate analysis in a large retrospective cGVHD cohort confirmed and extended this observation by showing that the time to stop systemic immunosuppression permanently was longer for recipients of mobilized blood allografts, for male patients with female donors, when HLA-mismatching occurred in the direction of GVHD, for patients with hyperbilirubinemia, or for patients with multiple organ involvement at onset of cGVHD [27]. Time to discontinue immunosuppressive therapy in the absence of recurrent malignancy has been used as a surrogate end-point indicating resolution of reversible cGVHD manifestations. Among patients with cGVHD who do not relapse or die from other causes, the median time to discontinue immunosuppressive therapy differs according to graft source and is just over 3 years for mobilized blood and 2 years for marrow [27].

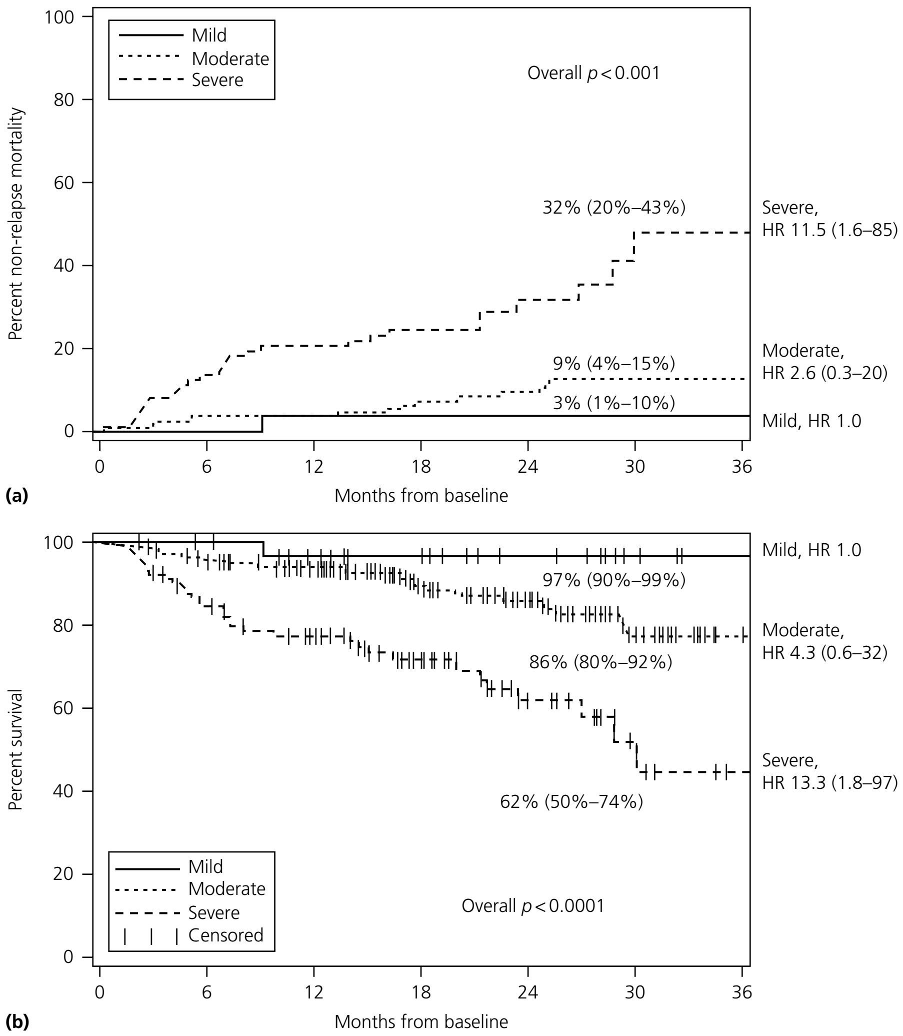

Figure 84.3 Cumulative incidence of non-relapse mortality (a) and Kaplan–Meier plot of overall survival (b) according to NIH global severity at enrollment, and showing 2-year estimates (95% CI). HR, hazard ratio; 95% CI, compared with mild cGVHD. Source: Arai et al 2011 [8]. Reproduced with permission of the American Society of Hematology.

Pathogenesis in humans

The pathophysiology of GVHD in described more fully in Chapter 13. The biology of cGVHD remains elusive even though recent murine and human studies have provided some new insights. Alloreactive T cells are key mediators as supported by observations that cGVHD usually does not occur after autologous or identical twin transplantation. The delayed onset of cGVHD suggests that inciting processes have a long evolution or latency. Thymic dysfunction may play a role in pathogenesis, as supported by older patient age being a predictor for cGVHD and that response rates in children to a given GVHD therapy are often higher than in adult patients. Most investigators consider cGVHD as a disease of immune dysregulation that involves donor-derived immune cells and host cell populations and tissues. Donor-derived T cells are central mediators and the process is both alloreactive (directed against recipient histocompatibility antigens) and autoreactive (directed against antigens present on both donor and recipient) [34]. Thus, cGVHD therapy has traditionally been with T-cell-based immunosuppression and when anti-T-cell globulin is given in the peri-transplant period, a reduced rate of cGVHD has been observed [35]. Once activated, the immune response in cGVHD is thought to proceed without the usual tolerance-promoting mechanisms of thymic or peripheral deletion and immunoregulation [36–38]. This pathologic immune response attacks target tissues directly through cytolytic mechanisms, secretion of proinflammatory and profibrotic cytokines, or promotion of B-cell activation and autoantibody production [34,39]. Inflammation leads to tissue damage, counter regulation and repair with fibrosis, or irreversible organ damage. Although autoantibodies have been detected in some patients with cGVHD, evidence for a causal relationship of these antibodies in cGVHD pathogenesis has not yet been demonstrated. One study suggested the potential involvement of stimulatory antibodies against antiplatelet-derived growth factor alpha receptor (PDGFR), in the sclerotic cGVHD phenotype, based on reported activities of these antibodies to induce fibrosis via induction of PDGFR phosphorylation, reactive oxygen species generation, and the increased expression of α-actin and collagen. Such observations have prompted recent interest in therapy with imatinib, a tyrosine kinase inhibitor of PDGFR, c-KIT, BCR-ABL, and discoidin domain receptor family members 1 and 2 (DDR1 and

DDR2) [40,41]. In addition to antibody production, B cells are involved in important antibody-independent mechanisms such as antigen presentation, cytokine production, and immune regulation, which are thought to be involved in cGVHD. Taken together, targeting B cells with agents such as anti-CD20 monoclonal antibodies (e.g. rituximab) or novel agents targeting B-cell-tropic factors (belimumab and anti-BR3 antibody) are of interest for cGVHD and are under investigation in other more common autoimmune diseases [39]. Finally, new insights about the role of $CD4^+CD25^+FOXP3^+$ regulatory T (Treg) cells in tolerance induction have generated enthusiasm for testing therapies that are thought to promote Treg cell expansion. In one study, the frequency of Treg cells in patients with cGVHD was about half that in the non-cGVHD group but suppressive function of these Treg cells remained normal [38]. This reduction was thought to be physiologically relevant given the low naturally occurring frequency of Treg cells in the blood of healthy individuals yet their critical role in controlling a wide range of immune responses. Perhaps best illustrating this point is the clinical severity of the immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, which shares a number of features with cGVHD and is caused by FOXP3 mutations that result in a functional deficiency of Treg cells [42]. Therapies such as extracorporeal photopheresis and mammalian target of rapamycin inhibitors (e.g. sirolimus) that promote Treg cell expansion are currently being explored as primary therapy of cGVHD rather than calcineurin inhibitors, which inhibit Treg cell expansion [43–45]. An ongoing randomized phase II/III trial of the BMT CTN (Protocol 0801) is testing the question of sirolimus versus calcineurin-inhibitor-containing therapy. In summary, owing to the diversity and unpredictability of cGVHD, contemporary therapy focuses on global suppression of all or many of the key components of this inflammatory cascade. Although an integrated understanding of cGVHD biology is still lacking, certain of its elements are becoming more evident.

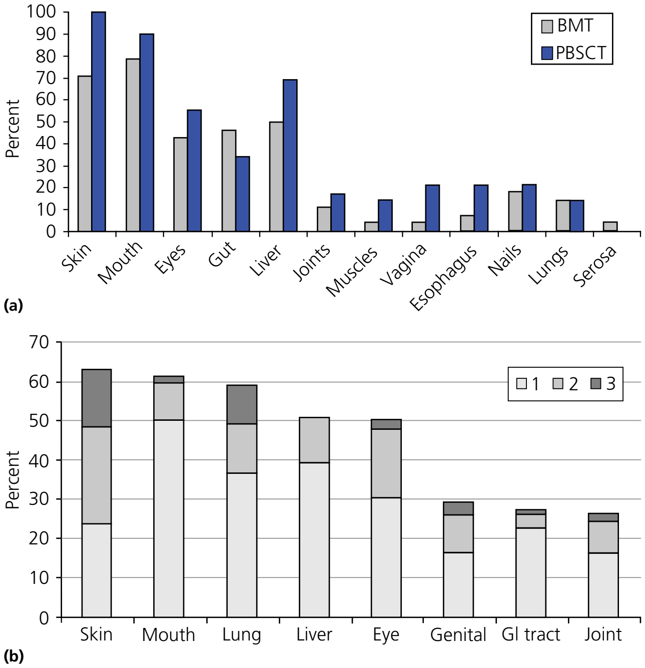

Figure 84.4 Sites affected by chronic GVHD. (a) After peripheral blood stem-cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Columns show the proportions of patients with sites affected by cGVHD at any time. Source: Flowers et al. 2002 [33]. Reproduced with permission of the American Society of Hematology. with permission. (b) Data from the prospective Chronic GVHD Consortium showing the proportion with involvement among incident and prevalent cases according to NIH organ severity scores. Source: Arai et al. 2011 [8]. Reproduced with permission of the American Society of Hematology.

Diagnosis of cGVHD

Manifestations of cGVHD usually present between 3 months and 2 years after allogeneic HCT but about half of all cases develop within the first 6 months. Chronic GVHD manifestations of the classic or overlap subtype can begin before or after post-transplant day 100. When manifestations of acute and cGVHD occur simultaneously, this is referred to as the overlap subtype of cGVHD. Manifestations of acute GVHD alone can develop, persist, or recur long after day 100, and while this has been referred to as "late" acute GVHD, the 2014 NIH Consensus prefers simply "acute GVHD" regardless of its timing. (Figure 84.1). Late acute GVHD is more common after RIC transplantation [46]. For example, liver transaminase elevations, skin erythema, or noninfectious diarrhea that develop while tapering off immunosuppression in the absence of diagnostic features of cGVHD are classified as late acute GVHD regardless of the time after transplantation. GVHD after DLI may present with manifestations of only acute, both acute and chronic ("overlap"), or classic cGVHD alone [47].

The most commonly affected organs reported in North Americans are skin, mouth, and eyes, with more than 50% of patients demonstrating these manifestations (Figure 84.4). Other common disease sites include the liver, lung, GI tract, musculoskeletal system, and female genital organs. Manifestations of cGVHD may be restricted to a single organ or typically 2–3 organs are involved. Obstructive lung disease and sclerotic features of cGVHD most often develop some time after the initial diagnosis of cGVHD.

Standardized criteria for physical signs, symptoms, and laboratory and diagnostic values are used to determine *diagnostic* or *distinctive* manifestations of cGVHD (Table 84.1 and Figures 84.5–84.21). A color atlas and classification of important cGVHD manifestations is available at http://www.asbmt.org/?page=MeasureCGVHD. Features that are neither diagnostic nor distinctive cannot be used to establish a diagnosis of cGVHD because they are either *common* to acute and chronic GVHD, or else represent *other features* that also lack specificity for cGVHD. Therefore, a diagnosis of cGVHD first requires the distinction from acute GVHD as recognized by the presence of one or more of erythema, maculopapular rash, pruritus, or acute involvement of the GI tract or liver. The second requirement is the presence of at least one diagnostic manifestation (i.e. found only in cGVHD) or at least one distinctive manifestation that is highly suggestive of cGVHD and confirmed by pertinent biopsy or other relevant tests (Table 84.1).

Lastly, other possible diagnoses that could account for the manifestations in question (e.g. infection, drug effect, others) should be excluded. For example, the oral lesions of candidiasis, herpes simplex or human papilloma virus, and squamous cell carcinoma always need to be considered.

Histological confirmation is necessary in the absence of diagnostic clinical features, or distinctive features that are unable to be confirmed by other pertinent tests [e.g. bronchiolitis obliterans based on pulmonary function and high-resolution chest computed tomography (CT) scan].

Table 84.1 Classification of manifestations for the clinical diagnosis of cGVHD

Organ or site	Diagnostic (sufficient to establish a chronic GVHD diagnosis)	Distinctive (insufficient alone to establish a chronic GVHD diagnosis)	Common (seen with both acute and chronic GVHD)
Skin	Poikiloderma Lichen planus-like Sclerotic Morphea-like Lichen sclerosis-like	Depigmentation Papulosquamous lesions	Erythema Maculopapular Pruritus
Nails		Dystrophy Longitudinal ridging, splitting or brittleness Onycholysis Pterygium ungus Nail loss (usually symmetric and affects most nails)	
Scalp and body hair		New alopecia not due to chemoradiotherapy Scaling	
Mouth	Lichenoid Hyperkeratotic plaques Diminished oral cavity opening from sclerosis	Xerostomia Mucocele Mucosal atrophy Pseuodmembranes[a] Ulcers[a]	Gingivitis Mucositis Erythema Pain
Eyes		New onset of sicca (dryness, gritty, or painful[b]) Cicatricial conjunctivitis Keratoconjunctivitis sicca[b] Confluent areas of punctate keratopathy	
Genitalia	Lichen planus-like or lichen sclerosis-like Females: Vaginal scarring or genital agglutination Males: Phimosis or urethral scarring	Erosions[a] Fissures[a] Ulcers[a]	
GI tract	Esophageal web Esophageal strictures or stenosis in upper to mid third[a]		Anorexia, nausea, vomiting, diarrhea Failure to thrive/weight loss
Liver[c]			Bilirubin >2 × ULN[a] Alkaline phosphatase >2 × ULN[a] ALT >2 × ULN[a]
Lung	Bronchiolitis obliterans)[d] (biopsy confirmed)	Bronchiolitis obliterans)[d] (based on PFTs and CT scan imaging)	
Muscles, fascia, joints	Fasciitis Joint stiffness or sclerotic contractures	Myositis or polymyositis[b]	

Manifestations acknowledged as cGVHD if the diagnosis is already confirmed

Skin	Sweat impairment, ichthyosis, keratosis pilaris, hypopigmentation, hyperpigmentation
Hair	Thinning scalp hair not otherwise explained (typically patchy, coarse, or dull), premature graying, or alopecia not explained by endocrine or other causes
Eyes	Photophobia, periorbital hyperpigmentation, blepharitis (eyelid erythema with edema)
GI tract	Exocrine pancreatic insufficiency
Muscles/joints	Edema, muscle cramps, arthralgia, or arthritis
Hematology	Thrombocytopenia, eosinophilia, lymphopenia
Immune	Hypo- or hypergammaglobulinemia, autoantibodies (AIHA, ITP), Raynaud's phenomenon
Lung	Cryptogenic organizing pneumonia (COP/BOOP)
Other	Hypo- or hypergammaglobulinemia, autoantibodies (AIHA, ITP) Ascites, pericardial or pleural effusions, peripheral neuropathy, nephrotic syndrome, myasthenia gravis, cardiac conduction abnormality, cardiomyopathy

AIHA, autoimmune hemolytic anemia; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BOOP, bronchiolitis obliterans with organizing pneumonia; COP, cryptogenic organizing pneumonia; CT, computed tomography; ITP, immune thrombocytopenia; PFTs, pulmonary function tests; ULN, upper limit or normal.

[a] In all cases, infection, drug effects, malignancy, and other cause must be excluded.

[b] Diagnosis of cGVHD requires biopsy or radiographic confirmation (or Schirmer test or slit lamp examination for eyes).

[c] Because liver histology in acute and chronic GVHD is not distinguishable, the diagnosis of cGVHD cannot be made on the basis of biopsy alone and requires a distinctive manifestation in at least one other organ system.

[d] Criteria for diagnosing bronchiolitis obliterans: forced expiratory volume in 1 s/forced (or slow) vital capacity ratio <0.7 *and* forced expiratory volume in 1 s <75% of predicted *and* evidence of air trapping or small airway thickening or bronchiectasis on high-resolution chest CT (with inspiratory and expiratory images), *or* residual volume >120%.

Organs in which the diagnosis may not be straightforward

Gastrointestinal tract

Diagnostic features include *esophageal web*, *stricture*, or *concentric rings* that may present as progressive dysphagia and require documentation by endoscopy or barium contrast radiograph. The classical "wasting syndrome" of untreated cGVHD is now uncommon with current immunosuppressive therapy. When diarrhea and weight loss, with or without abdominal cramping, occur late after HCT, it is usually unclear whether these represent cGVHD, infection, medication toxicity, or, infrequently, exocrine pancreatic insufficiency, which is recognized as a manifestation of cGVHD provided that the diagnosis of cGVHD has otherwise been established. The temporal relationship between symptoms and the addition or tapering of immunosuppressive medications could favor, respectively, opportunistic infection or a flare of GVHD. Common offending medications include high-dose oral magnesium, mycophenolate mofetil, and narcotic-associated pseudo-obstruction. Antibiotic therapy may predispose to *Clostridium difficile* toxin enteritis that should always be excluded by enteric screening for this and other viral, protozoan, and bacterial pathogens. If the history suggests steatorrhea and the diagnosis remains unclear, it may be useful to screen for exocrine pancreatic insufficiency by microscopy of a Sudan-stained spot fecal specimen or by fat quantitation in a 72-hour stool collection. Because stool tests have limited sensitivity for detecting milder cases of pancreatic insufficiency, a trial of pancreatic enzyme therapy is reasonable and may be diagnostic. Fat-soluble

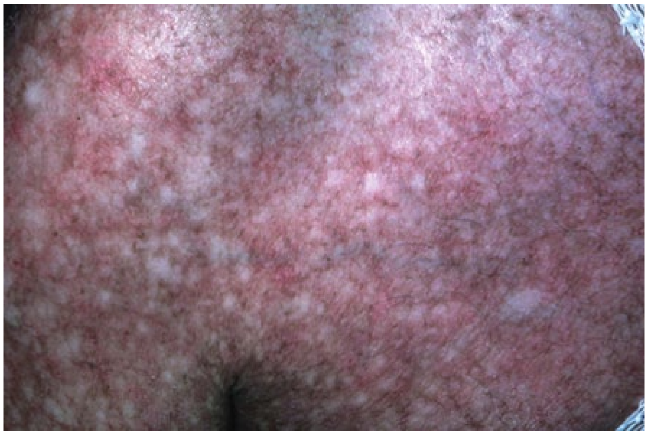

Figure 84.5 Poikiloderma, a diagnostic manifestation of cGVHD, which includes both atrophic and pigmentary changes. Note the combination of reticulated pattern, epidermal atrophy (cigarette-paper wrinkling of the skin surface), erythema, and brown pigment forming the borders of skin-colored or depigmented skin. (For a color version of this figure, please see the color plate section.)

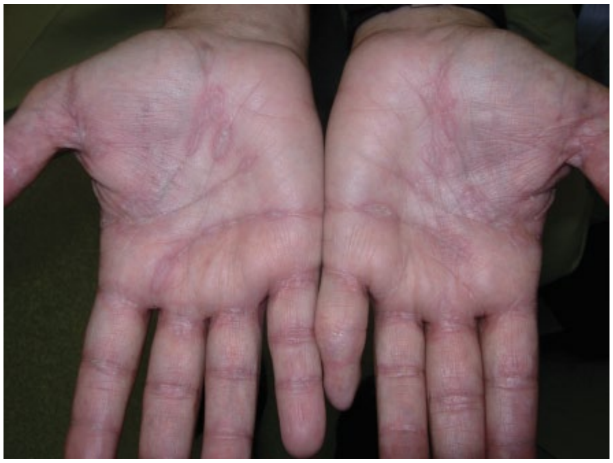

Figure 84.6 Lichen planus-like eruption involving the palmar surfaces, a diagnostic manifestation of cGVHD. Note the erythematous/violaceous flat-topped papules or plaques with or without surface reticulations or a silvery or shiny appearance on direct light. (For a color version of this figure, please see the color plate section.)

vitamin A, D, and E levels are low in severe cases, and replacement using water-soluble formulations may be necessary. Pancreatic insufficiency may be reversible after cGVHD has become inactive [48]. Lactose intolerance may be associated with intestinal GVHD and lactose avoidance or a trial of chewable lactase tablets with dairy products may be helpful if diarrhea consistently occurs with dairy products.

Lungs

The only diagnostic manifestation of lung cGVHD is biopsy-proven bronchiolitis obliterans (BO) (Figure 84.19). BO that is diagnosed clinically by pulmonary function and radiologic testing requires at least one other distinctive manifestation in a separate organ system to establish the initial diagnosis of cGVHD [2]. Patients with BO may be asymptomatic early in the disease process but frequently develop dyspnea, cough, or wheezing as they develop significant airflow obstruction. Pneumothorax, pneumomediastinum, and subcutaneous emphysema may be seen in advanced disease. BO is clinically diagnosed when all of the following criteria are met: forced expiratory volume in 1 s/forced vital capacity ratio <0.7 [or forced expiratory volume in 1 s (FEV_1)/slow vital capacity <0.7], *and* FEV_1 <75% of predicted with ≥10% decline over less than 2 years *and* evidence of air trapping or small airway thickening or bronchiectasis on high-resolution chest CT (with inspiratory and expiratory images), or residual volume >120%. The BO diagnosis also requires exclusion

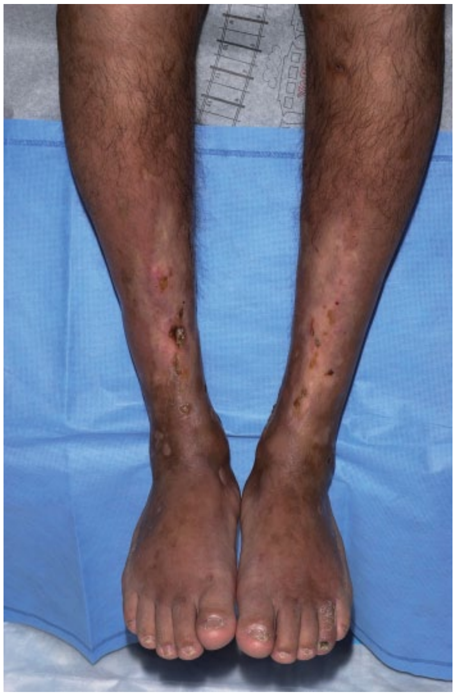

Figure 84.7 Diffuse deep sclerotic features involving the lower extremities, a diagnostic feature of cGVHD. Note the non-movable/hidebound subcutaneous tissues, erosions, and ulcerations, loss of hair follicles, and toenail dystrophy. (For a color version of this figure, please see the color plate section.)

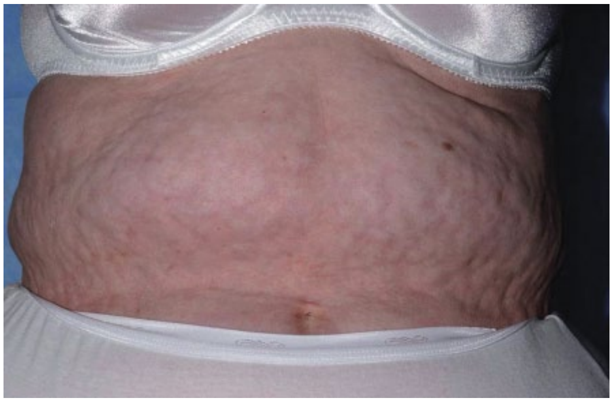

Figure 84.8 Alternative manifestation of deep sclerosis common in areas of excess subcutaneous tissue such as the abdomen, upper arms, and buttocks. Tissue is not hidebound but demonstrates prominent rippling, dimpling, or a cellulite-like appearance. (For a color version of this figure, please see the color plate section.)

of respiratory tract infection, and radiographic infiltrates should be evaluated by upper respiratory tract viral screen, consideration of sinus infection, sputum culture, and bronchoalveolar lavage. Restrictive pulmonary function test (PFT) abnormalities may be related to sclerosis of the chest wall or myopathy related to glucocorticoid therapy and other causes. Cryptogenic organizing pneumonia (COP) is most often associated with restrictive pattern PFT, but PFT results may show a normal pattern when the infiltrates are small (unifocal) (Table 84.2). COP may be due to GVHD or other causes but can be acknowledged as part of the cGVHD manifestations if cGVHD diagnosis is already confirmed. The diagnosis requires at least a bronchoalveolar lavage to exclude bacterial, viral, fungal, and other opportunistic pathogens; lung biopsy may also be required for diagnosis and to guide treatment. Therapy and prognosis of COP are different from those of BO, in that COP usually responds quickly to high-dose glucocorticoid therapy and is often reversible, whereas moderate or severe BO is generally irreversible and with variable response to corticosteroids.

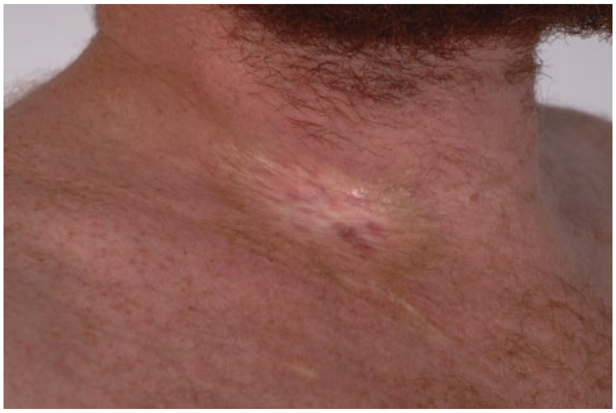

Figure 84.9 Morphea-like superficial sclerotic skin changes, a diagnostic feature of cGVHD. Note the localized, patchy areas of movable smooth or shiny skin with a leathery-like consistency, often with dyspigmentation. In this picture, there is a fibrotic, hypopigmented area in the center of the plaque with a slightly hyperpigmented border. (For a color version of this figure, please see the color plate section.)

Musculoskeletal system

Diagnostic features include fascial involvement often associated with sclerosis of the overlying skin and subcutaneous tissue (Figure 84.21). Fascial involvement may develop without overlying sclerotic changes of the skin and initially manifest as joint stiffness or skin dimpling when present near joints or as unilateral or bilateral extremity edema. Fasciitis is detected on examination by stiffness, restricted range of motion associated with joint contractures (Figure 84.22). Clinical myositis that is characterized by tender muscles or severe proximal weakness is a distinctive but non-diagnostic manifestation of cGVHD that is supported by the presence of elevated serum creatinine phosphokinase or aldolase, and sometimes with electromyographic findings. The differential diagnosis should consider the myopathy that may occur in patients taking statins, especially at higher doses when combined with calcineurin inhibitor therapy or sirolimus. Proximal myopathy is more commonly associated with corticosteroid therapy especially when tapering steroids. Muscle cramps are a common problem in patients with cGVHD even when serum electrolytes are normal. Arthralgia and arthritis are uncommon and are occasionally associated with the presence of autoantibodies.

Hematopoietic and immune systems

Hematopoietic abnormalities are common in cGVHD but also nonspecific and are not used to establish its diagnosis.. Thrombocytopenia ($<100 \times 10^9$/L), lymphopenia ($\leq 0.5 \times 10^9$/L), eosinophilia ($\geq 0.5 \times 10^9$/L), hypogammaglobulinemia, or hypergammaglobulinemia may be present. Autoantibodies may develop with autoimmune hemolytic anemia and immune thrombocytopenic purpura. Thrombocytopenia ($<100 \times 10^9$/L) at the initial diagnosis of cGVHD is a risk factor for NRM. Lymphopenia has been reported as a risk factor both for survival in cGVHD and for development of GVHD after DLI [49,50]. In the absence of other cGVHD manifestations and other causes, cytopenias may reflect an immune-mediated process and a trial of glucocorticoid or other immunosuppressive therapy may be of diagnostic and therapeutic value.

Renal

Two large retrospective studies have reported cumulative incidences of GVHD-associated nephrotic syndrome (NS) of 6.1% and 8%, suggesting that this manifestation may be more

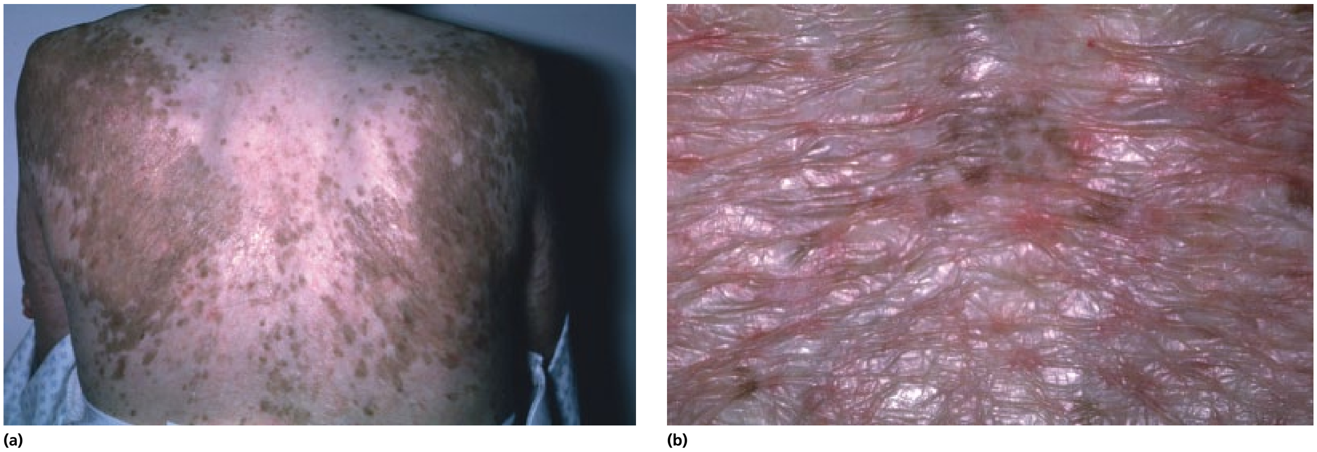

Figure 84.10 Lichen sclerosis-like lesion, a diagnostic manifestation of cGVHD. Note the discrete to coalescent gray to white movable papules or plaques, with a shiny appearance and leathery consistency (a) or cigarette paper-like textural changes (b). (For a color version of this figure, please see the color plate section.)

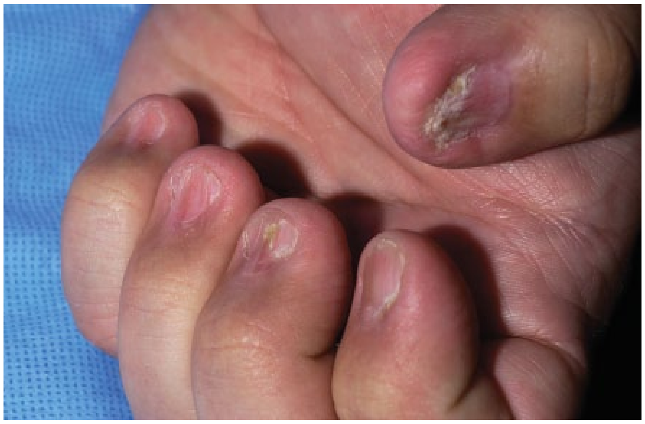

Figure 84.11 Nail dystrophy involving multiple digits is a distinctive feature of cGVHD, although it is insufficient alone to establish the diagnosis . Note the longitudinal ridging, splitting, and brittle nail features, and also periungual erythema. (For a color version of this figure, please see the color plate section.)

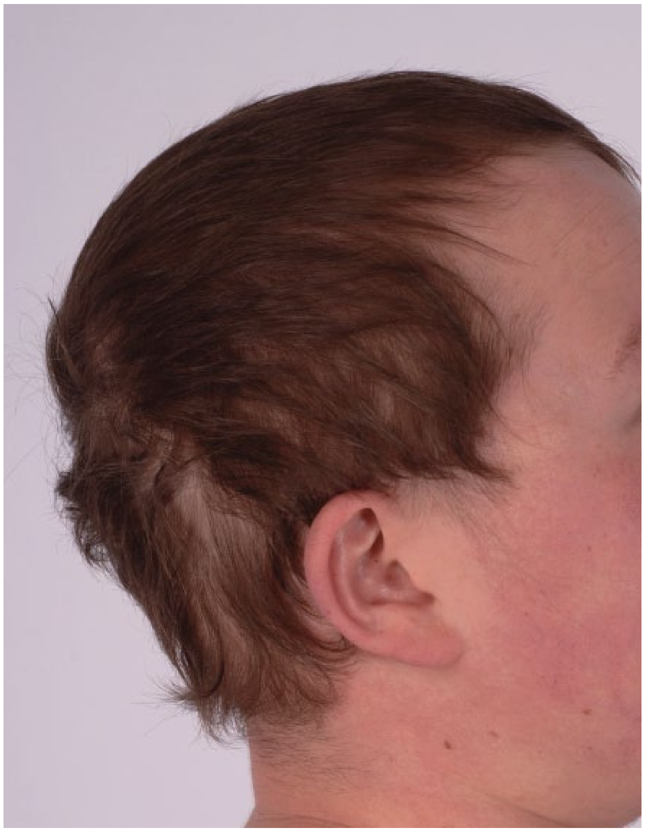

Figure 84.12 New onset of patchy alopecia after recovery from chemoradiotherapy is another distinctive manifestation of cGVHD (but is insufficient alone to establish the diagnosis). Patchy alopecia may involve loss of scalp or body hair. (For a color version of this figure, please see the color plate section.)

frequently encountered than previously thought. The first study found that all patients with NS had received RIC, whereas patients receiving high-dose conditioning at the same institution during the same period had no reported cases of NS. Patients who developed NS suffered significant morbidity, including

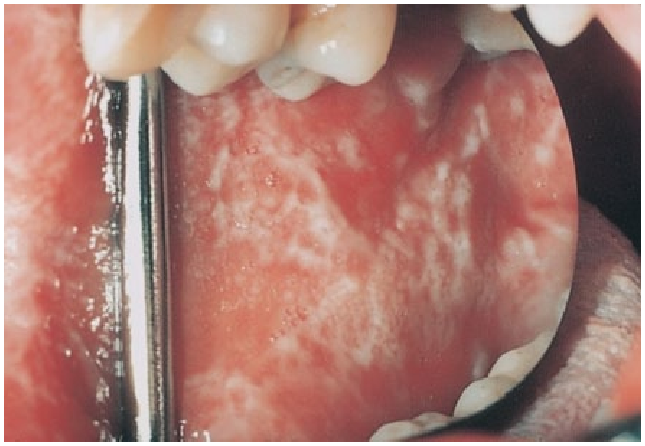

Figure 84.13 Lichenoid lesions of oral cGVHD showing crythema, white striae, plaque, and ulcer formation, day 394. (For a color version of this figure, please see the color plate section.)

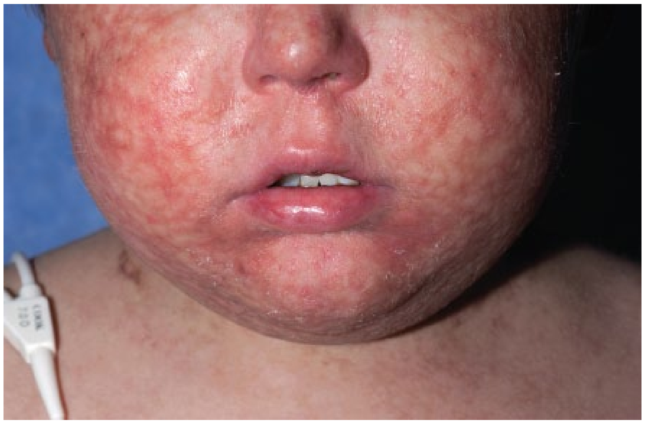

Figure 84.14 Decreased oral range of motion and microstomia in a patient with sclerotic features of cGVHD. This is a diagnostic manifestation. Note the erythema, edema, and atrophy of the vermillion lip and labial mucosa. (For a color version of this figure, please see the color plate section.)

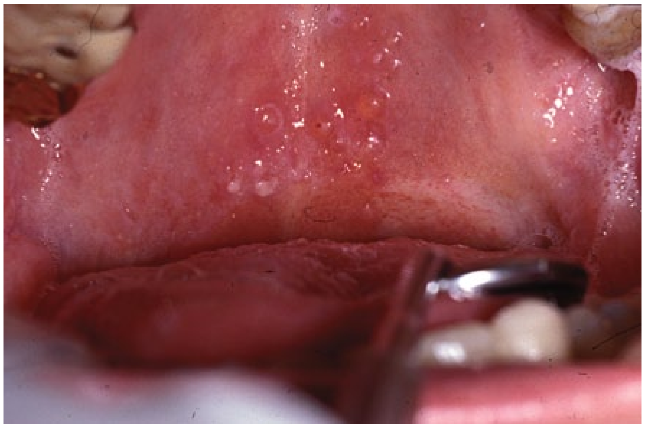

Figure 84.15 Numerous vesicle-like mucoceles (a distinct manifestation of cGVHD) are seen along the center of the soft palate. Patchy white lichenoid hyperkeratosis (diagnostic of cGVHD) and interspersed moderate erythematous changes are also evident across the soft palate. Additionally, note the foamy mucus saliva consistent with GVHD salivary gland dysfunction. (For a color version of this figure, please see the color plate section.)

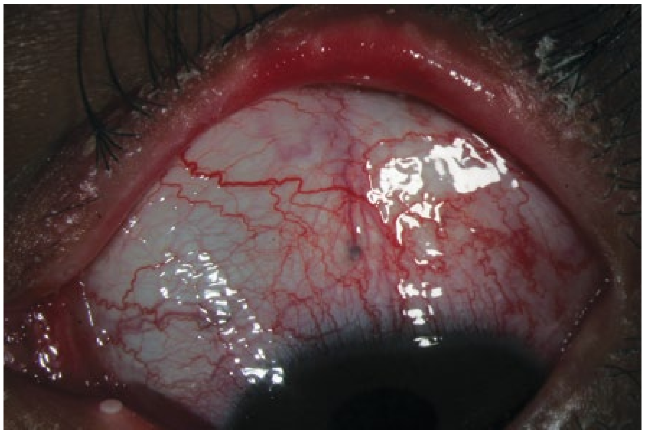

Figure 84.16 Keratoconjunctivitis sicca with blepharitis. The eyelid margins are thickened, edematous, and erythematous. Also note plugging of the meibomian gland orifices (along the eyelid margin) and significant conjunctival hyperemia/injection. A punctal plug can be seen at the lower left margin of the image. (For a color version of this figure, please see the color plate section.)

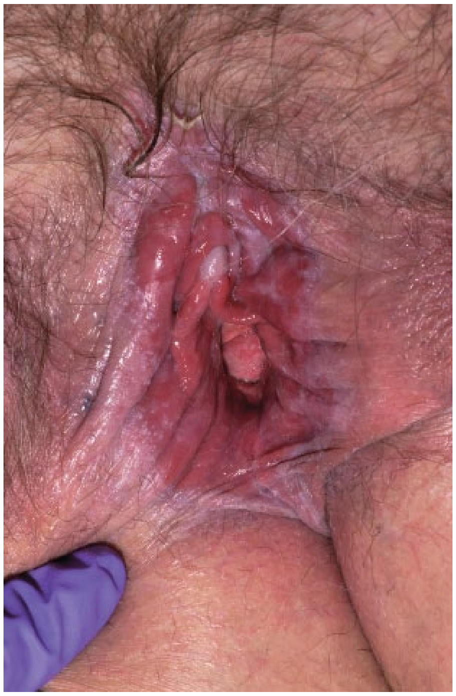

Figure 84.17 cGVHD involving the vulvovaginal tissues. Note lichen planus-like manifestations, which are diagnostic of cGVHD. Also seen are erythema and agglutination of the labial tissues. (For a color version of this figure, please see the color plate section.)

thromboembolic complications and progressive renal failure, and were unlikely to respond to immunosuppression [51]. The second study found no differences between conditioning regimens, but rather observed an association with hematopoietic cell source with increased probability of NS in patients receiving peripheral blood stem cells (PBSCs) versus bone marrow. All patients in this study responded to immunosuppressive therapy [52]. See also Chapter 96.

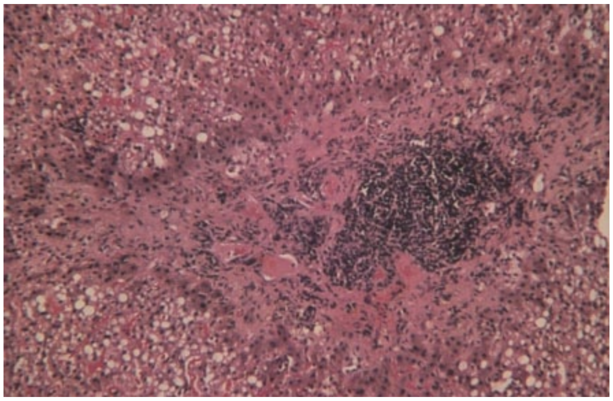

Figure 84.18 cGVHD of the liver, day 166. This biopsy shows a prominent plasmacytic infiltrate in the portal area and a paucity of interlobular bile ducts. The histological dichotomy between acute GVHD and cGVHD is less clear in the liver than in skin. However, increased portal inflammation and loss of bile ducts are more common in cGVHD. Despite the frequency of chronic hepatic GVHD, cirrhosis and liver failure are rare and may reflect superinfection with hepatitis C virus. (See also Chapter 23.) (For a color version of this figure, please see the color plate section.)

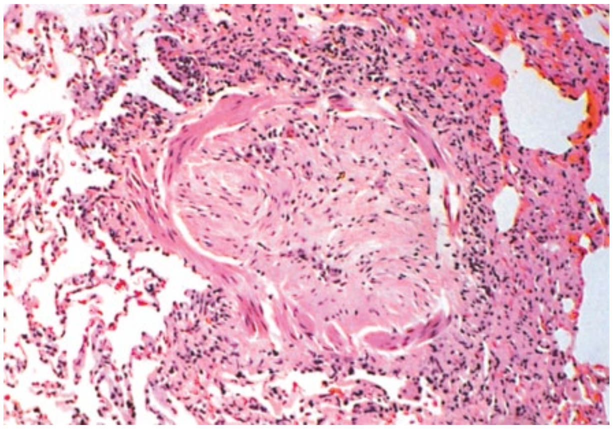

Figure 84.19 Bronchiolitis obliterans in a lung biopsy, day 396. The patient had cGVHD with severe obstructive pulmonary disease. Similar small airway inflammation with fibrosis is seen in lung allograft rejection. (See also Chapter 23 and Chapter 47.) (For a color version of this figure, please see the color plate section.)

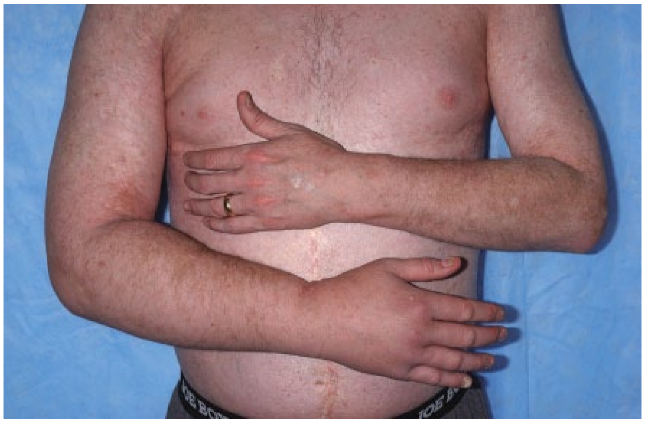

Figure 84.20 Edema in the extremities can be bilateral or, as shown, unilateral. Edema may be an early sign of evolving subcutaneous sclerosis and fasciitis. (For a color version of this figure, please see the color plate section.)

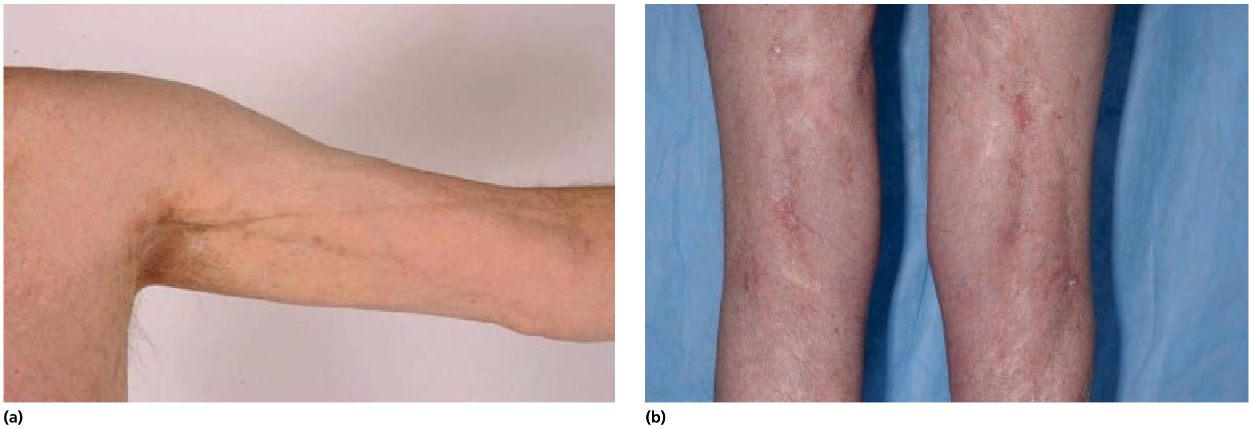

Figure 84.21 Manifestations suggestive of fascial involvement by cGVHD (a diagnostic feature) can include rippling or the groove sign. (For a color version of this figure, please see the color plate section.)

Table 84.2 Distinguishing bronchiolitis obliterans (BO) from cryptogenic organizing pneumonia (COP)

Feature	BO	COP
Symptoms	Dyspnea, cough, wheeze, or asymptomatic (early stages)	Fever, dry cough, dyspnea
Signs	Wheeze, crackles, or none	Crackles, tachypnea, or none
High-resolution CT scan	Air trapping with expiratory images, small airway thickening, bronchiectasis (advanced)	Infiltrates present, either focal or multifocal (but not diffuse)
FEV$_1$	<75% predicted with ≥10% decline over <2 years	Reduced or normal
FEV$_1$/FVC (or SVC)	<0.70	Normal
Residual volume	>120%	Reduced or normal
Response to steroids	Usually do not recover FEV$_1$ after steroid therapy	Often recover FEV$_1$ and FVC after high-dose steroid therapy but relapses do occur
Pulmonary prognosis	Poor	Good
Survival	Relatively poor	Relatively good

FEV$_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; SVC, slow vital capacity.

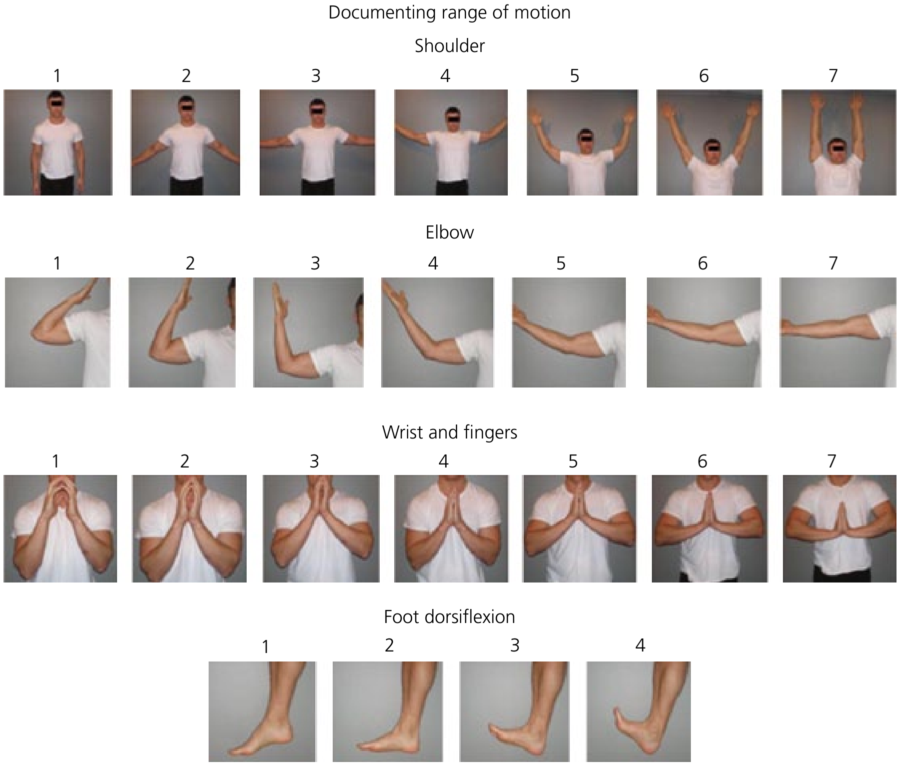

Figure 84.22 Documenting range of motion. Beginning with the full (normal) range of motion on the right, the images indicate progressively more limited range of motion as the numeric scores decrease toward maximally restricted joint motion. By row, from top to bottom, the tasks being attempted are full shoulder abduction with upper arms brought next to ears, full elbow extension with arms outstretched, "Buddha prayer" position, and full ankle dorsiflexion. Source: Carpenter 2011 [84]. Reproduced with permission of the American Society of Hematology.

Other manifestations

Serositis (pericardial and pleural effusions or ascites), peripheral neuropathy, myasthenia gravis, and cardiac function abnormalities occur rarely in cGVHD but are all diagnoses of exclusion unless the patient has additional features of cGVHD.

Organ severity staging of cGVHD

Historically, cGVHD was classified as limited (localized skin involvement and or liver dysfunction) or extensive (generalized skin involvement, liver histology showing aggressive hepatitis or involvement of any other target organ) [1]. This system has been replaced by NIH global severity grading (none, mild, moderate, severe), which in turn uses a 0–3 point scoring of cGVHD severity within each affected organ or site, taking functional impact into account (Figure 84.4b) [2]. The eight scored organs include skin, mouth, eyes, GI tract, liver, lungs, joints and fascia, and female genital tract. Before the 2014 NIH cGVHD Consensus, scoring did not consider whether the organ-specific manifestation was attributed to cGVHD versus another cause (e.g. diarrhea associated with magnesium therapy or *C. difficile* toxin). This point is emphasized by the ~60% incidence of lung involvement reported in the recent Chronic GVHD Consortium study that is out of keeping with the 10–15% incidence of BO found in earlier reports (Figure 85.4a and b). A score of 1 means that there is no significant impairment of function or activities of daily living (ADL), a score of 2 means impairment of ADL without major disability and a score of 3 indicates impairment of ADL with major disability. Scoring can be easily conducted in the clinic and the only mandated laboratory tests for its completion are liver function tests (Table 84.3).

The pulmonary NIH score uses symptoms plus the results of PFTs whenever possible, although evaluation can also be accomplished by simply assessing dyspnea symptoms and need for supplemental oxygen. When discrepancies exist between scores based on lung symptoms versus PFTs, the FEV$_1$ score should be used (Table 84.3). The diagnosis and scoring of bronchiolitis obliterans has been recently revised by the NIH 2014 cGVHD Consensus [13]. To increase the specificity of the cGVHD lung function score (LFS) for obstructive lung defects this score no longer includes the carbon monoxide diffusing capacity (DLCO) and just includes the FEV$_1$.

Table 84.3 NIH cGVHD 0–3 scale for scoring severity in each organ

	Score 0	Score 1	Score 2	Score 3
PERFORMANCE SCORE: KPS/ECOG/LPS	Asymptomatic and fully active (ECOG 0; KPS **OR** LPS 100%)	Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS **OR** LPS 80–90%)	Symptomatic, ambulatory, capable of self-care, >50% or waking hours out of bed (ECOG 2, KPS **OR** LPS 60–70%)	Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3–4, KPS **OR** LPS <60%)
SKIN Diagnostic/distinctive features ☐Present ☐Absent %BSA____	No manifestations	<18% BSA with disease signs but **NO** sclerotic features	19–50% BSA **OR** with superficial sclerotic features "not hidebound" (able to pinch)	>50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH Diagnostic/ distinctive features ☐Present ☐Absent	No symptoms	Mild symptoms with disease signs but not limiting oral intake significantly	Moderate symptoms with disease signs **WITH** partial limitation or oral intake	Severe symptoms with disease signs on examination **WITH** major limitation or oral intake
EYES	No symptoms	Mild dry eye symptoms not affecting ADL (requiring eyedrops <3× per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	Moderate dry eye symptoms partially affecting ADL (requiring eyedrops >3× per day or punctal plugs) **WITHOUT** vision impairment	Severe dry eye symptoms significantly affecting ADL (special eyewear to relieve pain) **OR** loss of vision caused by keratoconjunctivitis sicca
GI TRACT	No manifestations	Manifestations such as nausea, vomiting, anorexia, dysphagia, abdominal pain or diarrhea **WITHOUT** significant weight loss	Manifestations associated with mild-to-moderate weight loss (5–15%)	Manifestations associated with significant weight loss (>15%), requires nutritional supplement for most calorie needs **OR** esophageal dilatation
LIVER	Normal LFTs	Elevated bilirubin, AP, AST or ALT <2 × ULN	Elevated bilirubin >3 mg/day or bilirubin, enzymes 2–5 × ULN	Bilirubin or enzymes >5 × ULN
LUNGS Pulmonary function test results **FEV$_1$** **DLCO**	No symptoms FEV$_1$ ≥80%	Mild symptoms (dyspnea climbing one flight of stairs) FEV$_1$ 60–79%	Moderate symptoms (dyspnea walking on flat ground) FEV$_1$ 40–59%	Severe symptoms (dyspnea at rest; requiring O$_2$) FEV$_1$ ≤39%
JOINTS/FASCIA	No manifestations	Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	Tightness of arms or legs, **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	Contractures **WITH** significant limitation of ROM **AND** significant limitation of ADL (unable to tie shoes, button shirts, dress self, etc.)
GENITAL TRACT Diagnostic/ distinctive features ☐Present ☐Absent ☐Not examined	No symptoms	Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	Symptomatic with moderate signs on exam **AND** with mild dsypareunia or discomfort with gynecologic exam	Symptomatic **WITH** advanced signs (stricture, labial agglutination, or severe ulceration) **AND** severe pain with coitus or inability to insert a vaginal speculum

Abbreviations: ADL, activities of daily living, ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BSA, body surface area; ECOG, Eastern Cooperative Oncology Group; KPS, Karnofsky Performance Status; LPS, Lansky Performance Status; LFS, lung function score; LFTs, liver function tests; ULN, upper limit or normal.
Source: Adapted with permission from Filipovich et al. 2005 [2] (see Jagasia et al [13] for 2014 NIH Scoring updates that became available after this chapter was submitted for publication).

Global severity staging of cGVHD

The NIH cGVHD global severity score (none, mild, moderate, severe) is derived by combining organ-specific scores and has replaced the historical "limited" and "extensive" categories. Global scoring aligns better with patient and physician estimates of cGVHD severity [2]. Global scoring has treatment implications and considers the number of organs involved, the degree of involvement in affected organs/sites, and, importantly, the presence of mild or moderate lung involvement will upgrade global scores by one point (Table 84.4). The easily accomplished individual organ severity and global severity staging is considered useful for baseline or sentinel time points in the course of the cGVHD therapy in clinical practice or in clinical trials.

Although not initially intended for response monitoring in cGVHD intervention trials, emerging data from the Chronic GVHD Consortium suggest that NIH organ severity scoring (skin, oral, ocular, and joints) and global scores perform best in terms of predicting patient- and physician-perceived improvement or worsening over time and may also predict other key transplant outcomes such as NRM [4,8–10].

Histopathology and diagnosis of cGVHD

cGVHD is a clinical diagnosis. However, histological confirmation has an important role in the final assessment. Biopsies may be useful for establishing a diagnosis of cGVHD when no diagnostic manifestations of cGVHD are present, when alternative diagnoses are being entertained, or clinical assessment is difficult because of concomitant medical conditions. Among cGVHD referrals to the Johns Hopkins University, 7% of patients who did not have biopsies prior to referral and before starting cGVHD therapy had other diagnoses [53]. Specific details about the histopathology of cGVHD are covered in Chapter 26.

Management of cGVHD

Prevention

As in acute GVHD, preventive approaches have remained a desirable goal. Whereas the prevention of cGVHD provides obvious benefit in the transplantation of non-malignant diseases, the same cannot be said for malignant diseases because effective but non-targeted prevention may significantly abrogate crucial graft-versus-tumor (GVT) effects.

Among the aforementioned risk factors for developing cGVHD or prolonging its therapy, there are few modifiable factors such as choosing better matched donors, avoiding female donors for male recipients, and using marrow or cord blood rather than peripheral blood as a source of hematopoietic cells. However, these modifications have limited impact on cGVHD and more effective methods of prevention are needed. Although acute GVHD is the best predictor for developing cGVHD, strategies that have reduced acute GVHD rates have not reliably reduced the rates of cGVHD [20,54–56]. Other unsuccessful attempts to prevent cGVHD include addition of hydroxychloroquine, thalidomide, intravenous immunoglobulin, or preemptive treatment of subclinical cGVHD found in skin and lip biopsies [57–60]. Graft manipulation by T-cell depletion is successful in reducing acute GVHD but the effects on cGVHD are less evident and may depend on graft manipulation methods or source of stem cells [20,21].

Table 84.4 Indications for systemic therapy according to severity and high-risk features of cGVHD

Category	Maximum severity in all organs (scores according to Table 87.2)	No. of organs	High-risk feature[a]	Systemic treatment
Mild	1 (0 for lung)	≤2	Absent	No
Moderate (a)	1 (0 for lung)	≥3	Present	Yes[b]
Moderate (b)	2 (1 for lung)	Any	Present or absent	Yes[b]
Severe	3 (2 for lung)	Any	Present or absent	No

[a] High risk defined as thrombocytopenia (<100 × 10^9/L) or receiving glucocorticoids >0.5 mg/kg/day at the onset of cGVHD.

[b] The benefit of graft-versus-leukemia/tumor effect versus the risk of delaying systemic treatment for cGVHD needs to be weighted based on the risk of recurrent malignancy after transplantation.

A few promising approaches require further validation by other groups. The first approach was the randomized phase III trial conducted in Europe that compared standard GVHD prophylaxis in adults with or without pre-transplantation ATG-Fresenius. With follow-up now extended to 3 years, the cumulative incidence of extensive cGVHD was 12.2% in the ATG-Fresenius group versus 45% in the control group ($p < 0.0001$). A non-significant reduction in NRM without increased relapse risk led to an overall survival rate of 55% in the ATG-Fresenius group versus 43% in the control group [hazard ratio (HR) 0.84, $p = 0.39$] but the 3-year probability of survival free of immunosuppressive therapy was 53% versus 17%, respectively [61]. Two other approaches that are being compared in a randomized phase III trial (BMT CTN 1101] arose from the promising results of parallel phase II BMT CTN trials of double unrelated cord blood transplantation and non-T-cell-depleted haploidentical marrow transplantation with RIC and post-transplant cyclophosphamide. Although direct comparisons should not be made, cGVHD rates at 1 year were low: 13% after haploidentical BMT and 25% after double cord blood transplant [62]. The major obstacle to optimizing cGVHD prevention approaches remains our rudimentary understanding of cGVHD biology. The field has prioritized relevant correlative biology studies in an effort to generate this critically needed information.

Treatment

Therapy of cGVHD depends heavily on a coordinated multidisciplinary team that often includes transplantation specialists, a primary healthcare provider, organ-specific consultants, nurses, and ancillary services such as social services, vocational specialists, and patient and family support groups and systems. Principal components of cGVHD therapy include systemic immunosuppressive medications for an average of 2–3 years, integrated with ancillary therapy and supportive care. Ultimately, about 85% of patients who survive beyond 5 years after diagnosis are able to discontinue systemic therapy [27]. The goal of cGVHD therapy is to stop destructive immune dysregulation, alleviate symptoms, and prevent disease progression that may lead to irreversible disability or death. The ultimate goal is to establish tolerance and withdraw immunosuppressive therapy. It is also important to recognize that even after permanent withdrawal of immunosuppression, patients remain at increased risk for late complications of allogeneic HCT and require continued monitoring by providers familiar with the late effects of HCT and cancer therapy [63,64].

Primary systemic therapy

The need for cGVHD therapy may be guided by the NIH global severity score and the presence or absence of high-risk features for NRM (Table 84.4). For example, mild but symptomatic cGVHD of the skin and or mouth is often effectively treated with local therapies alone (e.g. topical steroids to the skin or steroid mouth rinses), yet these same features in a patient whose platelet count is <100 × 10^9/L at the time cGVHD is diagnosed would justify the addition of systemic therapy. However, systemic therapy should always be considered for patients with moderate or severe global severity. Good medical practice and judgment dictate flexibility in this recommendation and the underlying reason for transplantation (e.g. high-risk malignancy versus non-malignant disease) or current comorbid conditions (e.g. infection) into decisions on whether or not to treat with systemic immunosuppression.

To date, only six randomized phase III trials have ever been reported for initial therapy of cGVHD [65–70]. None of these studies demonstrated any benefit of additional agents (azathioprine, cyclosporine, thalidomide, mycophenolate mofetil, or hydroxychloroquine) over prednisone alone, except for lower rates of avascular necrosis when cyclosporine was added (13% versus 22%, $p = 0.04$). Hence the most widely used initial approach is cyclosporine or tacrolimus and prednisone initially at 1 mg/kg once daily [71,72]. Cyclosporine or tacrolimus is dosed twice daily to keep levels therapeutic. The starting prednisone dose has some flexibility, generally balancing cGVHD severity against the relative individual contraindications for high-dose prednisone. It is important to avoid split daily dose regimens for prolonged prednisone therapy, and once the patient is responding or showing signs of stable disease, generally after 2 weeks, prednisone is tapered to an alternate-day schedule over 4 weeks (Table 84.5) which avoids or minimizes many steroid-related complications [73]. Responses are evaluated at least every 3 months after alternate-day dosing is achieved, and after all reversible manifestations have resolved prednisone is gradually tapered by 10% every 2–4 weeks as tolerated. Examples of potentially irreversible manifestations include severe ocular sicca, joint contractures, and BO.

It is worth detailing further the results of the non-blinded phase III trial that compared prednisone alone with prednisone plus cyclosporine in 287 patients with standard-risk cGVHD without thrombocytopenia. Importantly, the cumulative incidence of NRM, overall survival, relapse, need for secondary cGVHD therapy, and discontinuation of immunosuppressive medications were not significantly different between the two arms [67]. However, survival without recurrent malignancy was better in the prednisone-only arm (71% versus 61%, $p = 0.03$). Hence there is no evidence that initial combination therapy of cyclosporine with prednisone improves control of cGVHD in standard-risk patients with platelet counts >100 × 10^9/L. The reason why combination therapy with prednisone and a calcineurin inhibitor continues to be favored is because of demonstrated steroid-sparing effects. However, in high-risk cGVHD with thrombocytopenia, the addition of cyclosporine exerted a survival benefit over prednisone alone based on the results of sequential phase II trials in the 1980s [65,74].

Table 84.5 Standard "9 months" glucocorticoid therapy for cGVHD.

Week	Dose of prednisone (mg/kg)
1–2	1.0 daily
3	1.0 alternating with 0.5 every other day
4	1.0 alternating with 0.25 every other day
5	1.0 alternating with 0.12 every other day
6	1.0 alternating with 0.06 every other day
7–20	1.0 every other day
21	0.9 every other day
22	0.8 every other day
23	0.7 every other day
24	0.6 every other day
25–40	0.5 every other day
	After resolution of all reversible manifestations, taper prednisone by 10% every 2–4 weeks as tolerated

Secondary therapy

Systemic therapy is changed in approximately 60% of patients during the first 3 years because of inadequate response to primary therapy for cGVHD [75]. In general, secondary systemic therapy is *not* indicated while there is continuing improvement in at least one manifestation of cGVHD without progression of any other manifestations. Secondary therapy is generally indicated whenever (1) clinical manifestations progress within 1 month in a previously involved organ or a new organ becomes involved, (2) manifestations show no improvement within 2 months on the originally prescribed medications, (3) daily prednisone doses cannot be tapered below 1.0 mg/kg within 2 months, or (4) manifestations worsen during later attempts to taper prednisone below 0.5 mg/kg/day [72]. Earlier initiation of secondary therapy would be appropriate in severe cGVHD. In other scenarios, a longer trial of initial therapy would be appropriate and examples include sclerotic manifestations where slow improvement is expected, or BO where improvement in the FEV_1 occurs slowly, if at all, and stability of the FEV_1 is generally considered a success. Cryptogenic organizing pneumonia, which may occur in either acute GVHD or cGVHD, is another exception since relapses are frequent with steroid tapers that follow the alternate-day schema of Table 84.5. Inability to tolerate higher doses of prednisone because of debilitating steroid myopathy, avascular necrosis, poorly controlled diabetes, or renal dysfunction due to cyclosporine-associated thrombotic microangiopathy are common reasons that prompt earlier changes to secondary treatment. Situations that lead to delayed initiation of secondary therapy are not unusual when the next-in-line agents have a high risk of toxicity, and especially when multiple therapies have already been exhausted.

A comprehensive review of published reports between 1990 and 2011 showed numerous deficiencies in studies of secondary systemic treatments for cGVHD [76]. A total of 60 studies were selected for review after excluding 26 studies that contained fewer than 10 patients, case reports, and phase III studies. Most studies relied on the unfounded assumption that any improvements after the study therapy must have been caused by the study therapy. Major deficiencies in ~90% of the studies were the lack of a suitable comparison group that could serve as a valid historical benchmark, the absence of a formal statistical hypothesis, and the absence of a consistent treatment due to variations in patient selection. Most studies failed to measure response at a fixed time point and simply selected best response at any time as the end-point. Lack of uniformity in the evaluation and reporting of cGVHD responses was highlighted by the 2005 NIH Consensus Conference [77] and validation of standardized response criteria that correlate with patient- and physician-perceived clinical improvement or worsening are only just starting to emerge [4,10–13]. One other problem in determining the efficacy of a therapy in cGVHD is that early responses may not necessarily translate into subsequent tolerance without recurrent malignancy [78]. All of these issues notwithstanding, physicians are often faced with having to choose (multiple) secondary therapies. The report by Martin et al. indicated that the seven most frequently studied therapies in descending order were photopheresis, mycophenolate mofetil, thalidomide, sirolimus or everolimus, rituximab, and methotrexate [76]. A list of 30 secondary therapies for cGVHD has been developed that includes supporting evidence and recommendations

Table 84.6 Considerations for use of the most published agents in cGVHD as second-line therapy or beyond

Extracorporeal photopheresis (ECP)	
Mechanism of action	Direct apoptosis of leukocytes (especially lymphocytes) and reinfusion of these cells seems to generate tolerogenic responses by reported mechanisms that include interference with dendritic cell maturation, modulation of cytokine production, and expansion of regulatory T (Treg) cells
Toxicity concerns	Limited: blood loss from the extracorporeal circuit, hypocalcemia depending on anticoagulant used, mild cytopenia, catheter-associated bacteremia, but overall infection risks not increased over standard therapy
Significant interactions	None
Infection concerns	Viral or fungal infection not increased
Convenience	Inconvenient; requires travel to ECP centers for up to many months. Complex schedule typically 2 per week (week 1), 2 per week (weeks 2–12), and 2 per month thereafter. Often requires central line placement for the procedure
Expense concerns	Very expensive
Mycophenolate mofetil (MMF)	
Mechanism of action	Non-competitive inhibitor of IMPDH, the rate-limiting step for de novo purine synthesis on which lymphocytes depend
Toxicity concerns	Dose-related cytopenia and gastrointestinal toxicity; consider risk:benefit carefully when GVHD includes GI tract. Enteric-coated mycophenolic acid (Myfortic) may be better tolerated
Significant interactions	May compound cytopenia when used with other myelosuppressive drugs
Viral infection concerns	Moderately high, especially if used with high-dose steroids in already lymphopenic patients
Convenience	Smallest pill formulations are 250 mg (MMF) or 180 mg (Myfortic) and IV formulation is available
Expense concerns	Moderately expensive
Comment	Randomized phase III study in cGVHD showed no benefit of adding MMF to standard therapy (prednisone plus CNI [69]) but no definitive data on its use as secondary therapy and without a CNI
Thalidomide	
Mechanism of action	Immunomodulatory, although specific actions are not fully understood. Under some conditions suppresses TNF-α
Toxicity concerns	Neutropenia, sedation, constipation, numbness, neuropathy frequent at >200 mg/day. Must counsel women of child-bearing potential and sexually mature males how to avoid birth defects
Significant interactions	Not usually clinically significant
Viral infection concerns	No considered to be a high risk
Convenience	50-mg capsule is smallest formulation; no IV form available
Expense concerns	Moderately expensive
Comment	May be most logical to try in the treatment of myeloma patients who have cGVHD
Sirolimus (rapamycin)	
Mechanism of action	Binds to FK-binding protein complex and blocks mTOR causing cell cycle arrest in G1; T cells are the most sensitive. Other mechanisms include blocking CD28 costimulation, prevention of dendritic cell antigen presentation and maturation, impairment of B-cell activation and immunoglobulin production
Toxicity concerns	Most clinically relevant are reversible cytopenia, hypertriglyceridemia, nephrotoxicity (HUS/TMA) and neurotoxicity (TTP) when combined with CNIs. Less common clinically relevant are transaminase elevations, edema, arthralgias, delayed wound healing and non-infectious pneumonitis.
Significant interactions	Sirolimus is a substrate for CYP3A4 and P-gp and attention to concomitant therapies that interact with CYP3A4 and P-gp is necessary. Critical interaction mandates an initial 90% sirolimus dose reduction when starting voriconazole (7% reduction for posaconazole, 25% reduction for fluconazole). Sirolimus may increase risk for rhabdomyolysis during HMG-CoA reductase inhibitor therapy. In patients who develop HUS, the CNI should be stopped and sirolimus dose should be adjusted to ensure trough level <10 ng/mL. If total bilirubin >2 mg/dL, sirolimus dose should be reduced by 30%.
Viral infection concerns	Moderate
Convenience	Oral formulations only: 0.5, 1, and 2-mg tablets, and 1 mg/mL oral solution. Target levels 3–12 ng/mL (<10 ng/mL if also on a CNI). Consider $T_{½}$ 57–62 hours when adjusting dose and checking levels
Expense concerns	Moderately expensive
Rituximab	
Mechanism of action	Mature B-cell-depleting chimeric anti-CD20 monoclonal antibody that suppresses antibody-dependent and, via T-cell collaboration, antibody-independent B-cell cGVHD-associated mechanisms
Toxicity concerns	Mostly well tolerated. The extent to which rituximab exacerbates cGVHD associated B-cell lymphopenia and hypogammaglobulinemia is unclear; infectious complications have been reported.
Significant interactions	Not usually clinically significant
Viral infection concerns	Low
Convenience	IV only; usually 375 mg/m^2 given weekly for 4 doses with possibility to repeat 4 doses given weekly or monthly (one group reported similar results if given at low dose, 50 mg/m^2) [92]
Expense concerns	Very expensive
Comment	Most successes reported for deep and superficial skin and autoimmune manifestations
Pentostatin	
Mechanism of action	Nucleoside analog that potentially inhibits adenosine deaminase. Broad activities in GVHD include marked reduction of CD4 and CD8 T cells, significant B-cell depletion and lowering of IgG levels.
Toxicity concerns	Myelosuppression, renal toxicity, neurotoxicity, and reversible elevation of liver function tests. It is advisable to reduce dose to 2 mg/m^2 if ANC <0.5 × 10^9/L, platelets <20 × 10^9/L, or if creatinine clearance 30–50 mL/min/1.73 m^2 (and hold if <30 mL/min/1.73 m^2). Do not give during severe infection. Stop or withhold therapy for neurotoxicity
Significant interactions	Not usually clinically significant.
Viral infection concerns	Moderate but apparently less that in the acute GVHD setting when pentostatin is dosed differently. Consider viral plasma PCR monitoring in patients with delayed numeric immune reconstitution.
Convenience	IV formulation only: 4 mg/m^2 every 2 weeks
Expense concerns	Very expensive
Comment	The largest study was in pediatrics [93].
Low-dose methotrexate	
Mechanism of action	Immunomodulatory and anti-inflammatory properties at low doses are mediated not through inhibition of DHFR and DNA synthesis but through release of adenosine, which binds to A_2 receptors and inhibits oxidative burst in activated neutrophils and the adhesion of neutrophils to endothelial cells. Adenosine modifies cytokine production by neutrophils, monocytes, macrophages, and T cells, decreasing production of TNF-α, IFN-γ, IL-6, and IL-8, and increasing production of IL-10 and IL-4. Methotrexate is converted intracellularly from monoglutamated derivatives to long-lasting polyglutamates, which explains the efficacy of the low-dose weekly regimen

(*Continued*)

Table 84.6 (*Continued*)

Toxicity concerns	Generally low. Myelosuppression uncommon. Liver toxicity uncommon. Cheilitis. Alopecia. Avoid in renal failure or in patients with large third-space fluid accumulations
Significant interactions	Not usually clinically significant
Viral infection concerns	Low
Convenience	Easy to prescribe (7.5 mg/m^2 per week), but important patient communication needed to avoid life-threatening overdose
Expense concerns	Inexpensive
Comment	Consider in mild fasciitis or arthralgias that are considered to be a cGVHD manifestation
Imatinib	
Mechanism of action	Tyrosine kinase inhibitor, PDGFR inhibitor
Toxicity concerns	Cytopenias, edema
Significant interactions	Inhibits CYP3A4 and may increase the concentration of statins, CNIs, and sirolimus
Viral infection concerns	Low
Convenience	Easy to prescribe. Oral formulation only: 100- and 400-mg tablets
Expense concerns	Very expensive and currently not often covered by insurance for the cGVHD indication
Comment	Most successes have been in sclerotic/fibrotic types of cGVHD based on early reports [40,41]
Hydroxychloroquine	
Mechanism of action	Antimalarial agent. Interferes with antigen processing and presentation. Reduces production of IL-1, IL-6, and TNF-α, and also decreases calcium signaling in T cells. Reduces proliferation and cytotoxicity associated with allorecognition
Toxicity concerns	GI symptoms, retinal damage and corneal opacities (need annual eye exams), myositis and polyneuropathy, rare cytopenias
Significant interactions	Not usually clinically significant
Viral infection concerns	Low
Convenience	Easy to prescribe but oral formulation only
Expense concerns	Relatively inexpensive
Comment	Randomized phase III study in children showed no benefit of adding hydroxychloroquine to standard therapy (prednisone plus CNI [70]) but no definitive data on its use as secondary therapy

IMPDH, inosine monophosphate dehydrogenase; BMT CTN, Blood and Marrow Clinical Trials Network; mTOR, mammalian target of rapamycin; HUS, hemolytic uremic syndrome; TMA, thrombotic microangiopathy; TTP, thrombotic thrombocytopenic purpura; EBV, Epstein–Barr virus; CMV, cytomegalovirus; ANC, absolute neutrophil count; CNI, calcineurin inhibitor; PDGFR, platelet derived growth factor receptor; P-gp, P-glycoprotein; $T_{1/2}$, terminal half-life.

for use by the Working Groups of the 2009 German–Austrian–Swiss Consensus Conference [79]. Because comparative data demonstrating superior efficacy for any particular agent over others are not available, therapeutic choices beyond primary therapy should be guided by the effects and toxicities of prior treatments, patient comorbidities, convenience, expense, familiarity of the physician with the agent, prior experience of the physician, and sensitivity to the patient's own preference. Key practical considerations are highlighted for some of the available agents in Table 84.6. Clinical practice aspects on how we treat cGVHD have been reported [80].

Ancillary therapy and supportive care

Chronic GVHD manifestations may affect multiple organs over the several years that it takes for tolerance to develop and for discontinuation of immunosuppressive therapy to occur. Current therapies often produce partial responses and GVHD flares occur frequently with attempts to taper systemic therapy. Late effects in multiple organs may arise through the interaction of pre-transplant chemoradiotherapy, cGVHD itself, infection associated with immune deficiency, and even the toxicities of cGVHD therapy. It follows that ancillary therapy and supportive care to prevent infections, optimize nutrition, ameliorate morbidity, and optimize functional performance and capacity are integral to cGVHD management (http://www.asbmt.org/?page=GuidelineStatements). The NIH consensus recommendations define "ancillary therapy" as any intervention such as topical corticosteroids, cyclosporine eye drops or any other non-systemic therapeutic intervention directed at control of symptoms. The term "supportive care" includes a broad spectrum of interventions that are directed at control of organ-specific or systemic symptoms. Supportive care includes antibiotics for prevention of infections, management of osteoporosis, metabolic problems, physical therapy, and a number of educational, preventive, and psychosocial measures. Ancillary and supportive care therapies are commonly added to systemic immunosuppressive therapy. Occasionally, their use may circumvent the need for systemic therapy or allow doses of systemic agents to be reduced. A summary of ancillary therapy and supportive care interventions guidelines is presented in the Table 84.7. Specific dispensary information is also available from the website http://www.asbmt.org/?page=GuidelineStatements.

Infection is the most common cause of mortality in patients with cGVHD and infection prophylaxis requires special emphasis. Recommendations supported by published evidence are more often derived from studies with patients who had conditions other than cGVHD. All patients with cGVHD are considered at risk for infection with encapsulated bacteria, particularly *Streptococcus pneumoniae*, but also *Haemophilus influenzae* and *Neisseria meningitidis*. Encapsulated organism prophylaxis should be given to all patients with cGVHD while systemic immunosuppressive therapy is being given. Optimal selection of prophylaxis considers local patterns of pneumococcal resistance and individual allergies or toxicities associated with available antibiotic choices that include penicillin, cotrimoxazole, second-generation cephalosporins, quinolones, and azithromycin. None of these strategies has been tested in randomized trials but historical data show fulminant septic events in patients without prophylaxis due to the profound immunosuppression. When pneumococcal resistance patterns permit, a daily cotrimoxazole regimen can provide prophylaxis against *Pneumocystis jirovecii* pneumonia (PJP), pneumococcus, and to some extent toxoplasmosis and nocardia.

Although no studies have evaluated the degree of protection provided by vaccination against encapsulated organisms in patients with cGVHD, expert consensus opinion advocates their use beginning as early as 3–6 months after transplant [82]. since the T-cell-dependent conjugated vaccines are more effective that the older polysaccharide vaccines and the risk of adverse outcomes with vaccination is low. Nonetheless, some centers require an arbitrary numeric level of immune reconstitution if vaccinations for pneumococcus, *H. influenzae* type b and *N. meningitidis* are given before 1 year after transplantation [e.g. serum immunoglobulin G (IgG) level >400 mg/dL, CD4 T cells >200/μL and CD19 B cells >20/μL].

Table 84.7 Summary of ancillary therapy and supportive care interventions

Organ system	Preventive intervention	Treatment intervention
Skin and appendages	UVA/UVB photoprotection Surveillance for malignancy	Intact skin: topical emollients, corticosteroids, antipruritics, and others (e.g. PUVA, tacrolimus) Erosions/ulcerations: microbiologic cultures, topical antimicrobials, protective films or other dressings, debridement, hyperbaric oxygen, wound care specialists
Mouth and oral cavity	Maintain good oral/dental hygiene. Consider routine dental cleaning and endocarditis prophylaxis. Surveillance for infection and malignancy	Topical high- and ultrahigh-potency corticosteroids and analgesics. Therapy for oral dryness with salivary stimulants (sugar-free gum, cevimeline, pilocarpine)
Eyes	Photoprotection Surveillance for infection, cataracts, increased intraocular pressure	Artificial tears, ocular ointments, topical corticosteroids or cyclosporine, punctal occlusion, humidified environment, occlusive eye wear, moisture chamber eye glasses, cevemiline, pilocarpine, tarsorrhaphy, gas-permeable scleral lens or bandage contact lens, autologous serum tears, microbiologic cultures, topical antimicrobials, doxcycline for blepharitis
Vulva and vagina	Surveillance for estrogen deficiency, infection (herpes simplex virus, human papilloma virus, yeast, bacteria), and malignancy	Water-based lubricants, topical high- and ultrahigh-potency corticosteroids or calcineurin inhibitors, dilators, surgery for extensive synechiae/obliteration, early gynecology consultation
GI tract and liver	Surveillance for infection (viral, fungal)	Rule out other potential etiologies. Dietary modifications, enzyme supplementation for exocrine pancreatic malabsorption, gastroesophageal reflux management, esophageal dilatation, ursodeoxycholic acid
Lungs	Surveillance for infection (*Pneumocystis jirovecii*, viral, fungal, bacterial)	Rule out other potential etiologies (e.g. infection, gastroesophageal reflux). Inhaled corticosteroids, bronchodilators, supplementary oxygen, pulmonary rehabilitation Consideration of lung transplantation
Hematopoietic	Surveillance for infection (cytomegalovirus, parvovirus)	Rule out other potential etiologies (e.g. drug toxicity, infection) Filgrastim, immunoglobulin for immune cytopenias
Neurologic	Calcineurin inhibitor drug level monitoring Seizure prophylaxis including blood pressure control, electrolyte replacement, anticonvulsants	Occupational and physical therapy, treatment of neuropathic syndromes with tricyclic antidepressants, selective serotonin reuptake inhibitors or anticonvulsants
Infectious disease	Prophylaxis against *Pneumocystis jirovecii*, encapsulated bacteria, shingles Consider prophylaxis against molds during treatment of cGVHD with prednisone and a history of proven or probable mold infection	Organ-specific antimicrobial agents Empiric parenteral broad-spectrum antibacterial coverage for fever
Immune reconstitution	Consider immunizations only with killed vaccines (influenza, conjugates against *H. influenzae* type B, tetanus/diphtheria/pertussis, pneumococcus and meningococcus, hepatitis A and B, polio) Immunoglobulin replacement based on levels through day 365 post-transplant	Consider immunoglobulin replacement based on levels for recurrent sinopulmonary infections
Musculoskeletal	Surveillance for decreased range of motion, bone densitometry, calcium levels and 25-OH-vitamin D. Consider MR imaging to detect avascular necrosis in patients with large joint pain Physical therapy, calcium, vitamin D, and consider bisphosphonates (or hormone replacement therapy if appropriate) if on prednisone	Physical therapy Consider bisphosphonate therapy for osteopenia, osteoporosis and early avascular necrosis Consider referral to orthopedic surgeon for avascular necrosis (immobilization brace, limited weight bearing with crutches, core decompression, osteochondral allografting, joint resurfacing, total joint arthroplasty)
Endocrine	Surveillance for fasting hyperglycemia, hyperlipidemia, and, if clinically indicated, consider cortisol stimulation testing to rule out secondary adrenal insufficiency	Diabetes management Lifestyle intervention and lipid-lowering agents Adrenal replacement hydrocortisone dosing

MR, magnetic resonance; PUVA, psoralen and ultraviolet irradiation.
Source: adapted from Couriel et al. 2006 [81]. (see Carpenter et al [http://www.asbmt.org/?page=GuidelineStatements] for 2014 NIH Ancillary and Supportive Care updates that became available after this chapter was submitted for publication.)

Vaccination with TIV (trivalent influenza vaccine) but never the LAIV (live attenuated influenza vaccine) is advised for all recipients who are at least 6 months out from HCT and, in the midst of an influenza epidemic, as early as 4 months after HCT. Some experts advocate empirical treatment of influenza with neuraminidase inhibitors during influenza outbreaks, using prediction rules based on symptoms and signs, although there is no evidence to support this practice [81]. Any live virus, including the live attenuated MMR (measles/mumps/rubella), varicella, and zoster vaccines, should not be given. Household contacts should not be given oral polio vaccine. Additional details about post-transplant vaccination are covered in Chapter 106. Universal administration of IVIG after HCT has not been shown to confer clinical benefit and should be avoided. Intravenous immunoglobulin supplementation may be considered for patients >90 days after HCT who have recurrent sinopulmonary infections and serum IgG levels <400 mg/dL.

Invasive mold infections are a significant risk for patients with severe cell-mediated immunodeficiency caused by cGVHD and its treatment. Although controversies remain about appropriate therapy duration, dosing and levels, drug interactions, and resistant breakthrough infections, many centers prescribe primary prophylaxis with posaconazole or voriconazole for patients considered at high risk for invasive mold infections such as those with cGVHD and on higher doses of steroids (>0.5–1.0 mg/kg/day) [82,83]. Most centers also prescribe secondary prophylaxis during systemic therapy for cGVHD; selection of an appropriate prophylactic drug is based on the specific pathogen that caused the previous invasive mold infection and the response to antifungal therapy during that episode. See also Chapter 86.

All patients who are receiving systemic therapy for cGVHD should receive and continue prophylaxis against PJP until 6 months after stopping immunosuppressive therapy according to consensus guidelines [82]. First-choice PJP prophylaxis is cotrimoxazole with alternatives being dapsone, atovaquone, and aerosolized pentamidine.

Some experts use long-term antiviral prophylaxis to prevent recurrent herpes simplex and varicella zoster virus infection among HCT recipients with severe, long-term immunodeficiency. If a patient with cGVHD is exposed to varicella (either primary or post-vaccination illness), VZV IG, if available, should be given within 96 hours, or valacyclovir may be given until 22 days from exposure. Patients who begin cGVHD therapy who have lymphopenia and/or a history of cytomegalovirus (CMV) reactivation during the first 3 months are at higher risk of late CMV reactivation and death [84]. Some centers continue to monitor CMV viremia after day 100 by weekly to every other week polymerase chain reaction (PCR) testing based on individual risks, followed by preemptive therapy. See also Chapter 87.

Monitoring

Because cGVHD therapy spans 1–3 years or longer, and its manifestations are by nature protean, close serial monitoring of all organ systems is recommended to promote early detection and intervention [81]. This first involves a directed interval medical history and physical examination and review of medications. It is worth remembering that the reported cumulative incidence of sclerotic manifestations is 20% at 3 years [85] and joint involvement, as defined by NIH joint/fascia score ≥1, is present in about 30% of patients with cGVHD, often affecting the wrists or ankles [86]. Comprehensive cGVHD assessments with documentation of NIH scores are advised every 3 months if disease tempo is aggressive or uncertain and every 6 months if more stable to allow subtle manifestations to be detected, monitored carefully, and/or treated early with the hoped for goal of avoiding progression to highly morbid, difficult to treat, and, fairly often, irreversible forms of cGVHD. This can be accomplished effectively in clinical practice by following a streamlined and reliable method [87]. Standardized medical photographs can be invaluable if taken at a predefined (days 80–100) baseline, at initial cGVHD diagnosis, and every 6 months if skin and/or joints are involved until resolution of reversible manifestations or discontinuation of therapy. Digital images can be stored electronically and made accessible for serial comparisons of the current state of a patient's skin lesions and/or limited joint mobility. Combined use of the NIH joint score and the photographic range of motion scale (P-ROM, Figure 84.22) are recommended tools to assess joint GVHD; the NIH score better reflects joint improvement and the P-ROM scale better reflects joint worsening from baseline (Y. Inamoto and P.A. Carpenter, unpublished data).

Routine blood monitoring includes the cord blood cell and differential, chemistry panel, therapeutic drug levels, IgG level, lipid profile, 25-OH-vitamin D, thyroid function, and ovarian and testicular function. Annual bone mineral density testing is appropriate for patients receiving glucocorticoids or abnormal prior tests. Pulmonary function test monitoring is advised to determine the NIH lung score, or to follow the LFS (composite score of FEV_1/DLCO) for those with known BO. Spirometry with lung volumes and corrected diffusing capacity is usually performed every 3–6 months, based on cGVHD disease tempo, to monitor for airflow obstruction. If airflow obstruction is new and moderate or severe and of uncertain tempo, or progressing rapidly, then limited spirometry is followed as often as monthly until the FEV_1 stabilizes.

Consultation with subspecialists in dental/oral medicine, gynecology, ophthalmology, rehabilitation medicine, pain, and supportive care might also be indicated. The scope and frequency of monitoring should be individualized as clinically indicated and ranges from monthly to at least yearly. More frequent monitoring is advised for those with active cGVHD and especially during the high-risk periods such as during tapering of medications. Especially challenging cases might benefit from referral for consultation at a major national center that has multidisciplinary expertise in cGVHD.

Graft-versus-leukemia effect

A landmark registry study by Horowitz et al. investigated GVL effects among 2254 patients with acute leukemia in first remission or chronic myeloid leukemia in first chronic phase, most of whom received HLA-identical sibling bone marrow after high-dose conditioning [88]. Patients who had only cGVHD or both acute GVHD and cGVHD had lower relative risk (RR) of relapse than patients without GVHD (RR 0.43, $p = 0.01$ and RR 0.33, $p = 0.0001$, respectively). In the same analysis, relapse rates in patients who received identical twin grafts or T-cell-depleted grafts were at increased risk of disease relapse (RR 2.09, $p = 0.005$ and RR 1.76, $p = 0.002$, respectively).

Using similar methodology in a large study cohort to examine GVT effects in patients after high-dose conditioning and allogeneic HCT for advanced non-Hodgkin lymphoma, Bierman et al. found no evidence that relapse risk was affected by acute GVHD or cGVHD, T-cell depletion, or identical twin grafts, suggesting that clinical expression of GVL/T effects may depend on the underlying disease and/or the number or nature of prior therapies [89]. Lee et al. analyzed the effects of cGVHD severity among 3472 patients who received high-dose conditioning and allogeneic HCT from related or unrelated donors for acute or chronic myeloid leukemia [25]. This large study showed that patients with mild, moderate, or severe cGVHD had lower relapse rates than patients without cGVHD, but there was no evidence that increasing cGVHD severity was linked to decreasing relapse rates. Equally important, NRM was not increased by the presence of mild cGVHD but was increased by severe cGVHD. Hence patients with mild cGVHD had the best disease-free survival, those without cGVHD had disease-free survival that was similar to that in patients with moderate cGVHD, and patients with severe cGVHD had the worst disease-free survival. These findings have practical implications and support pursuing effective treatments of moderate and severe cGVHD after myeloablative conditioning.

There are surprisingly few data on GVT effects after RIC and HCT, a setting where curative effects of HCT are almost entirely immunologically dependent. Baron et al. analyzed GVT effects in 322 patients given HLA-matched related or unrelated donor grafts after RIC transplantation and noted a higher probability of achieving complete remission in patients who developed extensive cGVHD (HR 1.7, $p = 0.07$) [90]. Grade II–IV acute GVHD had no significant impact on the risk of disease relapse/progression. Acute GVHD was associated with an increased risk of NRM and decreased probability of progression-free survival. In contrast, extensive cGVHD was associated with reduced risk of disease relapse/progression (HR 0.4, $p = 0.006$) and increased probability of progression-free survival ($p = 0.003$). These findings suggest that cGVHD may contribute substantially to the success of RIC transplants for malignant disease.

Recently, Inamoto et al. explored the influence of NIH-defined cGVHD and immunosuppressive therapy (IST) on risk for recurrent malignancy after high-dose conditioning and allogeneic HCT [91].

Rates and hazard ratios of relapse and mortality were analyzed according to GVHD and IST as time-varying covariates. Adjusted Cox analyses showed that acute GVHD and cGVHD were both associated with statistically similar reductions in risk of relapse beyond 18 months after HCT but not during the first 18 months. In patients with GVHD, resolution of GVHD followed by withdrawal of IST was not associated with a subsequent increase in risk of relapse. In patients without GVHD, withdrawal of IST was associated with a reduced risk of relapse during the first 18 months, but the risk of subsequent relapse remained higher than in patients with GVHD. Therefore, the association of GVHD with risk of relapse changes over time after HCT. In patients without GVHD, early withdrawal of IST might help to prevent relapse during the first 18 months, but other interventions would be needed to prevent relapse at later time points.

Future directions

Since the previous edition of this book, there has been considerable progress in the prospective validation of many of the new NIH chronic GVHD assessment tools. With the establishment of the North American Chronic GVHD Consortium and BMT CTN, the field is now much better organized and well poised to conduct multicenter observational and therapy intervention trials effectively. The emerging understanding of cGVHD biology is still rudimentary and lags significantly behind the recent developments in acute GVHD. Strategies for acquisition of tissue, blood, saliva, and other human samples should be aggressively implemented in transplantation and cGVHD therapy studies to help improve our understanding of cGVHD biology, develop better diagnostic and treatment strategies, and find more effective and less toxic drugs that do not interfere with GVT effects. The development of validated and practical cGVHD response criteria and a cGVHD activity score are paramount and urgent unmet needs. The straightforward 0–3 NIH individual organ severity scoring, while initially intended as a baseline assessment tool, holds promise as a standalone response assessment tool that correlates well with patient and physician perceived improvement and worsening in a number of organs [4,10,12].

It is hoped that greater diligence regarding good study design principles and the use of improved and validated response assessment tools will be able to help identify better promising candidate treatments in robust multicenter phase II studies that could be taken forward in phase III studies of secondary or primary therapy. The results of these improved communal efforts are expected to lead to better survival and elimination of disabling cGVHD in the future.

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CHAPTER 85

Bacterial Infections

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Introduction

Susceptibility to infection has posed one of the most formidable challenges in the clinical management of patients undergoing hematopoietic cell transplantation (HCT) from the earliest days of this treatment. A variety of advances in infection control have permitted major strides in the supportive care of transplant recipients, and these have translated into improved outcomes. Increased understanding of the pathogenesis of infectious syndromes, introduction of new antimicrobial agents, adoption of empirical antibiotics during aplasia before engraftment, development of novel strategies to prevent and treat infections, and recognition of the contributory role of infectious pathogens to the morbidity of other transplant complications, especially graft-versus-host disease (GVHD), have all been responsible for improved survival rates. Advances including peripheral blood hematopoietic cells as alternative sources of bone marrow cells have resulted in faster engraftment of both platelets and neutrophils, leading to shorter lengths of stay, fewer days of fever, and less antibiotic use. The increasing use of reduced-intensity transplants, which are associated with less gastrointestinal and overall chemotherapy-induced toxicity, are also associated with reduced infectious complications. However, shifts in the patterns of opportunistic pathogens, changing antimicrobial susceptibility, differences in host immunodeficiency with implementation of new immunosuppressive regimens for GVHD, introduction of new conditioning regimens for use in the treatment of solid tumors, and the increasing use of alternative donors continue to pose new and different challenges and opportunities for the management of infectious complications.

Biology of bacterial pathogens

Bacteria are found in a great variety of environments. They are classified as prokaryotic microorganisms distinguished by absence of a membrane enclosing their DNA and absence of other membrane-bound organelles. More than 2500 species of bacteria are known. Classification consists of the broad grouping of medically significant bacteria based on their staining properties using the Gram stain (positive or negative), morphology (cocci and bacilli), and oxygen tolerance (aerobic or anaerobic). Other properties, including DNA and RNA homology, ribosomal RNA homology, biochemical properties, specialized growth requirements, and antigenic attributes, have also been used for further characterization.

In general, bacteria have cell walls made of peptidoglycan, a molecule unique to bacteria and thus a target for many antimicrobial agents. The cell wall provides the basic shape of the organism and confers rigidity. The Gram stain detects differences in the cell wall structure of bacteria. These differences are useful in distinguishing both antibiotic susceptibility and the potential for interacting with host defenses. Gram-positive bacteria have walls that consist of thick layers of peptidoglycan, teichoic acids, teichuronic acids, and other polysaccharides. Inhibition of peptidoglycan synthesis is the mechanism of action of a variety of antibiotics, including penicillins, cephalosporins, and glycopeptides. The cell wall of Gram-negative organisms consists of an outer membrane composed of protein, lipopolysaccharide (LPS), and phospholipid, and a thin inner layer of peptidoglycan. Protein components of the membrane, known as porins, serve as permeability conduits for hydrophilic molecules. The size and number of porins determine permeability to various molecules, including antibiotics.

Several properties are important in the ability of a given bacterial species to colonize or invade the human host (Table 85.1). These include adherence to host cells, the capacity to acquire iron from the host, elaboration of toxins, and successful competition with other microbial colonizers for nutrients. Adhesins on the surface of bacteria interact with specific receptors on host tissue targets. Differences in either the adhesin molecules or host cell receptor binding determine tissue tropism. Various structures exist on the surface of medically important bacterial pathogens, which are important virulence factors. A capsule of polysaccharide can afford protection against phagocytosis. Immunologic diversity of such capsules within species is common (the basis for serotyping), and can permit evasion of the normal host defenses since opsonizing antibodies directed against the specific antigens of the capsule are important for phagocytosis. In Gram-negative bacteria, pili are important means by which bacteria adhere to epithelial cells. Gram-positive bacteria may have surface proteins (e.g. the M protein of group A streptococci or staphylococcal protein A) that serve as virulence factors.

Bacterial products such as superantigens may provoke host responses that are deleterious to the maintenance of host physiology. Exotoxins produced during exponential growth and released by both Gram-positive and -negative bacteria interfere with normal host cell metabolism and contribute to host toxicity. Certain toxins, known as bacteriocins, are elaborated to enhance an organism's competition with other microorganisms within the normal flora to establish an ecologic niche. The production of certain extracellular enzymes, such as hyaluronidase, neuraminidase, elastase, and collagenase, facilitates overcoming anatomic barriers and promotes spread past tissue barriers. Proteins may be produced that facilitate

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Table 85.1 Bacterial virulence factors

Molecule	Effects on host
Exotoxins	Interfere with cellular physiology
Endotoxins	Elicit sepsis syndrome
Bacteriocins	Establish ecologic niche in host flora
Exoenzymes	Disrupt anatomic barriers, facilitate tissue invasion
Proteases	Degrade humoral immune responses and complement
Polysaccharide capsules	Resist humoral immunity and phagocytosis
Plasmids, phages, transposons	Confer antibiotic resistance, multiple virulence properties, rapid adaptation to environment
Regulatory factors	Allow rapid adaptation to environmental changes
Biofilms	Form barrier to exclude antibiotics

Table 85.2 Targets for various antibiotics

Bacterial target	Antibiotics
Peptidoglycan synthesis	Penicillins, cephalosporins, glycopeptides
Lipopolysaccharide	Antibodies to endotoxins or cytokines mediating sepsis syndrome
Penicillin-binding proteins	Penicillins, cephalosporins, carbapenems
β-Lactamases	Clavulanic acid, sulbactam, tazobactam
Ribosomes	Aminoglycosides, macrolides, tetracyclines, chloramphenicol, oxazolidinones, streptogramins
RNA polymerases	Rifamycins
Folic acid synthesis	Sulfonamides, trimethoprim
DNA gyrase	Fluoroquinolones
Bacterial membrane	Daptomycin
Depolarization	Oxazolidinones, linezolid; streptogramins, quinupristin/dalfopristin

entry into host cells. Disruption of host humoral responses by proteases permits traversing of mucosal barriers and survival in extracellular tissue and serum.

Avoidance of phagocytosis or killing of microorganisms once ingested is the role of a variety of virulence factors. The polysaccharide capsule of many organisms is designed both to resist humoral immunity and to impede phagocytosis. Bacterial LPS (endotoxin) serves as an important barrier against bile salts, lytic enzymes, DNA, and heavy metals. LPS also contains a carbohydrate moiety (the O antigen) that resists complement binding and differs antigenically from strain to strain. The generation of such antigenic diversity is a mechanism that bacteria use to evade host humoral immune responses. LPS induces the sepsis syndrome by causing the release of a variety of proinflammatory cytokines, such as tumor necrosis factor-α, interleukin 1, interferon-γ, and various colony-stimulating factors by monocytes and macrophages, and by triggering a variety of host enzymatic mechanisms, including complement, clotting, fibrinolytic, and kinin pathways.

A number of gene products on extrachromosomal elements, such as plasmids, phages, insertion elements, and transposons, provide bacteria with mechanisms to adapt rapidly to a competitive environment, conferring antibiotic resistance and multiple virulence properties, which can be shared between a wide variety of unrelated microbes. A variety of regulatory proteins provide bacteria with the capacity to respond favorably to rapid shifts in the environment, such as temperature, iron, and pH. These proteins facilitate adherence and invasiveness and modify the expression of other proteins on the cell surface to optimize the organism's competitive stance. Antigenic variation of these proteins is the mechanism used by bacteria to evade host immune responses. These characteristics of bacteria offer a range of bacterial targets for antimicrobial agents (Table 85.2).

The host microbial flora itself may act as a barrier against opportunistic pathogens. Ordinarily, complex bacterial communities reside on the body's surfaces and within the gastrointestinal tract without deleterious effects. However, when the interactions are altered by the use of antimicrobial agents or other infection control measures, this natural barrier may be compromised, and overgrowth of pathogens that might otherwise not be capable of establishing a niche can occur. The normal commensal flora prevent the establishment of opportunistic pathogens by competition for the same nutrients or receptors on host cells through the production of toxic products, fatty acids, or other metabolites that inhibit growth of potential competitors and stimulation of the host immune system.

Some bacteria routinely cause disease in susceptible hosts who have normal defenses, and these certainly can cause morbidity in the HCT recipient. However, more commonly, infectious morbidity in the HCT recipient is caused by bacterial organisms (such as many Gram-negative bacteria, α-streptococci, and *Staphylococcus epidermidis*) that ordinarily are not pathogens. Such organisms, known as opportunistic pathogens, exploit deficits in host defenses that occur as a result of the transplant procedure or nosocomial procedures, or they exploit microbial shifts caused by infection control measures. Whether or not an opportunistic pathogen causes disease is a reflection of an interplay between its own inherent virulence, whether it has access to the host in sufficient numbers, and the state of the host defenses. Thus, effective infection control measures must address issues related to minimizing exposure to potential pathogens, the strategic use of antimicrobial agents to suppress microbial burden, and, where possible, bolstering host defenses.

Antibiotic resistance

A number of pathogens are problematic by virtue of documented resistance, including *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus faecium*, vancomycin-resistant *Staphylococcus aureus*, and methicillin-resistant *S. aureus* (MRSA). The mechanisms through which drug resistance is most commonly conferred are described below.

One of the most successful attributes that bacteria have to enhance survival is the capacity for genetic variability. This capacity is frequently used by bacterial organisms to foil clinicians' attempts to control infection by antibiotics. Through point mutations, rearrangement of entire sequences of DNA to different locations within the bacterial genome (e.g. transposons), or the transfer of DNA from one bacterial organism to another (e.g. via plasmids), development of antibiotic resistance can occur in a variety of ways (Table 85.3).

A common mechanism is inactivation of antibiotics by enzymes. For example, staphylococci and many Gram-negative bacteria produce a class of enzymes known as β-lactamases that render inactive β-lactam antibiotics, the group of molecules that includes the penicillins, the cephalosporins (e.g. cephalothin, cefuroxime, ceftazidime, cefepime, and cefoxitin), carbapenems (e.g. imipenem and meropenem), and monobactams (e.g. aztreonam). β-Lactamase genes found on transposons or plasmids permit the spread of high-level resistance widely to other classes of drugs such as the fluoroquinolones and trimethoprim–sulfamethoxazole (TMP–SMX). Unfortunately, prevalent use of the β-lactam class of antibiotics, particularly third-generation cephalosporins, has resulted in selection

Table 85.3 Mechanisms of antibiotic resistance

Mechanism	Examples	Genetic basis	Types of bacteria	Antibiotics affected
Inhibition of antibiotic by enzymes	β-Lactamases	Chromosomal, plasmids, transporons	Staphylococci, many Gram-negative bacteria	Penicillins, cephalosporins
	Acetyltransferases	Plasmids	Many Gram-negative bacteria	Aminoglycosides, chloramphenicol
	Phosphotransferases	Plasmids	Many Gram-negative bacteria	Chloramphenicol, aminoglycosides
	Nucleotidyltransferases	Plasmids	Many Gram-negative bacteria	Aminoglycosides
Reduced membrane permeability	Mutations involving porins	Chromosomal	Many Gram-negative bacteria	β-Lactams, aminoglycosides, carbapenems, nalidixic acid, fluoroquinolones
Increased efflux of antibiotics	New proteins	Chromosomal, plasmids, transporons	Many Gram-negative bacteria and staphylococci	Tetracyclines, macrolides, fluoroquinolones
Altered targets	Changes in ribosomal binding sites	Chromosomal, plasmids	Gram-positive and Gram-negative bacteria	Tetracyclines, macrolides, aminoglycosides, lincosamides
	Changes in cell wall	Chromosomal, plasmids	Gram-positive bacteria	Vancomycin, other glycopeptides
	Changes in penicillin-binding proteins	Chromosomal, plasmids	Gram-positive and some Gram-negative bacteria	Penicillins, cephalosporins
	Changes in dihydropteroate synthetase	Plasmids	Many Gram-negative	Sulfonamides
	Changes in dihydrofolate reductase	Chromosomal, plasmids	Gram-negative bacteria	Trimethoprim
	Changes in DNA gyrases	Chromosomal, plasmids	Gram-negative bacteria	Nalidixic acid, fluoroquinolones
Exclusion barrier	Biofilm formation	Chromosomal	*Staphylococcus epidermidis*, *Pseudomonas aeruginosa*	Multiple

pressure to give advantage to resistant organisms and further encourage spread. Extended-spectrum β-lactamase (ESBL)-producing organisms are an increasing cause of treatment failure. The ESBL-producing organisms are most often noted in *Klebsiella pneumoniae* and *Escherichia coli* species. Immunocompromised patients infected with these organisms should be placed on Gram-negative isolation precautions and treated with carbapenems. Carbapenem-resistant pathogens have been reported, including the *K. pneumoniae* carbapenemase (KPC) OXA carbapenemase, which is most commonly reported in *A. baumannii* isolates, and metallo-β-lactamases that are found in *Stenotrophomonas maltophilia*, *Acinetobacter* spp., *P. aeruginosa*, and *Flavobacterium* species. Carbapenem-resistant pathogens have been most commonly reported in Europe and the eastern part of the United States [1].

Reduced entry of the antibiotic into bacterial organisms by alteration of permeability, thereby restricting drug access to target sites, can be caused by mutations involving different porins. Such changes are potential mechanisms for resistance to nalidixic acid, fluoroquinolones (such as norfloxacin, ciprofloxacin, levofloxacin, and ofloxacin), aminoglycosides, and β-lactams, among others. Alternatively, resistance to some antibiotics may occur by an increase in efflux of antibiotics from the bacterial organism. This can take place by mutations in chromosomal genes or the acquisition of new genes from plasmids that lead to new membrane proteins, which reduce the retention of certain antibiotics, such as tetracyclines in Gram-negative bacteria and macrolides (such as erythromycin, azithromycin, and clarithromycin) in Gram-positive bacteria.

Alteration of the target of the antibiotic is another strategy. Multiple examples are notable. Changes in ribosomal binding sites may lead to resistance to tetracyclines, aminoglycosides, or macrolides, while changes in the amino acid residues on the constituents of cell-wall building blocks may confer resistance to antibiotics such as vancomycin. Mutations in the genes that encode for penicillin-binding proteins alter susceptibility to β-lactams, such as the penicillins and cephalosporins. A variety of target enzymes (as shown in Table 85.3) that can be changed by mutations can potentially lead to resistance to sulfonamides, trimethoprim, nalidixic acid, and fluoroquinolones, among other antibiotics.

The emergence of widespread antibiotic resistance can impede the best efforts of clinicians to control infections by various bacterial pathogens. Clearly, a cornerstone of any infection control program is the wise use of antibiotics. Repeated pleas by the Centers for Disease Control and Prevention (CDC) and other authoritative bodies for clinicians to practice antimicrobial stewardship have regrettably failed to slow the continuing emergence of drug resistance. However, several strategies have been offered [2–4]. Patients colonized or infected with resistant organisms should be isolated [3,4]. Each institution should maintain a program of surveillance for resistance to detect shifts in susceptibility and trends of problem organisms. Selected antimicrobial agents may need to be restricted to reduce the prevalence of specific resistant organisms. Certain antibiotics initiated empirically for suspected organisms (e.g. vancomycin) should be discontinued once it has been determined that they are not needed. Implementation of an antibiotic cycling program, where a particular class of antibacterial is withdrawn from use for a defined period of time as a strategy to limit bacterial resistance, has also been evaluated. Results with this approach are inconsistent. Other initiatives include limiting susceptibility reporting such that the more powerful antibacterials are not listed, and as such are less likely to be selected for treatment. Such efforts will reduce selection pressures and spread of antibiotic resistance among microbial populations [5].

Compromised host defenses

Host defenses are compromised by a variety of factors following HCT. The degree and type of compromise of host defenses change over time [6]. Three periods have been described: (1) early recovery, corresponding to the first several weeks after transplantation, the pre-engraftment phase; (2) mid-recovery, corresponding to the second and third months after transplant, the early post-engraftment phase; and (3) late recovery, corresponding to the interval beyond 3 months. Table 85.4 lists the predominant deficits that are present at various intervals.

Early recovery (phase I)

The cytoreductive agents used in conventional high-dose conditioning regimens damage rapidly dividing cell populations, particularly bone marrow progenitors and mucosal epithelial cells. For several weeks after HCT, pancytopenia and damage to the mucosal barriers are predominant deficits in the host defenses against infectious pathogens.

Table 85.4 Host defenses compromised by hematopoietic cell transplantation (HCT) that make patients vulnerable to bacterial infections

Early recovery
Neutropenia
Oral and gastrointestinal mucosal damage due to cytoreductive therapy
Skin barrier compromised by CVCs
Mid-recovery
Skin and gastrointestinal mucosal damage due to GVHD
Decreased cellular immunity due to GVHD and immunosuppressive therapy
Skin barrier compromised by CVCs
Decreased cellular immunity due to viral infections, especially CMV
Late recovery
Decreased cellular immunity; persistent with chronic GVHD
Nonspecific suppressor cells with chronic GVHD
Reduced opsonization
Decreased reticuloendothelial function
IgG subclass deficiencies

CMV, cytomegalovirus; CVCs, central venous catheters; GVHD, graft-versus-host disease; IgG, immunoglobulin G.

The duration of neutropenia varies according to the number of stem cells used to effect reconstitution, the stem-cell source, the occurrence of certain viral infections, especially cytomegalovirus (CMV), the use of certain immunosuppressive agents after transplantation as part of the GVHD prophylaxis that are also myelosuppressive, the use of agents to purge the marrow stem cells ex vivo from contaminating tumor cells, and the use of cytokines to stimulate recovery. In the autologous transplant setting, the number of prior treatment regimens, the prior use of radiotherapy, and the use of certain chemotherapeutic agents that are known to damage hematopoietic progenitors, such as nitrosoureas, melphalan, lenalidomide, and fludarabine, negatively influence the ability to obtain stem cells, leading to delays in engraftment. In the allogeneic transplant setting, human leukocyte antigen (HLA) disparity between donor and recipient, T-lymphocyte depletion of the graft, the use of umbilical cord as a stem-cell source, and inadequate conditioning of the recipient to eliminate host immune cells can also impede robust engraftment.

The duration and the depth of neutropenia independently influence the risk for infection [7]. Although the risk for bacterial infection increases incrementally as the circulating absolute neutrophil count (ANC) falls below $1000/\text{mm}^3$, the risk becomes substantial below $500/\text{mm}^3$. Bacteremia and life-threatening bacterial infection occur mostly at neutrophil counts below $100/\text{mm}^3$. Indeed, in HCT recipients, 90% of the bacterial infections occur at neutrophil counts below $100/\text{mm}^3$.

The degree of mucosal damage varies according to the preparative regimen. Busulfan, etoposide, melphalan, cytarabine, cyclophosphamide, and total body irradiation (TBI) are associated with varying degrees of mucositis. Although stomatitis is readily observable, damage to the mucosa of the entire gastrointestinal tract occurs. Attempts to quantify reliably the degree of mucosal damage are primitive, and have hampered efforts to correlate the degree of mucosal damage with infection risk along the same lines that the neutrophil count and the risk for infection have been quantified. However, the use of the D-xylose absorption test, a measure of mucosal functional integrity, has been evaluated in leukemic patients. An association between the degree of D-xylose absorption and the risk for infection has been noted [8].

Reactivation of herpes simplex virus (HSV) type 1 (which occurs in approximately 70% of seropositive patients, usually during the first or second week after HCT unless acyclovir prophylaxis is given) can lead to diffuse or localized ulceration of the mucosa of the oral cavity and the lower esophagus. This change has been associated with bacteremia from organisms that reside on the buccal mucosa. Reactivation of HSV type 2 occurs in seropositive patients and can result in urethral, labial, perineal, and perianal skin and mucosal breakdown. The use of cytotoxic agents after HCT, such as methotrexate or cyclophosphamide to prevent GVHD, can also result in exacerbation of mucositis and delays in healing, as can the corticosteroids.

The nearly universal use of indwelling central venous catheters (CVCs) today poses a risk for infection, both early and late after HCT. Such catheters compromise the integrity of the integument as a physical barrier to potential pathogens residing on the skin. Marrow puncture sites and peripheral venous catheters can also compromise the skin barrier.

The use of reduced-intensity conditioning (RIC) HCT is increasing, utilizing the immune system rather than chemotherapy to achieve disease control. The RIC regimens that are used limit the development of neutropenia, and mucositis is also significantly diminished. As a consequence, this new type of HCT has demonstrated differences in infection patterns compared with high-dose allogeneic HCT [9–12]. The incidence of early bacteremias (days 0–30) is low, and these account for a low rate of bacteremia during the first 100 days.

Mid-recovery (phase II)

Following engraftment, patients enter a period of profound deficiency of cellular and humoral immunity. The degree and duration of immunodeficiency are influenced by the type of HCT, the degree of donor and recipient histocompatibility, whether T-lymphocytes have been eliminated from the allogeneic marrow graft, the use of immunologic or pharmacologic purging of autologous marrow, the post-transplant immunosuppressive treatment given as GVHD prophylaxis, the occurrence of certain viral infections (especially CMV), and the occurrence and severity of GVHD. The integrity of mucosal barriers may continue to be compromised by recurrent HSV infections, the use of cytotoxic agents, and intestinal involvement by GVHD. Indwelling venous catheters typically remain in place during this period, adding to the patient's vulnerability to infection from skin bacterial flora.

Late recovery (phase III)

With time, there is gradual recovery of both cellular and humoral immunity (see Chapter 15). Generally, immune recovery is more rapid after autologous than allogeneic HCT. Among autograft recipients, those given high-dose preparative regimens (as typically used for acute leukemia) are more immunodeficient, and for longer intervals, than those given less intensive, RIC regimens, such as those used for solid tumor therapy. Immune recovery following peripheral blood autotransplantation is more rapid than after autologous marrow transplantation. Recipients of CD34-selected grafts have a slower pace of T-cell immune reconstitution [13]. Immune recovery after matched-related HLA-identical transplantation appears to be more rapid than after HLA-disparate or HLA-matched unrelated donor transplantation.

The occurrence of GVHD also influences the tempo of immune recovery and is associated with dysregulated immune responses (see Chapter 83). If chronic GVHD (cGVHD) occurs, cellular and humoral immunodeficiency may persist for months and even years [14]. Reticuloendothelial function can also be severely impaired, especially in patients with cGVHD. Immunoglobulin deficiencies can occur. Even in the face of normal levels of the isotypes, immunoglobulin G (IgG) subclass deficiencies,

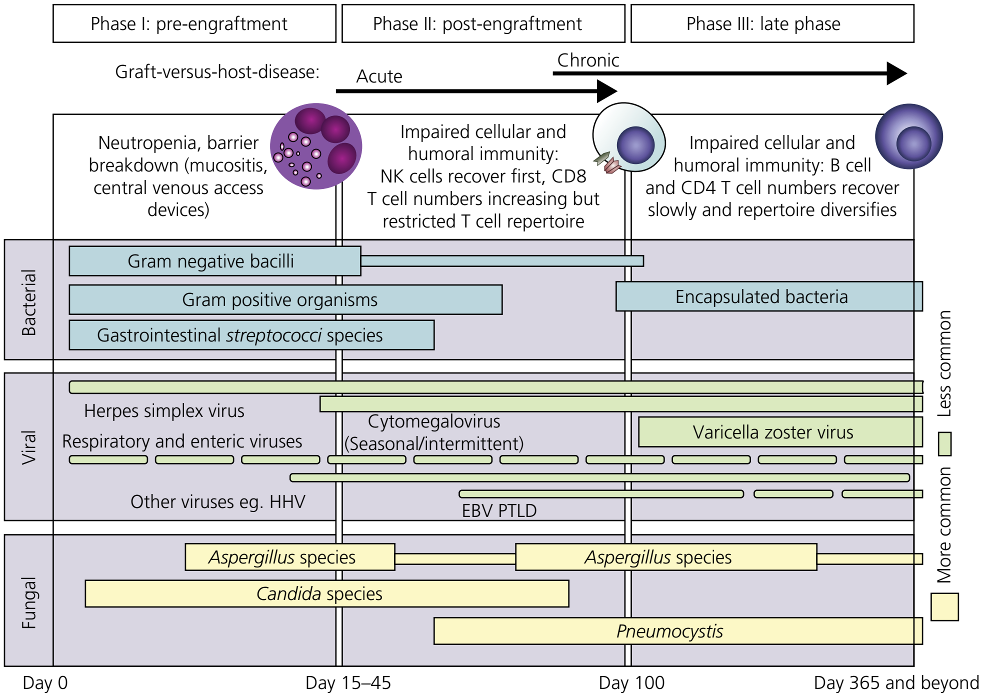
Phase I: pre-engraftment
Phase II: post-engraftment
Phase III: late phase
Graft-versus-host-disease:
Acute
Chronic
Neutropenia, barrier breakdown (mucositis, central venous access devices)
Impaired cellular and humoral immunity: NK cells recover first, CD8 T cell numbers increasing but restricted T cell repertoire
Impaired cellular and humoral immunity: B cell and CD4 T cell numbers recover slowly and repertoire diversifies
Bacterial
Gram negative bacilli
Gram positive organisms
Gastrointestinal streptococci species
Encapsulated bacteria
Viral
Herpes simplex virus
Cytomegalovirus
Varicella zoster virus
Respiratory and enteric viruses
(Seasonal/intermittent)
Other viruses eg. HHV
EBV PTLD
Fungal
Aspergillus species
Aspergillus species
Candida species
Pneumocystis
Less common
More common
Day 0
Day 15–45
Day 100
Day 365 and beyond

Figure 85.1 Phases of opportunistic infections among allogeneic HCT recipients.

especially of subclass II, can be present, rendering patients vulnerable to severe infection by encapsulated bacteria.

By 1 year, immune recovery is nearly complete in recipients of matched-related transplants without GVHD and off immunosuppressive medications. However, in matched unrelated donor and mismatched family-related donor transplants, immune recovery may lag substantially. Even in the absence of overt GVHD, vulnerability to recurrent sinopulmonary infections can persist for much longer periods than for those seen after HCT using grafts from HLA-matched siblings [15]. Responses to immunizations may be impaired up to 1 year or longer.

Spectrum of bacterial infections

Just as the defects in host immunity vary over time, so does the spectrum of infections. In Figure 85.1, the various infectious syndromes that occur at different times after HCT are portrayed [6]. As noted previously, relative to viral and fungal infections, bacterial infections predominate during the early recovery phase, whereas viral and fungal infections predominate during the mid- and late recovery phases. However, there are important differences that vary over time, as noted in Tables 85.5 and 85.6.

Early recovery (phase I)

Among neutropenic patients with fever, approximately 50% will have an infection that can be documented, and in the remainder no cause can be definitively identified. Because signs and symptoms of infection are attenuated during neutropenia, and untreated bacterial infections may be rapidly life threatening, neutropenic fevers should operationally be regarded as infectious until proven otherwise.

Bacterial pathogens account for more than 90% of the first infections during neutropenia. Gram-negative bacteria are the most virulent bacterial pathogens during neutropenia, and historically have been major causes of morbidity and mortality. Over the past 25 years,

Table 85.5 Bacterial and fungal infectious syndromes encountered early after hematopoietic cell transplantation (HCT), during the pre-engraftment phase (early recovery or phase I)

Syndrome	Relative frequency[a]	Relative life-threatening potential
First fever		
Staphylococci		
Staphylococcus epidermidis	+++	+
Staphylococcus aureus	+	+++
α-Hemolytic streptococci	+	++
Gram-negative bacilli	+	+++
Subsequent fevers		
Antibiotic-resistant Gram-negative bacilli	+	+++
Staphylococci		
Staphylococcus epidermidis	+++	+
Staphylococcus aureus	+	+++
Fungi	++	+++

[a] Increasing frequency and life-threatening potential depicted by increased number of "+" signs.

Table 85.6 Relative frequency[a] of bacterial infections encountered after engraftment (mid- and late recovery, or phases II and III)

Infection	Allogeneic HCT with no GVHD	Allogeneic HCT with GVHD	Autologous HCT
Mid-recovery (phase II)			
Staphylococci	++	++	++
Fungi	++	+++	+
Gram-negative bacilli	–	+	–
Late recovery (phase III)			
Encapsulated bacteria	–	++	–
Fungi	–	+	–

[a] Increasing frequency depicted by increased number of "+" signs; "–" indicates uncommon infections.

GVHD, graft-versus-host disease; HCT, hematopoietic cell transplantation.

the percentage of infections caused by Gram-negative organisms has steadily decreased from 70 to 30%. However, over the past decade several centers have reported an increase in the proportion of Gram-negative bacterial infections [16,17]. The most common Gram-negative bacteria have been *E. coli*, *Klebsiella* spp., and *P. aeruginosa*. Several other organisms, such as *Acinetobacter* spp., *Stenotrophomonas* spp., *Alcaligenes xylosoxidans*, *Serratia* spp., *Legionella*, and *Burkholderia cepacia*, have also been increasingly identified [18]. The portal of entry for many organisms is the damaged mucosa of the gastrointestinal tract. Perianal fissures or skin breakdown are other potential sources, especially for *P. aeruginosa.* Occasionally, venous catheters can also serve as an entry site for Gram-negative bacteria.

Gram-positive bacteria have emerged as major pathogens, and bacteremia rates for Gram-positive organisms now exceed those for Gram-negative bacteria [16–18]. Gram-positive bacteria currently account for 70% of bacterial infections, compared with 30% 30 years ago. This increase is in large part attributable to the nearly universal use of indwelling CVCs. The widespread use of antibiotics with Gram-negative coverage to decontaminate the gut and reduce Gram-negative infections has also contributed to the proliferation of Gram-positive infections. Occasionally, Gram-positive organisms may also invade the host via the gastrointestinal tract. *S. epidermidis*, *S. aureus*, α-hemolytic streptococci, and enterococci are the most common organisms [19].

The portal of entry for α-hemolytic streptococci is frequently a breach in the oral mucosa. Thus, patients with stomatitis due to chemotherapy or radiotherapy, or with HSV-induced mucosal ulcerations, are particularly at risk. Several large series have reported bacteremia rates of 15–25% in HCT populations [20–23]. Where speciated, *Streptococcus mitis*, an organism that normally resides on the buccal mucosa, is the most common α-hemolytic *Streptococcus*. Although Gram-positive bacteria are less virulent than Gram-negative bacteria, approximately 10% of α-hemolytic streptococcal bacteremias are associated with a toxic shock-like syndrome that can be fatal even with prompt antimicrobial therapy [21,22,24]. The use of fluoroquinolones in several series has been associated with an increased risk for streptococcal infection [25,26]. *Corynebacterium jeikeium* infections are associated with infected marrow needle puncture sites or infected peripheral venous catheters, often with associated thrombophlebitis. Typically, they are resistant to multiple antibiotics and frequently require catheter removal [27].

After institution of antibiotics during first fever, the microbial flora changes. Superinfections, as manifest by recurrent or persistent fever during the second or subsequent week of neutropenia, are more heterogeneous in etiology (Table 85.5). Gram-negative bacteria, especially those that are resistant to the antibiotic regimen used to treat the first febrile episode, are of paramount concern [28]. These bacteria account for only roughly 10% of superinfections, but are highly virulent and have rapid life-threatening potential if appropriate antibiotic modification is not made.

The most common etiologic pathogen for bloodstream infections is *S. epidermidis*, which accounts for roughly half of superinfections. These organisms are less virulent than Gram-negative bacteria and are quickly isolated from blood cultures of bacteremic patients. Hence clinicians can wait until multiple blood cultures are positive to ensure that the isolate is a true pathogen rather than a harmless contaminant (operationally defined as a single positive culture). Less common is *S. aureus*; however, *S. aureus* is highly virulent, and requires prompt and vigorous antibiotic treatment. Other potential causes of superinfection include fungal pathogens and *Clostridium difficile*-associated diarrhea (CDAD).

Table 85.7 Causes of fevers of obscure origin after engraftment during mid- and late recovery (phases II and III)

Cytomegalovirus
Central venous catheter infections
Occult sinusitis
Hepatosplenic candidiasis
Pulmonary or disseminated *Aspergillus* infection

Mid-recovery (phase II)

With recovery of the neutrophil count, most bacterial infections resolve and antimicrobial agents can be discontinued. Fever of obscure etiology is occasionally noted in the mid-recovery period (Table 85.7). Sinusitis is a common cause of fever of uncertain etiology during this period [29]. Frequently, it occurs without specific focal signs or symptoms, but can readily be detected by either radiographs or computed tomography (CT) scans of the sinuses [30], and further investigated by nasal endoscopy. Another potential cause of fever is an occult infection of the CVC. Blood cultures are helpful but may be unrevealing initially. Removal of the catheter is occasionally required to exclude this possibility in a patient with persistent obscure fever, and is discussed under treatment of intravascular catheter-related infections.

Patients who have undergone allogeneic HCT are more susceptible to infections during the mid-recovery period than are autograft recipients [31], particularly if acute GVHD occurs and more intensive immunosuppression is necessary (Table 85.6). Although viral and fungal infections predominate during this period, occasionally Gram-negative bacteremias can occur as a result of disruption of the gastrointestinal mucosa barrier from acute GVHD. Also, Gram-positive bacteremia frequently occurs related to infections associated with the CVCs [19,32].

Late recovery (phase III)

Over time, with gradual immune recovery, the risk for infection progressively declines. Autograft recipients have a very low risk for bacterial infection late after the procedure. If the indwelling CVC remains in place, however, infections associated with the catheter can continue to occur, especially if the catheter continues to be used or if fibrin sheaths develop. In the absence of cGVHD, allogeneic transplant recipients have a progressively declining risk for bacterial infection. However, patients with cGVHD are highly susceptible to recurrent bacterial infections, especially from encapsulated bacteria, including *Streptococcus pneumoniae*, *Hemophilus influenzae*, and *Neisseria meningitidis*, due to low CD4 lymphocyte counts, poor reticuloendothelial function, and low levels of opsonizing antibodies [14,15,33,34]. The continuing use of immunosuppressive therapy, especially corticosteroids, with their deleterious effects on phagocytosis, renders the patient susceptible to recurrent infection. As noted earlier, patients who are the recipients of mismatched or matched unrelated donor transplants are, even in the absence of cGVHD, vulnerable to recurrent infections [15], especially sinopulmonary infections.

Treatment strategies

First fever during neutropenia

With the recognition that most febrile episodes during neutropenia are infectious in origin, awareness of their life-threatening potential, and knowledge that most first infections are due to bacteria, evaluation should be prompt and thorough. Special attention should be directed to the oral cavity, skin, lung, catheter sites, and perianal area. Cultures of suspected sites of infection should be obtained, and in all patients at least two sets of blood samples should be submitted for bacterial and fungal cultures. Each set consists of an aerobic and anaerobic blood culture bottle. Patients with respiratory signs or symptoms should have a chest X-ray in addition to physical examination and routine blood cultures. Patients with signs and symptoms of a urinary tract infection or an abnormal urinalysis should have a clean catch urine specimen sent for analysis and culture.

Two or three blood cultures should be obtained either simultaneously or separated by 30–60 minutes [35]. According to the Infectious Diseases Society of America (IDSA) guidelines for the management of febrile neutropenia, at least one of these cultures should be from a peripheral venous puncture, and the other can be from an intravenous line [36]. Many centers do not draw peripheral blood cultures, preferring to draw two sets of cultures from the indwelling catheter. There appears to be no value in obtaining more than a single set of cultures per 24-hour period for patients with neutropenic fever. Preferably 20–30 mL per culture set for an adult is recommended [37], with proportionately smaller volumes for children: 1–2 mL blood per culture for neonates, 2–3 mL for infants aged 1 month to 2 years, 3–5 mL for older children, and 10–20 mL for adolescents are recommended.

Numerous satisfactory manual or instrumental culture systems are commercially available (reviewed in [35]). Most systems perform well, but no one medium or system is capable of detecting all microorganisms. Several systems are suitable for special considerations. There are products to minimize the inhibitory influence of antibiotics for patients who are already receiving antibiotics, and systems that are useful for detecting filamentous fungi or mycobacteria (such as the lysis centrifugation system). A recent technique, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) is speeding the ability to identify rapidly (within minutes) the organisms at the species level from colonies grown in cultures and also some antibiotic resistance phenotypic patterns (reviewed in [38]). Each technique has its strengths and shortcomings. In general, it is advisable that each transplant team meets periodically with the director of the hospital microbiology laboratory to review the blood culture system in place at that institution and the infections encountered in the HCT patients, in order to accommodate specific situations where special media would be advisable.

After evaluation, antibacterial agents should be instituted promptly on an empirical basis [36]. This strategy has reduced the morbidity and mortality associated with infection from more than 50% to less than 10% in the last 30 years, and has become universally adopted as the standard of care for management of the initial fever during neutropenia. A variety of antibiotic regimens have been evaluated in controlled clinical trials. It remains debatable as to whether one regimen is superior to another. What is generally agreed upon, however, is that antibiotics should be begun promptly at the first sign of fever, without waiting for isolation of an organism in blood or other cultures, even in the absence of signs or symptoms of infection, since the host is unable to mount an effective inflammatory response.

When choosing an antibiotic, a number of factors must be considered, such as the local susceptibility patterns, and also the susceptibility of organisms to available antibiotics. Other important considerations include toxicity of the individual agents (particularly in individuals with impaired organ function), allergy history (severity of allergy and class of agent that caused the allergy previously), the likelihood of the emergence of drug resistance, the risk for superinfection (both the frequency and the type of superinfection), and cost. A consensus committee of the IDSA first provided guidelines for the management of fever during neutropenia in 1990; these were updated in 1997, 2002, and 2010 [36]. Although developed primarily for hospitalized patients with neutropenia following conventional chemotherapy, they have been updated to include outpatient management of febrile neutropenia and are also suitable for HCT patients. These guidelines are evidence based, ranking both the strength and the quality of evidence of each recommendation.

The IDSA's suggested strategies for first fever are indicated in Figure 85.2 [36]. The first decision concerns risk assessment at first fever. Patients categorized as low risk are those expected to have a short duration of neutropenia (<7 days) or to have no or few comorbidities. High-risk patients are those expected to have prolonged (>7 days) profound ($\leq 100/\text{mm}^3$) neutropenia and/or to have comorbid conditions that include hemodynamic instability, new pulmonary infiltrates, new-onset abdominal pain, oral or gastrointestinal mucositis that interferes with swallowing or causes severe diarrhea, or neurologic or mental status changes [36]. In general, the risk-status defines where the patient will be managed – inpatient or outpatient. With the increasing role of RIC regimens followed by HCT and rapid recovery seen with autologous peripheral blood cell transplants, some HCT patients are managed exclusively as outpatients and could be appropriately managed using the "low-risk" pathway in Figure 85.2. Indications and candidates for the outpatient management of febrile neutropenia are discussed under patient management of neutropenia.

High-risk patients

In high-risk hospitalized individuals with bacteremia, the choice of antibiotic should provide adequate coverage against *Pseudomonas aeruginosa* and other Gram-negative pathogens. The primary strategy in the absence of known drug-resistant Gram-negative pathogens, hypotension, or pneumonia is treatment with a single-agent broad-spectrum antibiotic, or monotherapy [36]. Successfully studied agents include the third-generation cephalosporin ceftazidime [39–43], the fourth-generation cephalosporin cefepime [41,42,44–51], the antipseudomonal penicillin piperacillin–tazobactam [44,47,52–54], and carbapenems including imipenem [40,44,48,49] and meropenem [55,56]. A number of studies have compared the outcomes of monotherapy with those achieved with combination therapy in the management of neutropenic fever. The majority of studies, including several meta-analyses, have not demonstrated any difference in outcomes between the approaches [57–60]. A meta-analysis of monotherapy versus combination therapy demonstrated lower rates of bacterial superinfection and fewer treatment failures among the monotherapy recipients [59].

Disadvantages of monotherapy include limited Gram-positive activity, particularly with ceftazidime, and lack of efficacy against viridans streptococci. Cefepime and the carbapenems have excellent activity against viridans streptococci and pneumococci. None, however, has activity against MRSA, which must be considered in hospitals with high MRSA rates. Other organisms that are poorly covered by monotherapy include vancomycin-resistant enterococci (VREs), penicillin-resistant *S. pneumoniae*, coagulase-negative *S. aureus*, ESBL-producing organisms, and carbapenemase-producing *P. aeruginosa* bacteria.

In the era of increasing bacterial resistance to antimicrobial agents, particularly VREs, clinicians need to implement strategies to reduce unnecessary antibiotic consumption. Cefepime in particular has reduced the need for the addition of vancomycin to the

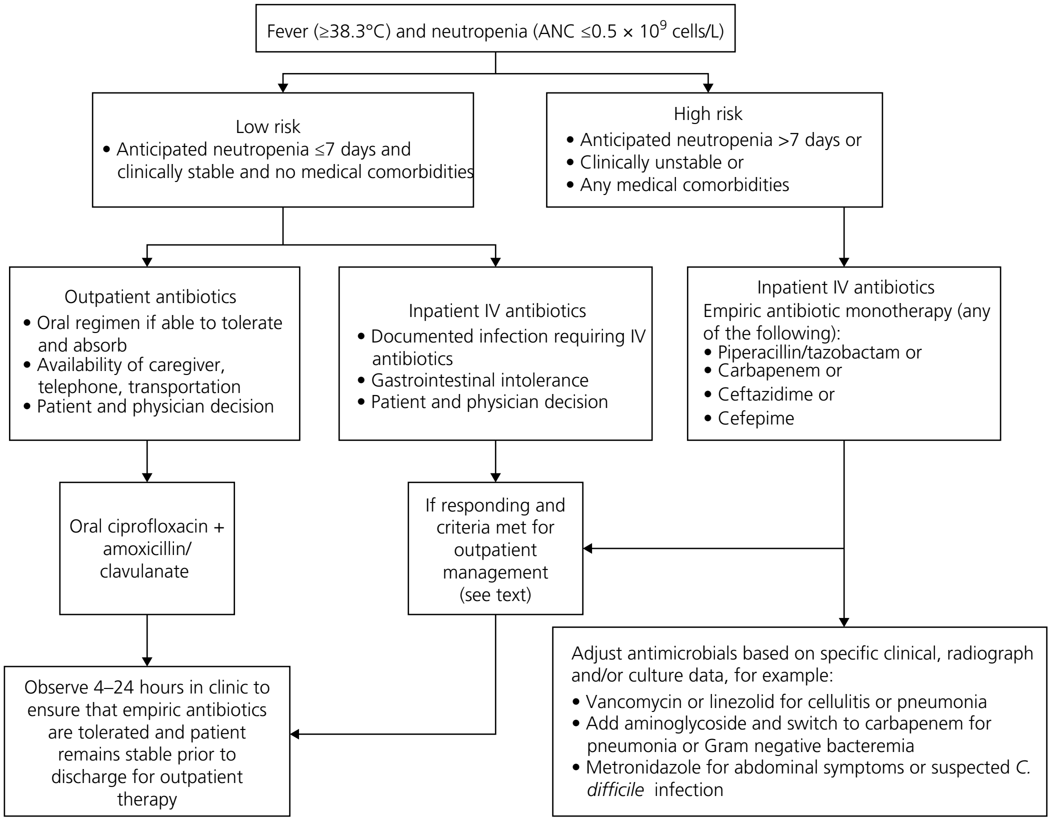
Fever (≥38.3°C) and neutropenia (ANC ≤0.5 × 10⁹ cells/L)
Low risk
• Anticipated neutropenia ≤7 days and clinically stable and no medical comorbidities
High risk
• Anticipated neutropenia >7 days or
• Clinically unstable or
• Any medical comorbidities
Outpatient antibiotics
• Oral regimen if able to tolerate and absorb
• Availability of caregiver, telephone, transportation
• Patient and physician decision
Inpatient IV antibiotics
• Documented infection requiring IV antibiotics
• Gastrointestinal intolerance
• Patient and physician decision
Inpatient IV antibiotics
Empiric antibiotic monotherapy (any of the following):
• Piperacillin/tazobactam or
• Carbapenem or
• Ceftazidime or
• Cefepime
Oral ciprofloxacin + amoxicillin/ clavulanate
If responding and criteria met for outpatient management (see text)
Adjust antimicrobials based on specific clinical, radiograph and/or culture data, for example:
• Vancomycin or linezolid for cellulitis or pneumonia
• Add aminoglycoside and switch to carbapenem for pneumonia or Gram negative bacteremia
• Metronidazole for abdominal symptoms or suspected C. difficile infection
Observe 4–24 hours in clinic to ensure that empiric antibiotics are tolerated and patient remains stable prior to discharge for outpatient therapy

Figure 85.2 Algorithm for initial management of febrile neutropenic patients. Source: Youssef et al. 2007 [34]. Reproduced with permission of Elsevier.

empirical regimen when compared with ceftazidime [61]. In one study, vancomycin was added to the empirical regimen in 51% and 80% of cases, respectively [62]. When appropriateness of vancomycin use was evaluated against IDSA guidelines, patients receiving cefepime had less inappropriate use compared with ceftazidime patients (28% versus 58%; $p < 0.001$) [62]. One of the main concerns of monotherapy is the emergence of antimicrobial resistance. ESBL-producing organisms have been shown to reduce the utility of ceftazidime monotherapy [63,64]. Fourth-generation cephalosporins have not been subject to the same problem, and in fact ceftazidime-resistant strains of *Enterobacter cloacae* remain susceptible to cefepime [64]. Some institutions that have adopted an "antibiotic cycling" program, or have changed their preference of cephalosporin from ceftazidime to cefepime, have seen a reversal in the resistance patterns to ceftazidime of inducible Enterobacteriaceae [65].

Imipenem and meropenem have also been extensively evaluated in the management of febrile neutropenia [40,44,48,49,55,56]. In trials comparing carbapenems with third- and fourth-generation cephalosporins, there were no appreciable differences in time to recrudescence of fever, days of therapy, or breakthrough infections. One potential concern with the routine use of carbapenems is the lack of coverage against *Stenotrophomonas maltophilia*, an organism that is increasingly isolated in HCT patients. Depending upon individual institutions' susceptibility patterns, carbapenems are a suitable choice for monotherapy. Piperacillin–tazobactam has also been evaluated in the empirical treatment of neutropenic fever. Several studies have demonstrated efficacy comparable to or better than that with other broad-spectrum antibacterials such as cefepime [44,47,52,53]. The most recent large randomized trial demonstrated non-inferiority compared with cefepime among patients with hematologic malignancies or undergoing autologous HCT, leading to the addition of piperacillin–tazobactam to the current IDSA guidelines for the management of neutropenic fever [36]. Among patients with documented penicillin or cephalosporin allergies, suitable alternatives include ciprofloxacin plus clindamycin or aztreonam plus vancomycin [36].

The other class of antibiotics that has been evaluated less extensively is the fluoroquinolones such as ciprofloxacin [66–68] and levofloxacin. Ciprofloxacin has been studied, with varying results. One potential concern is the development of resistance, based on its widespread prophylactic use as part of "gut decontamination." If a patient develops a fever while on an oral fluoroquinolone, changing to intravenous fluoroquinolone monotherapy is a poor choice since the cause of fever may be an organism not susceptible to fluoroquinolones.

In previous IDSA guidelines, two-drug combinations (aminoglycoside plus either an antispeudomonal penicillin, cefepime, ceftazidine, or carbapenem) were recommended as an initial treatment choice for the treatment of neutropenic fever. In general, the evidence suggests that two-drug combinations have similar response rates to monotherapy regimens but are associated with greater toxicity. As such, they are no longer recommended, except where antibiotic-resistant pathogens are prevalent or documented.

For patients at high risk for infections caused by antibiotic-resistant pathogens such as MRSA, VRE, ESBL-producing organisms, and KPCs, modification to the initial empirical treatment regimen should be considered, particularly when the patient's condition is unstable or there is a suspected and/or documented infection with these pathogens [36]. Previous infection and colonization with one of these organisms or treatment in a hospital with a high rate of endemicity are risk factors for infection. Modification to the initial empirical treatment is made according to the suspected pathogen. Where MRSA is suspected, the early addition of vancomycin, linezolid, or daptomycin can be considered. For suspected VRE, the early addition of linezolid or daptomycin is appropriate. Early treatment with a carbapenem is appropriate where ESBL-producing organisms are anticipated and the early use of polymyxin–colistin or tigecycline can be considered where there is a high rate of KPCs, as KPC-producing organisms are resistant to all β-lactams [36]. Routine evaluation of institutional antibiograms will help guide the need for changes to antibiotic management by tracking changes in infections pathogens and susceptibility profiles.

The addition of agents with Gram-positive activity, such as vancomycin, linezolid, or daptomycin, to the initial empirical antibiotic therapy for neutropenic fever is not recommended in the IDSA guidelines [36]. Although infections caused by Gram-positive pathogens are common in patients undergoing HCT, these infections rarely cause rapid clinical deterioration, and are easily controlled once they are identified. Although the concern about severe sepsis from alpha streptococci in patients with severe mucositis led some clinicians to advocate the use of vancomycin, the introduction of fluoroquinolones with some activity against Gram-positive organisms, such as levofloxacin, has reduced that concern and therefore does not justify routine addition of vancomycin. More importantly, there is also concern regarding the emergence of resistance pathogens, such as *Enterococcus* and *S. aureus*, if vancomycin is overused [69,70]. Certainly, the emergence of VRE in many centers has led to calls for restricted use of this drug from the CDC and multiple infectious disease committees [71]. VRE infections are discussed below. The role of glycopeptides as part of the empirical antibacterial strategy has been evaluated in three separate meta-analyses [70,72–74]. In these evaluations, the addition of a glycopeptide to the initial antibacterial regimen for febrile neutropenia did not result in differences in treatment failure or all-cause mortality, but was associated with an increase in adverse events. Inclusion of vancomycin in first-line therapy can be considered in a number of circumstances. Use outside these indications should be curtailed. When vancomycin is used, serum trough concentrations of >10 mg/L should be targeted as there is evidence that trough concentrations <10 mg/L in patients with proven *S. aureus* infection can produce strains that are VISA-like, and in some conditions higher trough concentrations i.e., >15 mg/L are preferred [75].

The role of linezolid as part of the initial treatment for neutropenic fever is unclear. Linezolid has demonstrated comparable efficacy to vancomycin when added to the initial empirical treatment of patients with febrile neutropenia with proven or suspected infection due to a Gram-positive pathogen [76]. Broad use of agents that are traditionally restricted to severe infections such as VRE is not wise, particularly as the development pipeline for new antibacterials is so limited. There are reports of linezolid-resistant coagulase-negative staphylococci and *S. aureus*, linked to increased linezolid utilization [77,78]. Further, linezolid is known to cause bone marrow suppression, a toxicity that is best avoided where possible in HCT recipients.

Tigecycline has not been systematically evaluated as part of the initial empirical treatment in febrile neutropenia patients, and as such cannot be recommended as part of the initial regimen. Similarly, daptomycin has not been evaluated as initial empirical treatment of febrile neutropenia. There are retrospective registry data for daptomycin in the treatment of documented Gram-positive infections in cancer patients [79].

Low-risk patients

Low-risk patients can receive antibiotics in either the outpatient or inpatient setting. Patients who have a caregiver and access to a telephone and transportation, and can absorb oral medications, can be prescribed oral antibiotics and discharged home, after taking the first doses of medication in the clinic/hospital and being observed for 4–24 hours [36], with close follow-up. Antibiotics of choice in this setting include a combination of oral ciprofloxacin with amoxicillin–clavulanate. Other choices that have been evaluated less rigorously include levofloxacin or ciprofloxacin monotherapy, or a combination of levofloxacin plus clindamycin [36]. If a patient has been taking prophylactic fluoroquinolones, they are not candidates for oral empirical treatment with a fluoroquinolone.

Antibiotic modification during neutropenia

The response to the initial antibiotic regimen dictates subsequent management decisions. If the patient defervesces and is culture negative, the initial regimen can be continued without modification until resolution of neutropenia [ANC ≥500/mm^3 and rising]. For patients who remain persistently febrile by day 2–4 after initial empirical therapy, the approach to treatment modification is based on the patients' risk category.

Low-risk (Figure 85.3) [36]

Low-risk culture-negative patients who are persistently febrile and are responding to the initial treatment regimen can continue on the initial treatment without modification until the ANC is >0.5 × 10^9/L. For those patients who started intravenous antibiotics, a transition to oral antibiotics can be considered if the gastrointestinal tract mucosa is intact and absorption can be assured. Low-risk culture-negative patients who remain persistently febrile and are clinically unstable should be hospitalized for broad-spectrum antibiotics. Antibiotic therapy should subsequently be modified according to culture results and/or the infection site. Antibiotics should be continued for 7–14 days for a documented infection, or longer, that is, until the ANC is >0.5 × 10^9/L and rising.

High-risk (Figure 85.4) [36]

High-risk culture-negative patients who remain persistently febrile, but are stable clinically with no signs of progressive infection, should continue the initial therapy without modification. Antibiotic therapy should be continued until the ANC is >0.5 × 10^9/L and rising. For those patients in whom Gram-positive coverage was initiated as part of the initial treatment regimen, and remain culture negative, consideration should be given to stopping the Gram-positive therapy. High-risk culture-negative patients who are persistently febrile and hemodynamically unstable should be re-evaluated carefully. Consideration of drug-resistant pathogens should occur, and may necessitate a change in treatment based on local susceptibility patterns and resistance trends.

If fever persists or recurs five or more days after initiation of antibiotics and the patient is likely to remain profoundly neutropenic, the patient is at high risk for fungal infection (Figure 85.4). Consideration should be given to initiation of antifungal therapy, as discussed in Chapter 86, and the patient should be evaluated clinically and radiologically. Other causes of persistent or recurrent fever include *C. difficile*, neutropenic enterocolitis, another bacterial infection, or a non-infectious source. Provided that the patient is hemodynamically stable, continued fever alone should not prompt significant antibacterial modifications in the absence of positive culture data.

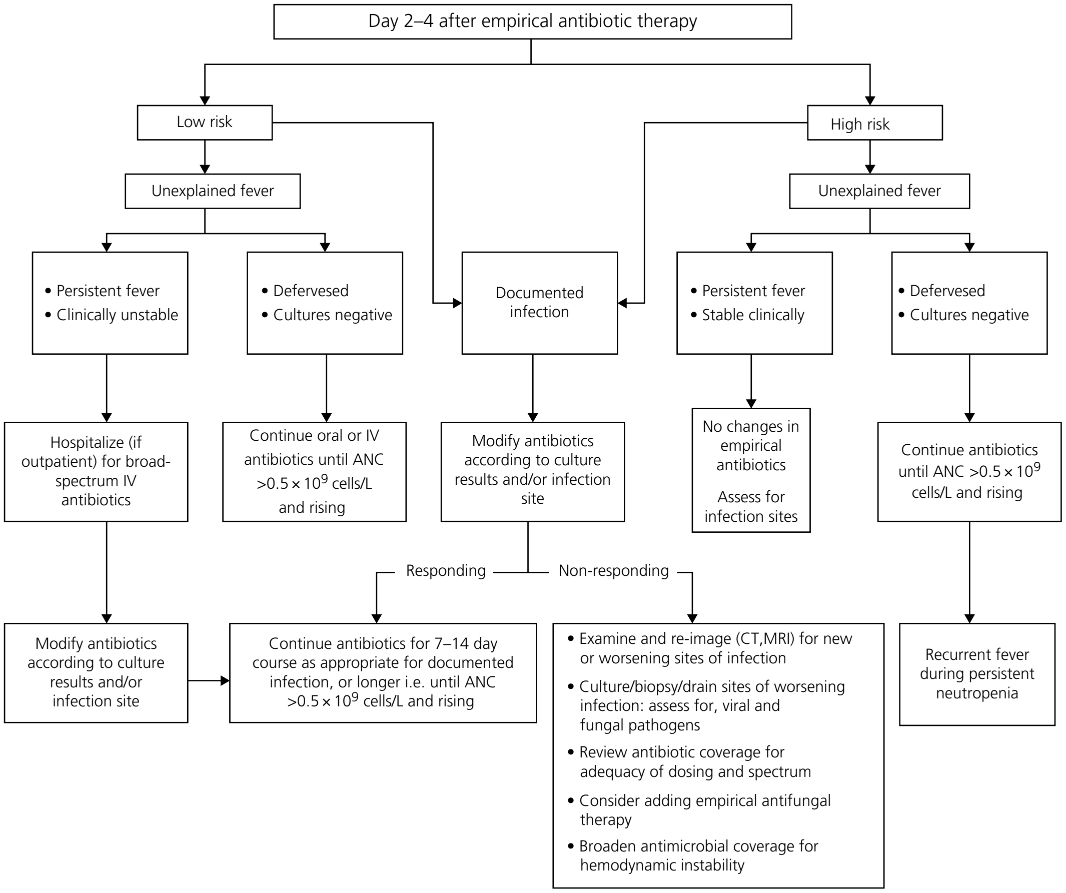
Day 2–4 after empirical antibiotic therapy
Low risk
High risk
Unexplained fever
Unexplained fever
• Persistent fever
• Clinically unstable
• Defervesed
• Cultures negative
Documented infection
• Persistent fever
• Stable clinically
• Defervesed
• Cultures negative
Hospitalize (if outpatient) for broad-spectrum IV antibiotics
Continue oral or IV antibiotics until ANC >0.5 × 10⁹ cells/L and rising
Modify antibiotics according to culture results and/or infection site
No changes in empirical antibiotics
Assess for infection sites
Continue antibiotics until ANC >0.5 × 10⁹ cells/L and rising
Responding
Non-responding
Modify antibiotics according to culture results and/or infection site
Continue antibiotics for 7–14 day course as appropriate for documented infection, or longer i.e. until ANC >0.5 × 10⁹ cells/L and rising
• Examine and re-image (CT,MRI) for new or worsening sites of infection
• Culture/biopsy/drain sites of worsening infection: assess for, viral and fungal pathogens
• Review antibiotic coverage for adequacy of dosing and spectrum
• Consider adding empirical antifungal therapy
• Broaden antimicrobial coverage for hemodynamic instability
Recurrent fever during persistent neutropenia

Figure 85.3 Guidelines for patient reassessment after 2–4 days of empirical antibiotic therapy. Source: Youssef et al. 2007 [34]. Reproduced with permission of Elsevier.

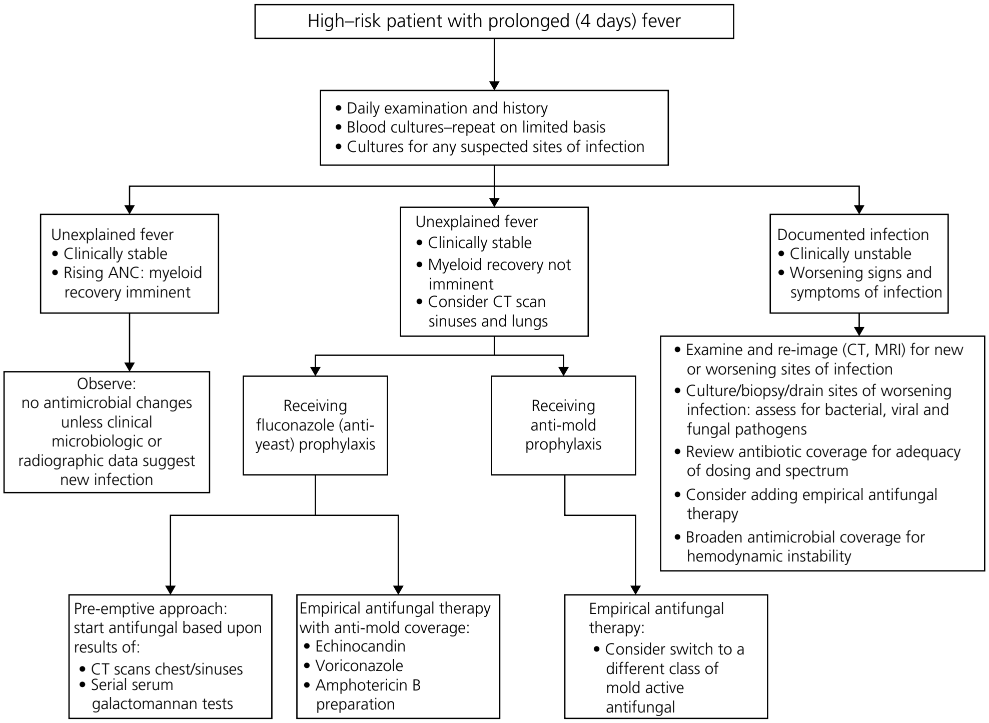
High–risk patient with prolonged (4 days) fever
• Daily examination and history
• Blood cultures–repeat on limited basis
• Cultures for any suspected sites of infection
Unexplained fever
• Clinically stable
• Rising ANC: myeloid recovery imminent
Unexplained fever
• Clinically stable
• Myeloid recovery not imminent
• Consider CT scan sinuses and lungs
Documented infection
• Clinically unstable
• Worsening signs and symptoms of infection
Observe: no antimicrobial changes unless clinical microbiologic or radiographic data suggest new infection
Receiving fluconazole (anti-yeast) prophylaxis
Receiving anti-mold prophylaxis
• Examine and re-image (CT, MRI) for new or worsening sites of infection
• Culture/biopsy/drain sites of worsening infection: assess for bacterial, viral and fungal pathogens
• Review antibiotic coverage for adequacy of dosing and spectrum
• Consider adding empirical antifungal therapy
• Broaden antimicrobial coverage for hemodynamic instability
Pre-emptive approach: start antifungal based upon results of:
• CT scans chest/sinuses
• Serial serum galactomannan tests
Empirical antifungal therapy with anti-mold coverage:
• Echinocandin
• Voriconazole
• Amphotericin B preparation
Empirical antifungal therapy:
• Consider switch to a different class of mold active antifungal

Figure 85.4 Algorithm for high-risk patients with persistent fever after 4 days of empirical antibiotic treatment. Source: Youssef et al. 2007 [34]. Reproduced with permission of Elsevier.

Where an infection has been documented, regardless of risk category, therapy targeted against the pathogens should be instituted. If signs of progressive infection occur, the patient should be re-evaluated, including examination and reimaging (CT or magnetic resonance imaging) for new or worsening sites of infection, culture/biopsy/drain sites of worsening infection, review of antibiotic coverage for dosing adequacy and appropriateness of coverage, consideration of adding empirical antifungal therapy, and broadening of antimicrobial coverage for hemodynamic instability (Figures 85.3 and 85.4) [36].

Fever following resolution of neutropenia

In the HCT population, patients occasionally remain febrile following neutrophil recovery. In this febrile, non-neutropenic population, where reassessment for undiagnosed infection is negative, antibiotics can be stopped after 4–5 days of an ANC over 500/mm^3, while evaluation to determine the cause of the fever is pursued. A common cause of fever that appears at the time of engraftment is the non-infectious entity known as engraftment syndrome.

Outpatient management of neutropenia

In recent years, a variety of considerations have led to the development of outpatient antibiotic regimens including the development of programmable infusion pumps, the widespread availability of home-care services, the desire for patients to receive more of their care in the outpatient setting, the introduction of highly effective and non-toxic antibiotic regimens, and an emphasis on cost containment. A variety of studies have identified factors that distinguish patients who are at low risk for serious morbidity from those who are at risk for septic shock and other complications of neutropenic fever [80–83]. Factors such as the presence of comorbid diseases, the development of fever as an inpatient, underlying hematologic malignancy, prolonged neutropenia (>10 days), and receipt of allogeneic HCT identify those at high risk. Low-risk patients include those with underlying solid tumors receiving conventional chemotherapy, those with a short anticipated duration of neutropenia (<7 days), and those with no comorbid conditions.

Following the stratification of the different clinical risk groups, further research has been centered on developing an internationally validated scoring system to identify low-risk patients at the onset of a febrile illness [82]. In a multivariate model, several characteristics have been identified that are considered independent predictive factors of favorable outcome. Integer weights were applied to these characteristics to develop a risk-index score, which was subsequently validated (Table 85.8) [82]. A risk-index score of 21 or more identified low-risk patients with a positive predictive value of 91%, a specificity of 68%, and a sensitivity of 71% [82]. This scoring system demonstrated lower misclassification rates and better sensitivity compared with earlier models. One of the limitations of the model is the applicability to the HCT setting. A small percentage of the patients studied were HCT recipients, but this model requires further validation in a larger cohort of transplant recipients. If applicable to this population, it could be possible to select low-risk patients for early intravenous-to-oral switch programs while remaining neutropenic.

Table 85.8 Scoring index for identification of low-risk febrile neutropenic patients at time of presentation with fever

Characteristic	Score[a]
Extent of illness[b]	
No symptoms	5
Mild symptoms	5
Moderate symptoms	3
No hypertension	5
No chronic obstructive pulmonary disease	4
Solid tumor or no fungal infection	4
No dehydration	3
Outpatient at onset of fever	3
Age <60 years[c]	2

[a] Highest theoretical score is 26. A risk index score of ≥21 indicates that the patient is likely to be at low risk for complications and morbidity. The scoring system is derived from the Multinational Association of Supportive Care in Cancer [82].

[b] Choose one item only.

[c] Does not apply to ≤16 years of age.

Source: reproduced from Klastersky et al. 2000 [82] with permission of University of Chicago Press.

Outpatient antibiotic therapy or early discharge of patients at low risk for serious sequelae has been evaluated in non-HCT patient groups [80,83,84]. A meta-analysis of randomized trials evaluating the efficacy of oral versus intravenous antibacterials for the treatment of febrile neutropenia in low-risk cancer patients has demonstrated the safety and efficacy of oral antibiotics in this setting. Neutropenic patients who are low risk for mortality can safely receive oral antibiotics in lieu of hospitalization and intravenous administration [85]. Careful selection of suitable low-risk patients is imperative. Outpatient antibiotic regimens have also been evaluated in autologous HCT patients (neither of these is truly treatment, rather prophylaxis) and appear to be fairly promising for some HCT patients at low risk for serious sequelae. Stable febrile and neutropenic patients who are undergoing RIC regimens are also candidates for outpatient therapy. Close monitoring of such patients is imperative.

Vancomycin-resistant enterococci (VRE)

Enterococcal infections are the third most common cause of hospital-acquired bacteremia in hospitalized patients [86]. Importantly, the most recently published National Nosocomial Infections Surveillance system report demonstrated that 28.5% of enterococcal isolates are resistant to vancomycin [87], and large series in allogeneic HCT patients have demonstrated vancomycin resistance in enterococcal bloodstream isolates of 66% in adults and 31% in children [88]. *Enterococcus faecalis* and *E. faecium* are the most frequently identified species, with *E. faecium* being the most common VRE infection, and a smaller number being caused by *E. faecalis* and *E. avium* and other species. Of great concern is the development of resistance of *Enterococcus* to multiple antimicrobial agents. Enterococci are intrinsically resistant to certain β-lactams (particularly cephalosporins and penicillinase-resistant penicillin), low-concentration aminoglycosides, clindamycin, fluoroquinolones, and TMP–SMX. Acquired resistance to high concentrations of β-lactams and aminoglycosides, glycopeptides (vancomycin, teicoplanin), tetracycline, erythromycin, fluoroquinolones, rifampin, chloramphenicol, fusidic acid, and nitrofurantoin is also well documented [89]. More recently, the emergence of VRE with reduced susceptibility to new agents has been documented and is concerning.

There are six vancomycin-resistant enterococcal phenotypes, namely VanA, VanB, VanC, VanD, VanE, and VanG [89,90], of which VanA and VanB are the most common and clinically relevant. VanA resistance, characterized by acquired inducible resistance to high concentrations of vancomycin [minimum inhibitory concentration (MIC) 64–1000 μg/mL] and teicoplanin (MIC 16–512 μg/mL), can be induced by the presence of either drug. VanA resistance is mediated by transposon Tn1546. Of concern, high-level VanA vancomycin resistance has been transferred from *E. faecalis* to *S. aureus* via the Tn1546 transposon [91]. VanB-type resistance is characterized by acquired inducible resistance to low-to-high concentrations of vancomycin (MIC from 4 to over 1064 μg/mL), but sensitive to teicoplanin (MIC 0.25–2 μg/mL). Both VanA and VanB VRE phenotypes are most common amongst *E. faecium* and *E. faecalis*, and less common among other *Enterococcus* spp. such as *E. gallinarum* and *E. casseliflavus*, which are typically associated with the VanC phenotype [89–91].

Colonization and infection with VRE are a concern in high-risk neutropenic patients [92–95]. Colonization rates in patients undergoing HCT have been reported to be between 5.5 and 40% [85,93,96], and have increased almost threefold in recent series [88]. Up to one-third of these colonized patients develop VRE bacteremia, which is associated with high mortality rates [88,97,98]. Several risk factors have been identified that are associated with colonization or infection with VRE [89,90,99,100]. Antibiotic exposure, particularly to vancomycin, cephalosporins, and antianaerobic agents such as metronidazole and imipenem, are reported to increase risk [88,90,101]. Other variables that increase risk are a longer duration of hospitalization, greater severity of underlying illness, hemodialysis, receipt of corticosteroids, parenteral nutrition, neutropenia, mucositis, presence of feeding tubes, and a close proximity to colonized or infected patients. A recent evaluation of 750 allogeneic HCT patients identified several other risk factors for VRE bloodstream infection including colonization with VRE prior to HCT [risk ratio estimate (RRE) 3.3], colonization with VRE after HCT (RRE 7.7), acute GVHD grade III/IV (RRE 3.5), and delayed engraftment [88]. The percentage of patients who developed VRE bloodstream infections increased by each week delay in engraftment (4.5% if engraftment by day 21, 6.3% if engraftment by days 22–28, 11.9% if engraftment by days 29–35, and 15% if engraftment by days 36–42) [88].

Enterococci are resilient organisms and are able to be transmitted easily via healthcare workers, contaminated medical equipment, and also inadequately cleaned rooms following occupation with a VRE-positive patient. It is essential that standard infection control measures, as suggested by the CDC guidelines for preventing opportunistic infections in HCT recipients [6], be strictly adhered to [102]. These measures include either use of alcohol-based gels (preferred unless there is visible soiling of hands where hand washing is preferred) or hand washing with antibacterial soap before and after entering HCT patient rooms, and disinfecting patient rooms and equipment, including structural surfaces (e.g. walls, floors, and bed frames) with an approved US Food and Drug Administration (FDA) or Environmental Protection Agency-registered disinfectant [6,102,103]. Patients must also be placed under contact precautions until all antibiotics are discontinued and three separate repeat cultures, each 1 week apart, are negative. The Healthcare Infection Control Practices Advisory Committee has published recommendations for the management of multidrug-resistant organisms in healthcare settings, which includes VRE [104]. These guidelines recommend the development and implementation of protocols to obtain active surveillance cultures from high-risk patients, including HCT candidates. Implementation of contact precautions from the time the surveillance cultures are sent pending the results is also recommended. Implementation of surveillance programs for MRSA and VRE appears necessary based on increasing infection rates across a number of institutions. Whether these guidelines can be easily implemented remains to be seen, with likely significant increases in resource consumption [103,104].

Initial treatment of VRE-infected patients includes draining any collections if present, which is less likely in severely neutropenic patients with an absence of neutrophils. All indwelling vascular access devices should be removed immediately. There are a number of drugs that have been used in the management of VRE infection with traditional approaches, including ampicillin, gentamicin, chloramphenicol [105], tetracycline, and nitrofurantoin [89]. Newer agents include quinupristin–dalfopristin [106–108], a combination of streptogrammins, linezolid, an oxazolidinone [108–114], tigecycline [112], and daptomycin [115–121], a cyclic lipopeptide, all of which have shown good response rates in VRE bacteremias. The new agents have not been prospectively compared in large studies for the treatment of bloodstream enterococcal infections.

Quinupristin–dalfopristin is approved by the FDA for the treatment of *E. faecium* infections. Clinical efficacy of quinupristin–dalfopristin was demonstrated against VRE infections in a prospective, noncomparative, emergency-use study. Clinical response rates of 69% were documented [122]. Despite the FDA indication, it is rarely used in clinical practice owing to limited activity against *E. faecalis* and an unfavorable side-effect profile that includes musculoskeletal adverse events.

Daptomycin, an agent with bactericidal activity against VRE, has been evaluated in several retrospective observational studies [114,117–121,123,124]. Treatment with daptomycin is associated with microbiological or clinical cures in 58–90% of patients. Most enterococcal isolates have MIC values of ≤2 mg/L, although there are a number of isolates with MICs ≥4 mg/L. Isolates with higher MICs may need to be treated with higher doses of daptomycin up to 12 mg/kg [125,126]. There are several reports of VRE with reduced susceptibility to daptomycin [127,128], although more than 99.5% of VRE isolates remained susceptible to daptomycin in surveillance studies [129]. Close attention to local susceptibility patters and antibiograms is essential to detect any changes and impact on the likelihood of a positive outcome.

Linezolid is also effective for the treatment of *E. faecium* infections, including concurrent bacteremia. It is a bacteriostatic against enterococci. Clinical and microbiological response rates of approximately 75 and 94%, respectively, have been observed [130]. Similar to the other new agents for the treatment of enterococci, there are several reports of reduced susceptibility of VRE isolates to linezolid [131–135]. Linezolid therapy has also been associated with hematologic toxicity, which limits longer courses of therapy [136,137], although some data suggest that it can be safely administered in the post-HCT timeframe [138].

The role of tigecycline as monotherapy in the treatment of enterococcal bloodstream infections is unclear owing to low serum concentrations at standard dosing and its bacteriostatic properties [112,139]. A phase III trial comparing tigecycline with vancomycin or linezolid for the treatment of VRE was limited by poor enrollment, and no conclusions on comparative efficacy can be drawn. Case reports of VRE infections resistant to tigecycline have also been reported [139–141].

Clostridium difficile-associated diarrhea (CDAD)

C. difficile, a Gram-positive, spore-forming, anaerobic bacillus, of endogenous or exogenous origin, can establish itself in the colon and proliferate. *C. difficile* infection results in a wide range of clinical conditions ranging from asymptomatic colonization to colitis and toxic megacolon. Approximately 1–19% of patients with *C. difficile* have severe disease resulting in colectomy, intensive care admission, or death [142]. The impact of CDAD on other complications in allogeneic HCT recipients was recently evaluated in a large epidemiologic study of approximately 1000 patients. The development of CDAD is associated with a risk for developing gastrointestinal GVHD and the presence of gastrointestinal GVHD is a risk factor for relapsed/recurrent CDAD [143].

C. difficile usually produces two toxins, toxin A and toxin B. Patients are classified as *C. difficile* positive when either toxin A or B is detected in the stool. There are several methods for detecting *C. difficile*, of which the toxin-specific enzyme-linked immunosorbent assay (ELISA) method is the most common. The ELISA test can detect both toxin A and B, or only toxin A, depending on the reagent used. Reagents that detect both toxin A and toxin B are preferred as a small number of cases (1–2%) of *C. difficile* involve strains of *C. difficile* that produce only toxin B. ELISA results in a relatively high false-negative rate [144] and, as a consequence, between 10 and 20% of patients need more than one stool sample to detect toxin. Good clinical practice involves sending multiple stool specimens (up to three), to increase the diagnostic yield by 5–10% [145]. The ELISA test has a sensitivity of 63–94% and a specificity of 75- 100% [146] and also has the advantage of a quick turnaround for results. A number of other tests have been used [including glutamate dehydrogenase, cell culture neutralization assays, anaerobic toxigenic culture, and real-time polymerase chain reaction (PCR)] [146–148], but many of these tests are less sensitive and take considerably longer to generate a result, delaying implementation of treatment (reviewed in [146]). More recently, laboratories have been adopting nucleic amplification tests (i.e. PCR) targeting *C. difficile* toxin. These tests are associated with high sensitivity and specificity [146,147]. Testing for *C. difficile* should only be performed on unformed diarrheal stool, the only exception being where ileus due to *C. difficile* is suspected [146].

In 2005, a hypervirulent epidemic strain of *C. difficile* was associated with a number of CDAD outbreaks in Canada, the United States, the United Kingdom, and Europe. This strain was associated with an increase in incidence of CDAD and also severity of the disease. The hypervirulent strain was identified as the North American Pulsed Field Type 1 (NAP1), restriction enzyme analysis type "B1," and PCR isotype 027 [149–152]. NAP1/B1/027 produces a binary toxin that is associated with more severe CDAD cases. The NAP1/B1/027 strain has an altered repressor gene, *tcdC*, which regulates toxin A and B production, resulting in 16–23-fold more toxin A and B than wild-type strains [149]. The presence of the NAP1/B1/027 strain is associated with a more severe disease course [152,153], increased relapse rates following conventional treatment [154,155], disease in uncommon populations [156,157], and a higher attributable mortality [152,158]. Most laboratories do not have capabilities to determine whether the *C. difficile* is the NAP1/B1/027 strain. Specimens can be sent to the CDC for testing. Today, the NAP1/B1/027 strain occurs relatively frequently, representing approximately one-third of patients in recent randomized clinical trials assessing a new treatment, and is more common in North America than Europe [159].

C. difficile has emerged as the most common cause of hospital-acquired diarrhea. *C. difficile* is thought to be the cause of 20–30% of all cases of antibiotic-associated diarrhea, and a higher percentage of colitis associated with antibiotic treatment [145,146]. Among HCT recipients, CDAD is reported to occur in 5–15% of patients with diarrhea [143,160–164]. The most widely recognized risk factor for the development of CDAD is antimicrobial therapy, an increased duration of therapy, the use of broad-spectrum antibacterials, and the use of multiple agents. Almost all antimicrobial agents have been associated with the development of CDAD, including clindamycin, penicillins, and cephalosporins. The fluoroquinolones emerged as a predominant risk factor for CDAD during an outbreak of NAP1/B1/027 CDAD in Canada [153]. Other risk factors for developing CDAD include older age, residence in a nursing home or long-term care facility, use of proton pump inhibitors [165,166], and contact with active carriers. In HCT recipients, risk factors for the development of CDAD include receipt of chemotherapy prior to HCT conditioning [143], TBI [164], receipt of cord blood stem cells [164], broad spectrum antibimicrobial use [143], VRE colonization [143], and acute graft-versus-host disease [143,164].

Where possible, treatment of CDAD involves discontinuation or streamlining of antibiotic therapy. Among HCT recipients, this is often not feasible owing to underlying infection. First-line treatment options for CDAD, as currently recommended by the Society for Healthcare Epidemiology of America (SHEA) and IDSA, include metronidazole and vancomycin, choice being dependent upon severity of disease [146]. Two older comparative trials [167,168], a recent systematic review [169], and a randomized trial demonstrated equivalent response rates in mild CDAD [170]. In severe cases of CDAD, vancomycin was associated with an improved response rate [170]. Failure rates associated with metronidazole therapy have increased from approximately 7% in the 1990s to 16–38% in more recent series [155,171]. No specific cases of metronidazole resistance have been documented in North America. Mild cases of CDAD should be treated with metronidazole 500 mg orally three times per day for 10–14 days [146]. It is significantly cheaper and is not associated with the VRE resistance issues that plague vancomycin therapy. If alternative treatment is necessary owing to intolerance or failure to respond to initial therapy, vancomycin 125 mg orally four times daily should be initiated. Severe cases of CDAD should be treated initially with vancomycin 125 mg orally four times per day for 10–14 days [146]. Severe cases are defined in the SHEA/IDSA guidelines as those with leukocytosis with a white blood cell (WBC) count of 15,000 cells/mL or higher or a serum creatinine level ≥1.5 times the premorbid level [146]. For those patients with severe complicated CDAD, defined as presence of hypotension or shock, the recommended treatment is vancomycin 500 mg orally four times per day plus metronidazole 500 mg intravenously every 8 hours. If an ileus is present, vancomycin can be administered rectally at a dose of 500 mg (diluted in 100 mL of normal saline) every 6 hours in addition to the oral/intravenous combinations listed previously [146].

Fidaxomicin, an oral non-absorbable macrocyclic antibiotic, was approved by the FDA for the treatment of CDAD in adults ≥18 years of age in 2011. The efficacy of fidaxomicin (200 mg orally twice daily) compared with vancomycin (125 mg orally four times daily) was evaluated in two large double-blind, non-inferiority, randomized studies, where the primary end-point was clinical cure, defined as resolution of diarrhea for the duration of treatment and no need for additional treatment up for 48 hours following treatment completion [159,172]. In both trials, the clinical cure rate was similar in both groups, as was time to resolution of diarrhea [159,172]. Recurrence of CDAD was more common among recipients of vancomycin compared with fidaxomicin, which translated into lower sustained responses with vancomycin in both trials [159,172]. A recent meta-analysis of these trials confirmed that fidaxomicin is superior to vancomycin in reducing CDAD recurrences [173]. In subgroup analyses, fidaxomicin appeared to be superior to vancomycin among patients who needed to continue concomitant antibiotics [159], patients ≥65 years of age [159,172], patients with severe CDAD [159,172], and patients infected with the non-027 strain of *C. difficile* [159,172]. Among patients infected with the hypervirulent NAP1/B1/027 strain, the efficacy of fidaxomicin was equivalent to that of vancomycin [159,172]. Fidaxomicin is well tolerated, with no differences in treatment-emergent adverse events compared with vancomycin. Despite the favorable efficacy and safety profile, the cost of fidaxomicin is prohibitive for widespread use. The cost of 10 days of therapy with fidaxomicin is approximately 125-fold higher than that with metronidazole and double that of vancomycin at standard doses. As a consequence, fidaxomicin is restricted in many institutions for use in patients who are known to be at high risk for CDAD recurrence or who have had multiple recurrent episodes of CDAD. SHEA/IDSA do not currently have recommendations for the use of fidaxomicin as their guidelines were published prior to FDA approval of fidaxomicin. Another approach is to take patients at high risk for developing a CDAD infection, and administer effective agents prophylactically. A phase IIIb trial to evaluate the safety and efficacy of fidaxomicin as prophylaxis against CDAD in adult HCT patients is currently enrolling to address this question.

Treatment failure in immunocompetent patients is often defined as failure to stop diarrhea after 4–5 days of therapy. This definition may not be applicable in immunocompromised patients, since neutropenic patients require a longer time to respond [174]. If clinical symptoms progress, alternative treatment options should be considered. Vancomycin must be administered orally, not intravenously. Oral therapy results in high concentrations in the stool, whereas intravenous therapy does not achieve therapeutic concentrations in the stool. If intravenous therapy is absolutely necessary, metronidazole can be administered intravenously, as bactericidal concentrations are achieved in the colon when administered via this route [146]. A recent study compared the efficacy of oral metronidazole, intravenous metronidazole, and oral vancomycin in hospitalized patients with mild CDAD [175]. Patients treated with intravenous metronidazole had the highest mortality rate, suggesting that intravenous metronidazole is inferior to oral metronidazole and oral vancomycin, and should not be recommended unless absolutely necessary [175].

Other treatment options that have been investigated for CDAD include tolevamer [176], rifaximin [177], nitazoxanide [178,179], tigecycline [180], and fusidic acid. Nitazoxanide has been compared with metronidazole for the treatment of CDAD, resulting in similar times to resolution of symptoms of colitis, similar complete response rates at the end of 7 days of treatment, and similar sustained clinical responses 31 days after starting treatment [178]. Tolevamer at two different dosage levels has been compared with oral vancomycin, resulting in similar times to resolution of diarrhea [176]. Tigecycline has been reported to be effective in the management of recurrent/refractory CDAD in several case reports, although there have been no large studies demonstrating its efficacy, hence it should be considered investigational in this setting at present [180]. Both fusidic acid and bacitracin have been used in the treatment of CDAD, with mixed success. Emergence of resistance limits their use.

Patients with CDAD should be treated with appropriate therapy for 10–14 days. Relapses are reported to occur following successful treatment in up to 25% of cases [145,155], and recent data suggest that the relapse rate in HCT recipients is comparable at 21.7% [143]. Relapses can be treated with another 10–14 days of therapy as used in the initial episode. Relapses are primarily due to reinfection with a new strain and are not associated with bacterial resistance [146], therefore the drug used for the initial infection can be used for the relapse. Current SHEA/IDSA recommendations include a slow, tapering schedule of oral vancomycin over a period of 6 weeks or, in appropriate candidates, intracolonic vancomycin. Use of metronidazole beyond the first recurrence is not recommended owing to the potential for cumulative neurotoxicity with long-term use [146]. The use of probiotic therapy to restore normal colonic fecal flora has also been investigated in non-immunocompromised individuals [181]. Administration of *Lactobacillus* or *Saccharomyces boulardii* is reported to protect against *C. difficile*-induced colitis. A recent meta-analysis of probiotics for the prevention of CDAD suggested that there is a role for probiotic therapy, as probiotics cause a large reduction in CDAD without any adverse events [181]. These approaches should be avoided in neutropenic patients owing to risk of active infection with the probiotic organism as opposed to colonic colonization. Finally, intravenous immunoglobulin (IVIg) therapy has been used with some success. The basis of decreasing transmission of *C. difficile* is good infection control. This is discussed later under preventive strategies.

Investigational approaches to the treatment and prevention of CDAD include fecal microbiota transplantation and the use of monoclonal antibodies to *C. difficile* toxin A and administration of antibodies specific to *C. difficile.* Preclinical studies suggest that such antibodies will augment the immune response to *C. difficile* toxins. A phase II randomized trial of two fully human monoclonal antibodies against *C. difficile* toxins A (CDA1) and toxin B (CDB1) has been completed. The trial demonstrated that infusion of CDA1 and CDA2, in conjunction with systemic therapy, reduced the rate of recurrence of CDAD and more specifically also reduced the recurrence of more virulent strains such as NAP1/BI.027 [182]. Phase III trials assessing the safety and efficacy of monoclonal antibodies against toxin A, toxin B, and toxin A and B are currently under way (MODIFY-1 and MODIFY-2). Fecal microbiota transplantation, a process whereby feces from a healthy donor are transplanted into the colon of a CDAD-infected patient, has demonstrated efficacy in several case series for the treatment of recurrent or refractory CDAD [183]. This approach is being evaluated in two randomized controlled trials, one of which, the Fecal therapy to Eliminate *Clostridium difficile* Associated Longstanding diarrhea (FECAL) study is completed and results are expected soon. The second trial, being conducted by investigators at the National Institutes of Health, was recently approved and will start enrollment in the near future. This approach has two limitations, the first being the willingness of patients to consider transplantation of feces from anther individual, and second the risk of disease transmission. Hence this approach is considered investigational and should be reserved for highly refractory cases. This approach has been used in non-neutropenic HCT candidates [184].

MRSA and CA-MRSA

Infections caused by MRSA are increasing, and not only nosocomially acquired but also community-acquired infections have been identified [185]. The MRSA strains found in the community are genetically different from strains typically found in healthcare facilities, resulting in subtle differences in clinical presentation and considerable differences in the antimicrobial susceptibility profile. Typical infections associated with healthcare-associated MRSA (HA-MRSA) strains include ventilator-associated pneumonia, surgical site infections, and central line-associated bloodstream infections. In contrast, community-acquired MRSA (CA-MRSA) strains primarily present as skin and soft tissue infections, but have been associated with more severe syndromes such as osteomyelitis and pneumonia [186,187]. There are also differences in antibacterial susceptibilities between HA-MRSA and CA-MRSA. HA-MRSA is not only resistant to penicillins, and other β-lactams, but is usually also resistant to other classes of drug, including fluoroquinolones, macrolides, and clindamycin. CA-MRSA, however, maintains susceptibility to fluoroquinolones, macrolides, clindamycin, tetracyclines, and TMP–SMX [188]. These issues need to be considered in HCT patients who move routinely between the inpatient and outpatient units. Historically, CA-MRSA has not been a concern, although it now should be part of the differential when patients are readmitted.

Vancomycin has been the reliable cornerstone of MRSA treatment for more than 20 years. Over the past few years, a number of vancomycin-intermediate *Staphylococcus aureus* (VISA) infections and vancomycin-resistant *S. aureus* infections have been reported, and the presence of VISA in the setting of complex infections has been underestimated [189]. Likewise, vancomycin failures in the absence of VISA and vancomycin-resistant *S. aureus* are increasingly reported [190]. In recognition of this information, the Clinical and Laboratory Standards Institute lowered the MIC interpretative criterion for *S. aureus* to 2 μg/mL to improve the likelihood of clinical success and improve resistance detection. In addition, adjustment of vancomycin dosing to achieve higher troughs is recommended in the vancomycin monitoring guidelines to minimize resistance selection and optimize clinical response for complicated infections [75]. Desired trough concentrations between 10 and 20 μg/mL are needed for most infections, and may be pushed to 15–20 mg/mL in complicated infections such as endocarditis and osteomyelitis. Despite these recommendations, data suggest that elevated vancomycin MICs (1–2 μg/mL) in concert with optimized vancomycin dosing may still result in treatment failure and in this setting combination therapy may be necessary, or even a change of therapy [191]. Of note, treatment failure occurs in both methicillin-susceptible *Staphylococcus aureus* (MSSA) and MRSA strains, suggesting there are other variables impacting patient outcomes beyond vancomycin therapy [192]. Owing to the complexity in managing MRSA infections, the IDSA developed a clinical guideline to assist clinicians with managing common clinical syndromes observed in MRSA infections (e.g. skin and soft tissue infections, central nervous system infections, bacteremia, pneumonia, endocarditis, abscesses), provide guidance for determining clinical response, define the role of alternate therapies, and address appropriate dosing [193].

The changing epidemiology of MRSA infections is concerning. CA-MRSA infections are being reported in healthcare facilities, and strains of MRSA typically associated with healthcare facilities are being reported in the community. Acute awareness of the change in infection patterns is necessary when selecting empirical treatment for HCT candidates. Likewise, the reduced efficacy of historically "workhorse" antibacterials must be considered among patients failing to clear infections. Alternatives to vancomycin, in the face of treatment failure, vary according to the type of infection. For MRSA bacteremia, daptomycin is the recommended alternative agent in a dose of at least 6 mg/kg/day, often increased to 8–10 mg/kg/day [193]. In contrast, patients with pneumonia should not receive daptomycin owing to inactivation by pulmonary surfactant, and instead linezolid (600 mg PO/IV twice daily) and clindamycin (600 mg PO/IV three times daily) are alternatives assuming susceptibility.

Neutropenic enterocolitis/typhilitis

Neutropenic enterocolitis (also known as typhilitis and necrotizing colitis) is a syndrome characterized by neutropenic fever (ANC <100/mm^3), abdominal pain (especially right lower quadrant pain), diarrhea, and radiographically observed bowel wall thickening [194,195]. Characteristic radiologic findings include one or more of the following: thickened bowel wall (>4 mm) with a predilection for the cecum, fluid-filled dilated bowel loops, right lower quadrant inflammatory mass, pericecal fluid or inflammatory changes in the pericecal soft tissues. Rebound tenderness may or may not be present. Neutropenic enterocolitis occurs in approximately 6% of adult patients receiving treatment for acute leukemia [194]. Pathologically, the structural integrity of the bowel wall is compromised, with microbial invasion, bowel wall necrosis, and edema; hemorrhage and perforation sometimes occur. Neutropenic enterocolitis has a high mortality rate. Recognition of clinical signs and symptoms, prompt evaluation, and institution of broad-spectrum antibacterial treatment (to cover Gram-negative and anaerobic bacteria) are imperative. In addition to bacteria, fungal organisms are often isolated, with *Candida* being the most common pathogen. Therefore, antifungal treatment with fluconazole, or another suitable azole, should be instituted in addition to antibacterial treatment [142].

Treatment of late bacterial infections

Recurrent sinopulmonary infections are frequently associated with cGVHD or immunoglobulin deficiency [6,14,15,33]. They can be treated successfully with a variety of antibiotics, including penicillin, macrolides, fluoroquinolones, and second-generation cephalosporins, depending on local susceptibility patterns [6]. If immunoglobulin deficiency is present (i.e. IgG <400 mg/dL), IVIg can be helpful [6], but it is very costly. An alternative strategy that can be considered is chronic antibiotic prophylaxis, but emergence of antibiotic resistance may limit the utility of such an approach.

Treatment of intravascular catheter-related infections

Intravascular catheters are used almost universally in HCT recipients. Tunneled CVCs such as Hickman, Broviac, and Groshong catheters, and also implantable devices such as the PortACath, are most commonly used. Peripherally inserted central catheter lines are also gaining greater use. The majority of infections are caused by Gram-positive organisms, particularly coagulase-negative *Staphylococcus*, and, less commonly, *S. aureus*, although Gram-negative infections with *Pseudomonas* spp., *A. baumannii*, and *Stenotrophomonas* spp. are increasingly documented.

The IDSA has developed, as mentioned previously, guidelines for the management of intravascular catheter-related infections [32], and these are useful in therapeutic decision-making processes. For those patients with a suspected CVC-related infection, the guidelines recommend that two sets of blood cultures should be drawn, with one set drawn percutaneously. This approach is most helpful if the blood sample drawn through the CVC is negative, as it excludes CVC-related infection. Once results identifying the organism are available, the choice of antibiotic therapy must be based on susceptibilities. Guidelines for managing tunneled CVC infections are outlined in Chapter 80 [36] and specifically address when to remove the catheter and the duration of antibiotic therapy. Patients should be educated on how best to access their lines if they are performing catheter care at home, to minimize the risk of catheter-associated infection. See Chapter 80 for further discussion of this topic.

Preventive strategies

General measures

The foremost principle of infection prophylaxis is minimization of the possibility that encounters with the healthcare team and exposure to the hospital environment place patients at greater risk for acquiring infection. Accordingly, clinical procedures, especially the placement of venous and urinary catheters, should be avoided when possible. General consensus guidelines for the prevention of infection in HCT patients have been published [6]. Hand hygiene with antiseptic gel (preferred with the exception of patients with *C. difficile* infection [6], or where there is considerable soiling of the hands) or washing by care providers is of utmost importance to avoid transmission of infectious agents from one patient to another or from staff to patients. Current guidelines recommend hand washing in the following circumstances: before and after direct contact with a patient; after contact with bodily fluids, non-intact skin, wound dressing; when moving from a contaminated to a clean body site during care of patients; where gloves are worn before and after placement of gloves [6]. Opinion is divided as to the need for decontaminating regimens to reduce the endogenous flora and the use of pathogen-free diets. However, abstinence from fresh fruits and vegetables is frequently advised, although studies have questioned the value of neutropenic diets [196,197].

Particular attention has been placed on the need for minimizing exposure to airborne organisms. Opinion again is divided as to the value of various isolation strategies. The consensus guidelines for the prevention of opportunistic infections in HCT recipients have recommended that all allogeneic HCT patients be placed in rooms with at least 12 or more air exchanges per hour and point-of-use high-efficiency particulate air (HEPA) filters capable of removing particles 0.3 μm or more in diameter be used [6]. The relative merits of laminar air flow versus HEPA-filtered rooms have not been established.

Knowledge that most infectious pathogens originate from the endogenous flora, and also the observations that in many instances these organisms are acquired after entry to the hospital environment, have led to investigation of the utility of bacterial and fungal surveillance cultures. Bacterial surveillance cultures have been found to be useful in detecting antibiotic-resistant bacteria [198]. Such surveillance culture programs have been useful primarily in the setting of prolonged neutropenia for detecting causes of superinfection. The disadvantages of routine use of surveillance cultures are the high cost, the enormous workload for the microbiology laboratories, and low predictive value [199]. Accordingly, routine surveillance of individual patients has not been used widely. Nevertheless, there is consensus that ongoing hospital-wide and unit-specific infection surveillance programs need to be in place to detect shifts in infectious pathogens and patterns of antibiotic resistance [198].

C. difficile is a major cause of diarrhea in HCT patients. Contaminated environmental surfaces and the hands of healthcare personnel are major sources of organism transmission to susceptible patients. Minimization of antibiotic exposure and attention to infection control measures to reduce person-to-person contact are of paramount importance in preventing outbreaks [200,201]. Most institutions utilize alcohol-based hand sanitizers for healthcare workers. Alcohol-based solutions do not effectively kill *C. difficile* spores; therefore, personnel should use soap (or antimicrobial soap) and water to wash their hands in units where there are infected patients. Healthcare personnel and visitors should use gloves and gowns when entering rooms of patients infected with *C. difficile*. Patients with CDAD should be isolated to a single room with contact precautions, and these contact precautions should be continued until the diarrhea has resolved [146]. Following patient discharge, the room should be cleaned with chlorine-containing agents or other sporicidal agents [146].

A variety of factors influence the risk for infection associated with CVCs: the type of catheter, site of insertion, catheter size, duration of use, degree of host immunodeficiency, type of precautions used during catheter insertion, and skill with which the catheter is inserted. The Hospital Infection Control Practices Advisory Committee has published guidelines for the prevention of infections related to intravascular devices [201]; these include training of the healthcare team that will place and maintain the lines, monitoring infection rates with different kinds of catheter over time, hand washing before and after handling catheters or their dressings, the use of barrier precautions during catheter insertion and care, the use of skin antiseptics before catheter insertion, the use of either sterile gauze or transparent dressings for covering the catheter site, change of dressings when they are soiled or wet, avoiding touching of the catheter exit site when the dressing is changed, the use of a single- rather than a multilumen catheter when possible, the use of a tunneled catheter if long-term use is anticipated, and the use of a subclavian rather than a jugular or femoral site unless necessary. Several controlled trials have evaluated the use of silver and antibiotic-impregnated catheters to reduce the risks for infection in non-HCT recipients [202–204]. This promising strategy was successful in the context of those clinical trials; however, the long-term effects in terms of antibiotic resistance are not known. Further, since the studies were not conducted with tunneled catheters, how applicable the results are to the HCT setting is yet to be tested.

Antibiotic prophylaxis during neutropenia

The frequency and severity of Gram-negative bacterial infections during neutropenia and concomitant gastrointestinal mucosal damage led to an early emphasis on suppressing the intestinal flora to prevent invasive infection. Early efforts to prevent bacterial infections employed the use of oral non-absorbable agents. Agents such as vancomycin, gentamicin, neomycin, colistin, and polymyxin B were advocated in various combinations. A variety of controlled trials have been conducted, and some have shown a beneficial effect. Unfortunately, difficulties with patient compliance, concerns about the emergence of resistance, the high cost of several of these regimens, and lack of evidence for a treatment effect on all-cause mortality and attributable mortality have led to poor acceptance. Interest in such regimens today remains, but due more to the potential for decreasing the elaboration of proinflammatory cytokines involved in the pathogenesis of GVHD than for infection control.

TMP–SMX has also been found to reduce both Gram-positive and Gram-negative bacterial infections in patients with hematologic malignancies and undergoing HCT [205,206]. Its broad-spectrum activity against a number of Gram-negative organisms is offset by its lack of activity against *P. aeruginosa*, a major pathogen in HCT patients. Moreover, concerns about delays in engraftment, patient tolerance, and selection of resistant organisms have limited its use. Because of these concerns and the paucity of data for HCT patients, this regimen has not been adopted widely.

The fluoroquinolones have been shown in controlled trials to reduce Gram-negative bacterial infections significantly, appear to be as or more effective than other oral antibiotic regimens, and have been evaluated in HCT patients [207,208]. Norfloxacin [209–212], ciprofloxacin [212–218], ofloxacin [219–221], perfloxacin [222,223], levofloxacin [224], and moxifloxacin [225] have been the most widely studied in HCT patients. Ciprofloxacin provides an advantage over norfloxacin in that oral administration achieves systemic blood levels; however, it is not active against most methicillin-resistant staphylococci (the majority of staphylococcal isolates). Levofloxacin offers greater activity against Gram-positive organisms, including *Staphylococcus* and *Streptococcus* spp., and therefore would be preferable to ciprofloxacin in patients expected to develop severe mucositis and at risk for streptococcal infections.

Concerns about the emergence of fluoroquinolone resistance have also been raised. The effect of fluoroquinolone prophylaxis on microbial resistance was addressed in a systematic review and meta-analysis of 56 randomized trials of antibiotic prophylaxis in neutropenic hosts [226]. Within the analysis, the trials were separated into two cohorts, namely those that compared fluoroquinolones with placebo/no intervention, and those that compared fluoroquinolones with other antibiotics. Many trials did not report colonization data, microbiological surveillance end-points, or routine susceptibility data. Of the fluoroquinolone versus placebo/no treatment trials reporting these end-points, there were no increases in colonization with organisms resistant to fluoroquinolones, and no difference in the proportion of patients developing infections caused by bacteria resistant to fluoroquinolones. Several microbiologically documented infections were observed in those trials, more of which were caused by fluoroquinolone-resistant organisms in the fluoroquinolone arm compared with placebo. A number of trials compared a fluoroquinolone with TMP–SMX. In those trials, fluoroquinolone use was associated with fewer fluoroquinolone-resistant bacteria in the fluoroquinolone arm than TMP–SMX-resistant bacteria in the TMP–SMX arm. Fewer fluoroquinolone-resistant infections were noted in the fluoroquinolone arm. That analysis demonstrated that there was no difference in the infection rate of fluoroquinolone-resistant pathogens when prophylactic fluoroquinolones were used. Limitations of the analysis were the small number of trials that reported colonization rates and microbiological surveillance results. Continued surveillance and analysis are necessary to evaluate local concerns.

The risk for streptococcal infections may also be greater among recipients of fluoroquinolone prophylaxis [26]. The addition of penicillin or rifampin to the fluoroquinolone can reduce the risk for streptococcal infection [216,227,228]. However, resistance to penicillin is also high in the community and it may not confer additional protection. Historically, preservation of the anaerobic flora has generally been believed to offer protection against more virulent pathogens. Little activity against anaerobes is a feature of the older quinolone, and this may be desirable in protecting against the more virulent pathogens.

Several groups have evaluated the role of antibiotic prophylaxis in neutropenic patients over the past two decades [207,208,229–233]. Early meta-analyses demonstrated that fluoroquinolones, although effective in preventing Gram-negative bacteremia, had no effect on days of fever or infection-related mortality [229,230]. Other analyses have similarly shown a reduction in the number of documented infections compared with placebo, particularly for Gram-negative pathogens [231,232]. Reduction in infection-related mortality due to bacterial causes has also been demonstrated [233]. More recently, a meta-analysis of 95 randomized clinical trials in 9283 afebrile neutropenic patients concluded that prophylactic antibiotics significantly reduced the risk for death compared with placebo or no treatment [relative risk (RR) 0.67, 95% confidence interval (CI) 0.55–0.81] [208,209]. Within the analysis, there were 55 trials that specifically addressed fluoroquinolone prophylaxis. Similar results were observed, with fluoroquinolone prophylaxis leading to decreases in all-cause mortality (RR 0.52, 95% CI 0.35–0.77) and infection-related mortality. Other important end-points including fever, and clinically and microbiologically documented infection rates, were also reduced.

The CDC/IDSA/American Society for Blood and Marrow Transplantation guidelines for the use of antimicrobial agents in neutropenic patients with cancer recommend the use of routine prophylactic quinolone therapy in this patient population only where the duration of neutropenia is anticipated to be 7 days or more [6].

Bacterial prophylaxis after engraftment

Morbidity and mortality from chronic GVHD are frequently due to infection. Antibiotic prophylaxis, although not providing universal protection against encapsulated organisms, substantially reduces the mortality from cGVHD [234,235]. Accordingly, all patients with cGVHD should be placed on daily antibiotic prophylaxis with penicillin, TMP–SMX or a suitable alternative guided by local susceptibility patterns for at least as long as active GVHD therapy is prescribed [6]. The host deficits that mediate vulnerability for infection persist even after successful completion of GVHD therapy and some centers continue antibiotic coverage longer, but duration of need has not been adequately studied. If penicillin is used, additional prophylaxis for *Pneumocystis jirovecii* (formerly *Pneumocystis carinii*) should also be administered. Even in the absence of cGVHD, patients with recurrent sinopulmonary infections and autografted recipients undergoing HCT with TBI-containing regimens [235] should be considered for prophylaxis to prevent infection from encapsulated organisms. Agents such as penicillin, cefaclor, cefuroxime, TMP–SMX, and the new-generation macrolides are particularly useful. Moreover, patients who have previously undergone splenectomy should be considered for antibiotic prophylaxis against encapsulated bacteria.

Adjunctive measures

Growth factors

Growth factors such as granulocyte colony-stimulating factor (G-CSF) or granulocyte–macrophage colony-stimulating factor (GM-CSF) to stimulate more rapid hematopoietic recovery, and to obtain an enriched hematopoietic cell product, have become widespread in the field of HCT, and are especially useful in autologous HCT (see Chapter 43). Unfortunately, although neutrophil recovery is hastened in most prospective randomized trials, the effects on preventing bacterial infections have been either nonexistent or marginal.

A meta-analysis of 34 randomized controlled trials of prophylactic G-CSF and GM-CSF demonstrated that colony-stimulating factors were associated with a small reduction in the risk of documented infections (RR 0.87, 95% CI 0.76 – 1.00, $p = 0.05$), with an absolute decrease in the risk of documented infection of 8%, which did not translate into improved infection rates or treatment-related mortality [236]. The meta-analysis included a number of subgroup analyses including differences between colony-stimulating factors, that is, G-CSF versus GM-CSF, and also differences between transplant types, that is, autologous versus allogeneic HCT. In those analyses, there was no difference in documented infections in trials that utilized G-CSF, whereas a benefit was demonstrated for

GM-CSF. There was no advantage to colony-stimulating factor use in the autologous HCT setting, whereas there was a significant reduction in documented infections among allogeneic recipients receiving colony-stimulating factors. Other outcomes that were evaluated included microbiologically and clinically documented infection, days of fever, days of parenteral antibiotics, and infection-related mortality. There was no difference in microbiologically documented infection regardless of analysis type. Clinically documented infection was no different among the groups analyzed. Days of fever were not statistically different in the overall analysis and in the G-CSF recipients, whereas GM-CSF recipients had fewer days of fever. There were fewer days of parenteral antibacterials administered in all analyzed groups with the exception of allogeneic HCT recipients [236]. Finally, infection-related mortality did not differ in any of the groups analyzed. Therefore, the benefit of colony-stimulating factors on documented infection and associated complications is small and should be weighed against the cost of these agents.

Current consensus guidelines from the American Society of Clinical Oncology (ASCO) recommend colony-stimulating factors following autologous HCT and not following allogeneic HCT [237]. Of interest is the use of hematopoietic growth factors as an adjunct to antibiotic therapy for established infection or for patients with persistent fever. However, controlled trials have failed to show improved outcomes in either HCT or non-HCT neutropenic patients other than shortening of neutropenia duration, antibiotic utilization, and hospitalization. The current ASCO guidelines do not recommend the routine use of colony-stimulating factors as adjunctive treatment with antibiotic therapy in patients with febrile neutropenia. The exception to this recommendation is in patients who are at high risk for infection-associated complications or those patients who are likely to have a poor clinical outcome based on known prognostic factors such as prolonged and profound neutropenia (>10 days and ANC $<0.1 \times 10^9$/L), being elderly (>65 years old), uncontrolled primary disease, pneumonia, hypotension, multiorgan dysfunction, invasive fungal infection, or being hospitalized at the time of fever [237]. The advantages, disadvantages, and indications for the use of these cytokines are discussed in more detail elsewhere (see Chapter 43).

Several other cytokines, including transforming growth factor-beta [238] and keratinocyte growth factor [239], have been shown in preclinical studies to enhance mucosal stem-cell regrowth. Clinical trials have shown that both recombinant human keratinocyte growth factor (palifermin) and repifermin are able to reduce the severity of mucositis [240,241]. Since mucositis is associated with infection [8,242], whether or not these molecules reduce the risk for infection was an important consideration. Palifermin treatment was found to be associated with a reduction in the incidence of febrile neutropenia (75% palifermin versus 92% placebo, $p < 0.001$), and a non-significant trend towards a lower rate of bacteremia [240]. Repifermin was associated with a lower rate of non-neutropenic fever; however, no difference in rates of febrile neutropenia was observed [241]. Other agents that have been evaluated for their ability to decrease mucosal toxicity and subsequent infections in cancer patients include GM-CSF mouthwash, glutamine, and low-level laser therapy. None of these agents has consistently demonstrated reductions in infection rates, antibacterial consumption, or length of stay of HCT recipients. Of note, the use of GM-CSF mouthwash in HCT recipients is discouraged in the new mucositis guidelines published by the Multinational Association of Supportive Care in Cancer.

Granulocyte transfusions

During the 1970s, granulocyte transfusions were frequently used to treat or prevent infections in severely neutropenic patients [243,244]. Unfortunately, they were found to have only marginal benefit in most individuals, perhaps in part due to the low number of granulocytes mobilized. Moreover, they often transmitted CMV from donors and morbidity frequently developed from the acquired CMV infection. Today, with modern antibiotic regimens, the need for granulocyte transfusions has diminished. However, they can be useful where bacterial infection from an organism resistant to multiple antibiotics is present or the infection progresses despite the administration of optimal antimicrobial agents [245,246]. The use of granulocyte donors who are serologically negative for CMV eliminates the risk of transmitting CMV.

The use of growth factors such as G-CSF or GM-CSF, alone or in combination with corticosteroids, to stimulate granulocyte production and mobilization into the peripheral blood has led to the potential for collecting large numbers of granulocytes from normal donors [245,247], and the use of these "enhanced" granulocyte transfusion products for the treatment of severe infection. It has yet to be demonstrated whether this approach offers significant clinical benefit. A Cochrane review in 2005 found that there is inconclusive evidence from available trials to support or reject routine use of granulocyte transfusions [246]. Since that review, several small series have been published, although only one was a randomized trial that was closed prematurely due to poor enrollment [248]. Randomized controlled trials are needed to establish definitively the role of granulocyte transfusions in the treatment of infections in patients with neutropenia or neutrophil dysfunction, and until these results are available granulocyte transfusions are not recommended for routine use. If granulocyte transfusions are prescribed, the recommended dose to be administered should be $\geq 2 \times 10^{10}$ polymorphonuclear cells/dose with corticosteroid stimulation or $\geq 4 \times 10^{10}$/dose with G-CSF continued until either the infection resolves or the WBC count is $>1 \times 10^9$/L [247].

An alternative approach is to collect G-CSF-mobilized granulocytes and administer them to patients prophylactically post-transplant, during the expected neutropenic window, to facilitate accelerated neutrophil recovery [249]. This approach has been shown to reduce the number of patients who develop fever while hospitalized, the number of febrile days, and the duration of antibacterial therapy in a small series. Additional research on the validity of such an approach is needed, and may be suitable only for patients at high risk for serious or antibiotic-resistant infections.

Immunobiologic agents

Several studies have shown that the use of IVIg reduces bacterial, fungal, and viral infections after allogeneic HCT [250–253], but others have not [254,255], including a controlled trial in autologous HCT patients [255]. The relative merits of IVIg as a general antimicrobial measure versus the specific antimicrobial agents mentioned above remain unclear. Generally, IVIg is well tolerated. Its disadvantages include its high cost, large fluid volumes, need for parenteral administration, and occasional side effects. Beyond day 90, IVIg given as a monthly infusion was of no benefit in reducing bacterial infections, and routine use is not recommended in consensus guidelines [6,251]. Moreover, there was no reduction in the incidence of bronchiolitis obliterans, overall survival, or survival from cGVHD, and serum IgG and

IgA levels in patients who received IVIg were lower than those in control subjects, suggesting that the use of IVIg might retard humoral immune recovery. Although there may be other reasons to consider the use of immune globulin (e.g. to reduce GVHD or interstitial pneumonia), its high cost and cheaper antibiotic alternatives do not make this approach attractive for the prevention of bacterial infections.

Activated protein C has been shown to be effective in the treatment of sepsis syndrome, which frequently complicates HCT and is associated with significant morbidity and mortality. This syndrome is the end result of the activation of inflammatory and coagulation cascades in response to infection [256]. Activated protein C is an endogenous protein that is important in maintaining circulatory homeostasis. In sepsis, concentrations are depleted, a factor that has been associated with poor outcomes in septic patients.

Activated protein C has several actions; those of most importance in sepsis are the anti-inflammatory, profibrinolytic, and anticoagulant activities. Drotecogin alfa, a recombinant human activated protein C, has been shown to reduce the relative and absolute risks of death in septic individuals [256,257]. In the pivotal FDA licensing trial, HCT recipients were excluded owing to increased risk from bleeding complications in an already thrombocytopenic population. Since FDA approval, some investigators have used this agent in high-risk thrombocytopenic HCT recipients, with mixed success [258]. The benefits of administration should be weighed against the potential risks of treatment.

Conclusion

The morbidity and mortality from infection have improved dramatically in recent years as insights into the deficits in host bacterial pathogenesis and epidemiology of infections, the introduction of more effective and less toxic antimicrobial regimens, and improved strategies of using various antimicrobial agents have translated into effective therapeutic approaches. Such advances have facilitated enormous strides in reducing transplant-associated mortality, thereby improving the acceptability of HCT as a treatment option for an ever-widening array of malignant and non-malignant diseases.

Variations in the epidemiology of opportunistic pathogens, the ever-continuing emergence of antimicrobial resistance among microorganisms, changes in host characteristics due to new preparative and immunosuppressive regimens, the application of HCT to new patient populations (older ages, different hematopoietic cell sources, and different underlying diseases), and the adoption of post-transplant immunoadjuvant therapies to reduce the risk for relapse in the autologous transplant setting require continuing vigilance, and will pose new challenges for the control of bacterial infections. Better diagnostic tools to detect pathogens more accurately and rapidly, and to detect them earlier in the course of infection, are needed. Newer antimicrobial agents that are safer and more effective, with a broader spectrum of activity, must be sought. The expanding array of biologic agents to modify pathogen virulence and host responses to infection offer the opportunity to reduce host susceptibility to infection, and will likely prove important adjuncts to antimicrobial agents directed against the growing repertoire of opportunistic pathogens.

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CHAPTER 86

Fungal Infections After Hematopoietic Cell Transplantation

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Introduction

Invasive fungal infections (IFIs) are among the leading causes of infectious morbidity following allogeneic hematopoietic cell transplantation (HCT) in adults and children, especially since the 1990s [1,2]. The rising incidence of these infections, the emergence of "new" fungi, the refinement of new diagnostic modalities, and the introduction of the broadest array of antifungal drugs in the history of medicine have resulted in a sustained renaissance in the study of IFIs.

There are three major classes of medically important fungi: yeasts, molds, and dimorphic fungi. One genus of yeast, *Candida* spp., and one genus of mold, *Aspergillus* spp. cause more than 80% of all IFIs in HCT recipients. *Candida* spp. are distinguished by their lack of branching hyphae, existing primarily as unicellular yeast. These are prominent mucocutaneous colonizers, but can cause disease when barriers are breached. Molds, such as *Aspergillus* spp., are most commonly transmitted via inhalation of conidia (spore) forms and have true, branching hyphae. The dimorphic fungi, such as *Coccidioides immitis/posadasii* and *Histoplasma capsulatum*, can grow as both molds (in the environment), and yeasts (in the body).

Much has been learned over the last two decades regarding the epidemiology and risks for IFI, and how to prevent and treat these infections effectively. A large review comparing over 2500 patients who received allogeneic HCT in Seattle over two different periods (1993–1997 vs. 2003–2008) showed lower risks for death caused by both *Candida* species and molds in the recent cohort [3]. Strides have been made in all arenas – screening for early diagnoses, prophylaxis, and improvements in the treatment of established disease. This chapter focuses on the clinically significant infections and how to prevent and treat them. Attention is drawn to infections caused by *Aspergillus* and *Candida* spp., which are the most important in people who have received HCT. Infections caused by other fungi, including *Pneumocystis jirovecii*, the agents of mucormycosis, are discussed briefly.

Infections caused by *Candida* species

Mycology and epidemiology

Candida spp. typically reside on the skin and mucous membranes of the gastrointestinal (GI) and genital tracts. There are numerous species of *Candida*, each of which differs with regard to its biology and predilection for colonization, accounting for variability in the risks and prevalence of disease caused by each. Risks for invasive infections typically cause breakdown in initial defenses, or they increase *Candida* colonization (or both). They include mucositis, neutropenia, graft-versus-host disease (GVHD), administration of broad-spectrum antibiotics and total parenteral nutrition, and central venous catheters.

Of the numerous species of *Candida*, *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis* are the most common organisms associated with invasive disease [23,24]. These organisms differ in their intrinsic ability to cause infection, and also their ability to exhibit resistance to antifungal drugs. *C. albicans* is by far the most "virulent" of the *Candida* species (and the best studied), in part due to its ability to create hyphae that can invade and adhere to mucous membranes and tissues [4]. Other species, such as *C. glabrata* and *C. krusei*, also cause disease, but at lower frequency; the exception to this rule is in the setting of fluconazole prophylaxis, which selects for colonization of these more resistant organisms. The incidence and species distribution of reported disease thus depend on institutional prophylaxis practices [5]. In a recent prospective surveillance study performed in 23 transplant centers in the United States (TRANSNET), *C. glabrata* was the most common organism identified to cause IFIs, followed by *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* [6]. Pediatric programs often report a relatively increased incidence of infections caused by *C. parapsilosis*, which are more commonly introduced exogenously via infusates and/or skin. *C. tropicalis* causes disease more frequently in people with leukemia, for reasons that are not entirely clear [7]. The bottom line is that these species are unique, and the frequency of infection is associated with specific host and treatment characteristics in different patients.

Pathogenesis and immunity

The association between neutropenia and IFIs has long been recognized; however, recent years have brought into focus the understanding that defenses against these commensals are redundant and complicated, with overall risks being a function of both acquired defects and innate immune variability.

The high incidence of mucosal candidal infection associated with human immunodeficiency virus (HIV) infection first highlighted the clinically significant role of the CD4 T cell in anticandidal defense. Studies demonstrated that invasive candidal infection follows adhesion of the organism to the cell surfaces, which induces proinflammatory cytokine responses. Various immune effectors are involved in the response to organism invasion. Neutrophils damage

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candidal pseudohyphae and phagocytose yeasts, and serve to modulate the innate immune response, largely functioning to dampen the proinflammatory response [8]. Both dendritic cells (DCs) and macrophages attach to and ingest candidal yeasts and hyphae, presenting fungal antigens to T cells [84]. Recovery from infection is dependent on CD4$^+$ T helper type 1 (T_H1) cytokine enhancement of monocyte and neutrophil function. Studies using antibodies and/or knockout mice have demonstrated that interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-18 are important in the defense against invasive candidiasis. The balance of cytokines and the nature of CD4 T cell responses are important in modulating risks for both mucosal and invasive disease.

There is a critical difference in the innate immune response to *Candida* yeasts versus hyphal forms, largely dictated by dectin-1 recognition of β-glucan, which is differentially expressed based on morphology [4]. Not only does this provide an explanation for mammalian ability to differentiate between potential "colonization" and "invasion," but it has also led to studies that are now explaining individual genetic risks after HCT. These suggest that a loss-of-function polymorphism in dectin-1 results in lower cytokine responses and increased *Candida* colonization of the GI tract in HCT recipients [9]. It has been postulated that increased colonization may predispose to development of GI tract GVHD, possibly through dectin-1 effects on T-cell immunity [10,11].

Clinical manifestations

Multiple clinical syndromes associated with *Candida* spp. have been described. Primary syndromes and secondary manifestations of invasive disease are discussed here.

Mucocutaneous infection

The classic mucocutaneous manifestation is thrush, which appears with white plaques in the mouth. *Candida* also causes angular cheilitis. Esophagitis and involvement of other areas of the GI tract can occur in the absence of thrush, and the associated complaints of dysphagia, substernal chest pain, nausea, vomiting, and upper GI bleeding are indistinguishable from esophagitis due to other etiologies. The stomach is the second most common GI site of candidal infection, followed by the large and small bowel. Findings at endoscopy include white plaques, single or multiple ulcers, erosions, and pseudomembrane formation. Mucocutaneous infection can lead to invasive disease, especially with concurrent damage to mucosal integrity.

Candidemia, or acute disseminated candidiasis

Following HCT, there is a higher incidence of dissemination to peripheral sites. Although the liver, spleen, and lungs are most frequently affected, virtually all other organs are at risk for seeding, including the kidneys, brain, heart (myocarditis or endocarditis), central nervous system (CNS), vascular endothelium, bone, and eye (endophthalmitis). Microabscesses are the hallmark of candidal tissue invasion. Hence disseminated disease may not be clinically apparent at the time of positive cultures.

Candida species can go to the eye and the skin as primary sites of hematogenous seeding. "Endophthalmitis" implies infection of the vitreous and/or aqueous humor, which typically occurs as chorioretinitis that can progress to produce vitreous and sometimes aqueous infection. Eye examinations should be performed on people with candidemia, as effective therapy may require surgical intervention. Skin lesions are also common, especially with infection caused by *C. albicans* and *C. tropicalis*. These are typically small, papular or pustular, and painful, and can occur in a widespread distribution, depending on the species and fungal burden (Figure 86.1).

Candida spp. are capable of attaching to the endothelium and to catheters, which may explain the presence of endovascular infections, such as endocarditis and thrombophlebitis. Thrombophlebitis is most commonly a post mortem diagnosis; however, the classic, albeit rarely seen, presentation includes a patient with localizing extremity edema and a history of protracted broad-spectrum antibiotic and/or total parenteral nutrition administration via a central venous catheter. Although rare, *Candida* spp. are the most common cause of fungal endocarditis.

Candida species can disseminate to the lungs via hematogenous spread, but these organisms rarely cause disease by airway invasion. For this reason, cultures of sputum or bronchoalveolar lavage fluid have limited diagnostic value. Computed tomography (CT) scans of the chest may show abnormalities suggestive of nodular disease. In one study, CT findings in HCT recipients with histopathologically proven pulmonary candidiasis included multiple nodules, seen in 15 of the 17 (88%) patients studied. In nearly half of the patients, the nodules were described as centrilobular with a tree-in-bud pattern, and in 75% of these scans the nodules were bilateral. A majority of

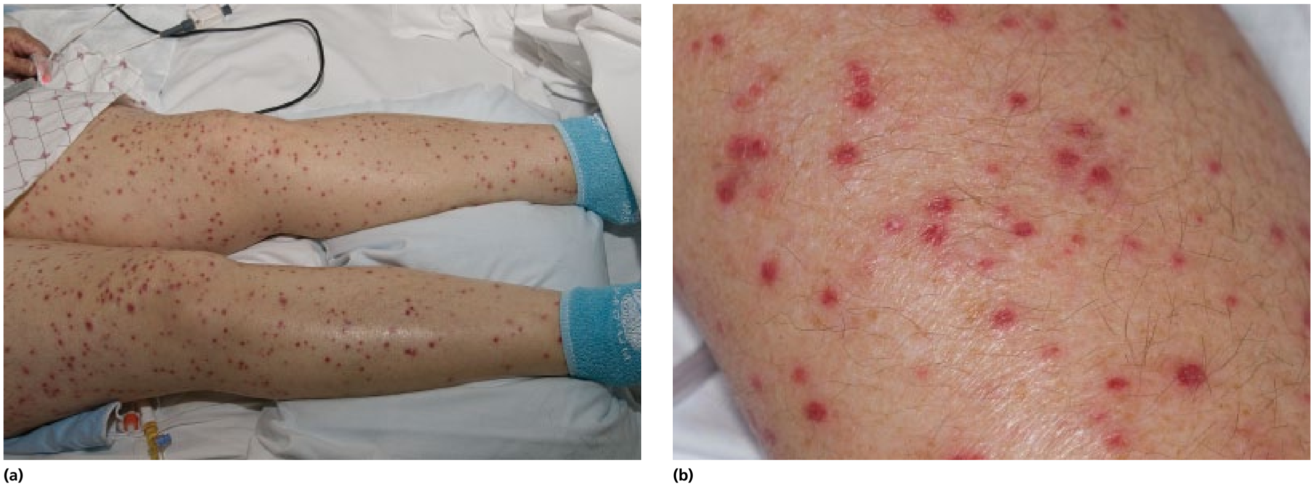

Figure 86.1 Skin lesions caused by *Candida tropicalis* in a patient who was neutropenic after therapy for AML.

patients had associated air-space consolidation, and even ground-glass opacities, pleural effusions, thickening of the bronchial walls, and cavitation [12]. Hence it is difficult to distinguish lesions caused by hematogenous dissemination of *Candida* spp. from organisms that invade through the airway (e.g. *Aspergillus* spp.).

Hepatosplenic candidiasis, or chronic disseminated candidiasis

Hepatosplenic candidiasis is probably the most common syndrome of disseminated infection, although it is infrequently diagnosed to a microbial level. The pathogenesis of hepatosplenic candidiasis is unique, as the organism is acquired through portal vasculature, in the setting of mucosal breakdown, *Candida* colonization, and neutropenia. Symptoms typically occur concurrent with progressive inflammation after neutrophil engraftment, with histopathology demonstrating more granulomatous inflammation; hence this syndrome is also referred to as "chronic disseminated candidiasis" [13]. People usually present with persistent fever, and may have abdominal pain, and elevated liver function tests, especially alkaline phosphatase. Recently, attention has focused on the potential development of an immune reconstitution syndrome in driving protracted symptomatology, as discussed in greater depth below.

Diagnosis and therapy

Diagnosis

The diagnosis of candidemia usually relies on the identification of *Candida* species from blood cultures. Blood culture sensitivity, even with new culture methodologies, is relatively poor; with this in mind, a person can have an invasive infection without having positive cultures.

Multiple non-culture-based assays have been studied for adjunctive diagnostic use, including methods to identify antigens (mannan, glucan), and also polymerase chain reaction (PCR) assays to detect nucleic acids. These assays may have utility in detecting early disease; when compared with blood cultures, a multiplex PCR designed to amplify unique sequences of *C. albicans* provided results in 8 hours and had a sensitivity of 96.9% [36]. Although PCR assays have been introduced in some reference centers, they are not widely available to most clinicians. The US Food and Drug Administration (FDA) cleared an assay that detects β-glucan for use to assist diagnosis of IFI; these assays have been more widely utilized in Japan. The FDA-cleared assay was initially evaluated in the setting of neutropenic fever, and in this setting it has approximately 80% sensitivity and specificity for diagnosis of candidemia [14,15]. In a small evaluation of HCT recipients, the assay became positive a median of 2.5 days after blood culture positivity, but the assay remained positive after cultures had cleared [16]. It is important to remember that circulating β-glucan can be present with multiple fungal infections and other states, with bacteremia and infusion of some drugs [e.g. intravenous immunoglobulin (IVIg)] triggering "false positives."

The diagnosis of hepatic candidiasis typically relies on imaging. With a reported sensitivity of over 90%, CT scans of the abdomen performed with intravenous (IV) contrast are more sensitive than ultrasound examinations. One prospective study examined the role of magnetic resonance imaging (MRI) scans in patients with suspected hepatosplenic candidiasis, revealing high positive and negative predictive values (85% and 100%, respectively, with a sensitivity of 100%). In 11 of 13 patients with hepatosplenic abnormalities, the lesions were less than 1 cm in diameter and were described as well-defined, high signal intensity foci on T1-weighted images [34].

Preventative therapy

Strategies to prevent candidiasis include (1) *primary prophylaxis*, defined as the administration of antifungal drugs in the absence of any evidence of infection, (2) *secondary prophylaxis*, defined as the administration of agents to patients with a history of prior IFIs, and (3) *empiric treatment*, defined as the administration of agents based on a defined but non-diagnostic clinical indication, but without proven IFIs. The prototypic empiric therapy is the use of antifungals in patients with fever during neutropenia. Here, primary prophylaxis is discussed only, given the complexity in interpreting the efficacy of other preventative strategies and widespread adoption of primary prophylaxis strategies in HCT recipients.

The first well-designed placebo-controlled multicenter trial that evaluated fluconazole for primary prophylaxis pre-engraftment showed that candidiasis rates were decreased within the active drug arm, although overall mortality did not differ [17]. In this study, approximately half of the subjects enrolled were recipients of allogeneic HCT and autologous HCT, hence risks and predicted mortality caused by infection were heterogeneous. A subsequent single-center study that enrolled a larger proportion of allogeneic HCT recipients showed improved survival in people who received fluconazole for a longer period (75 days) [18]. That survival benefit was sustained in long-term follow-up analysis [19]. The real benefit of long-term administration of fluconazole has been controversial, and most guidelines have suggested that fluconazole is useful primarily during the pre-engraftment period [20]. Looking back at this issue, it is interesting that the long-term follow-up analysis did observe a benefit of fluconazole associated with GI tract GVHD scores; perhaps some of the benefits could be explained by recent observations that *Candida* gut colonization exacerbates the development of GVHD in select individuals with dectin-1 polymorphisms [10,11,21]. Despite its excellent activity against most *Candida* spp., it is not useful in the treatment of infections due to select species, especially *C. glabrata* and *C. krusei*, which proportionally cause more invasive disease in people receiving fluconazole [22].

The major limitation of fluconazole is its lack of activity against molds. Hence the availability of well-tolerated, broad-spectrum agents renewed the search for a regimen to prevent these more lethal IFIs. Lipid formulations of amphotericin B, multiple broad-spectrum triazoles, and echinocandins have all been or are being studied for their utility as a prophylactic antifungal in various high-risk populations. All studies to date have confirmed our ability to prevent invasive candidiasis; however, the data regarding preventing proven mold infections and reducing fungal-related mortality are more controversial, as discussed in detail in the next section.

Treatment of invasive candidiasis

Multiple antifungal drugs have been made available and shown to be effective in treating invasive candidiasis. These drugs have different mechanisms of actions and different toxicities and drug interactions, as outlined in Figure 86.2 and Table 86.1. In general, the utility of each is specific to the patient with the infection, and the predicted (or measured) susceptibility of the causative *Candida* species.

The utility of fluconazole for the primary treatment of candidiasis is relatively limited in this population owing to extensive prior exposure that selects resistant organisms, and the lack of robust clinical trial data. Although studies have shown that fluconazole is not inferior to amphotericin B deoxycholate and echinocandins for treatment of candidiasis, these studies enrolled

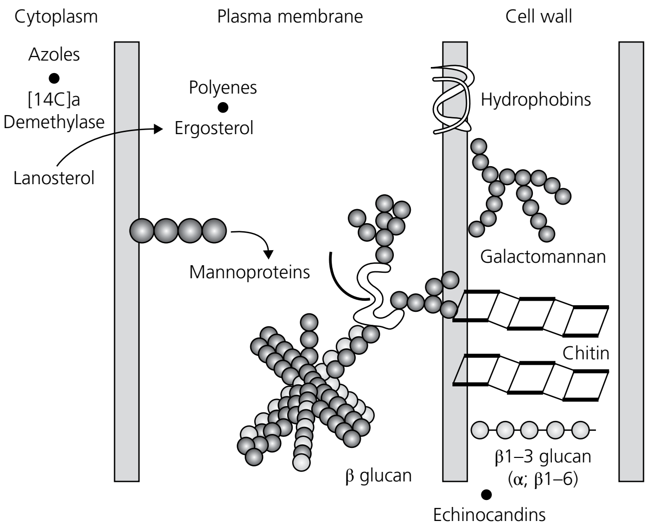

Figure 86.2 Schematic mechanisms of actions of three current classes of antifungal drugs.

Table 86.1 Systemic administered antifungal agents

Antifungal agent	Spectrum	Route	Most common toxicities	Drug interactions
Polyenes				
Amphotericin B	Yeasts and molds	IV	Infusion-related, nephrotoxicity, hypokalemia, hemolysis	Concomitant administration of other nephrotoxic agents increases risk for Ampho-induced renal failure
ABCD	Yeasts and molds	IV	Infusion-related, nephrotoxicity	Less potential for nephrotoxicity than Ampho B
ABLC	Yeasts and molds	IV	Nephrotoxicity	Less potential for nephrotoxicity than Ampho B
Liposomal amphotericin	Yeasts and molds	IV	Nephrotoxicity	Less potential for nephrotoxicity than Ampho B
Azoles				
Fluconazole	Yeasts	IV	Hepatic	Decreases fluconazole levels: rifampin Fluconazole increases levels of CSA, phenytoin, glipizide, glyburide, warfarin
		PO		
Itraconazole	Yeasts and molds	IV	Hepatic, hypokalemia, edema	Decrease itraconazole levels: increased gastric pH, rifampin, isoniazid, phenytoin, carbamazepine, cisapride
		PO capsules	GI, hypokalemia, edema (hepatic)	Itraconazole increases levels of: CSA, tacrolimus, steroids, digoxin, astemizole, cisapride, warfarin, vinca alkaloids, busulfan
		PO solution	GI, hypokalemia, edema (hepatic)	
Voriconazole	Yeasts and molds	IV	Visual disturbances, hepatic, neurologic, phototoxicity	Similar to fluconazole except that metabolism via CytP4502C19 introduces genetic variability in levels
		PO	As above	As above
Posaconazole	Yeasts and molds	PO	GI, hepatic	Similar to itraconazole
Echinocandins				
Caspofungin	Yeasts, less so molds	IV	Hepatic	Increases caspofungin levels: CSA
Micafungin	Yeasts, less so molds	IV	Hepatic	Increases levels of sirolimus, nifedipine, itraconazole
Anidulafungin	Yeasts, less so molds	IV	Hepatic, histamine-like reactions	CSA modestly increases anidulafungin levels

ABCD, amphotericin B colloidal dispersion; ABLC, amphotericin B lipid complex; Ampho B, amphotericin B; bid, twice daily; CSA, cyclosporine; GI, gastrointestinal; IV, intravenous; PO, oral administration.

largely immune-competent (non-neutropenic) patients [23–25]. Treatment guidelines generated by the Infectious Diseases Society of America (IDSA) recommend primary therapy with an echinocandin when cultures are not yet identified to the species level; a switch to an active azole drug (fluconazole or voriconazole) can be considered with stabilization, given the IV requirements of echinocandins [26]. This approach, with an increased utilization of echinocandins for the treatment of candidemia, has been endorsed in multiple medical centers. Data generated by systematic reviews suggest potential benefits, with echinocandin-based primary therapy, with the exceptions noted for organisms that have relative resistance to these drugs [27].

One of the treatment questions that has arisen with the introduction of multiple antifungal drugs concerns the utility of susceptibility testing to guide therapy. Although this question is largely beyond the scope of this chapter, it is useful for all clinicians to recognize that drug choice should be tailored to the predicted susceptibility of the causative species. For instance, *C. glabrata* is a species that may become resistant to fluconazole over time, largely by expression of cellular efflux pumps [28]; use of an azole for primary therapy of severe disease should be approached cautiously. *C. krusei* is never susceptible to fluconazole, but it is sensitive to voriconazole, and also other options (echinocandins). Other species, especially *C. parapsilosis*, appear to have innately decreased susceptibility to echinocandin drugs, and studies have shown that others can become resistant to echinocandins over time, by mutation of a gene involved in the drug target [29].

Current recommendations are to continue an active antifungal agent for at least 2 weeks after clearance of blood cultures [26]. These recommendations are based on the observation that the organism may seed other sites without presenting signs of infection, and this can become clinically apparent later, if antifungal therapy is discontinued prematurely. One site that is important to evaluate is the eye; as discussed above, chorioretinitis, with or without vitreal involvement, can be common, and noting this development is important both to guide appropriate choice of antifungal drugs and for consideration of surgical therapy. Both fluconazole and voriconazole have good distribution into the eye, but echinocandin drugs do not. Vitrectomy, or injection of an antifungal into the vitreous humor, are important considerations to resolve infection, so ophthalmology should be involved in the care of these patients.

Candidemia can originate from an intravascular catheter or secondarily seed a catheter in the setting of high fungal burden. For this reason, historic recommendations have been that catheters should be removed from infected individuals. It has been noted that in the absence of either randomized or clear observational data in this subset of patients who usually acquire infection through the gut, this recommendation may not be appropriate [30]. Current recommendations are to remove the catheter, *when feasible*. This obviously is a highly individualized decision in people with hemodynamic compromise. Resolution of symptoms (primarily fever) following the removal of the central venous catheter does not obviate the need for antifungal therapy.

Treatment of hepatosplenic candidiasis follows a similar rationale as that described above, and multiple drugs have utility, including amphotericin products, azoles, and echinocandins. There are a few things to note. First, it is rare to have microbiologic confirmation to the species level, so a regimen that is broader than fluconazole is usually a good place to start. That said, *C. albicans*, the species that can most effectively invade the gut into the portal vasculature, is the most common cause of disease and fluconazole may be appropriate maintenance therapy, especially for people who have no prior exposure to azole-based prophylaxis during neutropenia. Also, one of the major therapeutic issues in treating hepatosplenic candidiasis concerns the duration of therapy, as patients typically have protracted courses of fever. Prolonged symptomatology can lead to frequent changes in antifungal regimens and increased, unnecessary toxicities, as the fever can be largely driven by inflammation rather than persistence or growth of the fungus itself. For this reason, investigators have recently questioned the utility of anti-inflammatory agents in augmenting therapy in this setting. Adjunctive corticosteroid therapy has been preliminarily evaluated for this entity, frequently referred to as an “immune reconstitution syndrome,” but definitive trials have not been performed [31,32].

Table 86.2 Host and therapeutic risks for invasive aspergillosis (IA) after allogeneic HCT

Variable	“Early” IA[a]	“Late” IA[a]
Host	Advanced age	Advanced age
	Underlying disease: other than CML	Underlying disease: MM
	CMV seropositive, disease	CMV seropositive, disease
	Prior IA	Prior IA
	Polymorphisms in innate immune genes	Polymorphisms in innate immune genes
Transplant	Bone marrow, cord blood	Peripheral blood, T-cell depletion
	Myeloablative conditioning	Non-ablative conditioning
	Donor TLR4, dectin-1 polymorphisms	Donor TLR4 polymorphism, D$^+$/R$^-$
	Hospital exposure, airway colonization	Ambient exposure, season
Complications	Poor engraftment	GVHD and therapies
		CMV, respiratory virus infections
		Secondary neutropenia, lymphopenia
		High iron stores

CML, chronic myelogenous leukemia; CMV, cytomegalovirus; D$^+$, seropositive donor; MM, multiple myeloma; R$^-$, seronegative recipient; TLR, Toll like receptor.

[a] “Early” and “Late” defined as in earlier studies, at approximately day 40 cut-off.

Infections caused by *Aspergillus* species

Mycology and epidemiology

Aspergillus spores are thermotolerant and abundant in the environment. Exposure to spores likely occurs all the time, as they are found throughout Nature, particularly associated with decaying vegetation and soil, barns, compost piles, and plants; exposure is primarily through the airway, with normal inhalation or smoking (tobacco or marijuana). *Aspergillus* species, particularly *A. niger*, can also be ingested in foods and are found in peppers and spices. Filamentous fungi, including *Aspergillus*, have also been isolated from water supplies, likely as they can grow as biofilms [33].

Most cases of invasive aspergillosis (IA) are caused by *A. fumigatus*, although other species, particularly *A. flavus* and *A. terreus*, also cause disease. The marked predominance of *A. fumigatus* may be explained in part by its ubiquity in Nature, thermotolerance, and rapid growth rate, and also multiple “virulence” characteristics, as briefly discussed below. Recently, it was recognized that *A. fumigatus* is not one species, but contains multiple different “cryptic species” [34]. Although these organisms appear very similar and are frequently identified as *A. fumigatus* based on morphologic characteristics, they are genetically unique and exhibit differences in growth features that may be clinically important. For instance, *A. lentulus* typically exhibits higher resistance profiles to different antifungal drugs. This is discussed in depth in the therapy section below; it is important here only in clarifying that based on genetic analyses, many of the previously considered single species are considered “groups,” and some are being given new names [35–37].

Historically, IA was thought to be an infection that occurred primarily in hospitalized patients during neutropenia, and focus was placed on potential sources of nosocomial transmission, in “outbreak” settings. However, in the 1990s, important epidemiologic studies outlined the observation that increasing numbers of cases were occurring in HCT patients, with a bulk of diagnoses established after engraftment, during periods of severe GVHD or with T-cell depletion [1,38]. Thus, “late” cases illustrate the complexity of host defenses to these organisms. It also emphasizes the importance of environmental, non-hospital sources of exposure. In fact, environmental exposure, which appear to be geographically variable, possibly accounts for differences in observed rates of IA. Recent studies have shown that ambient quantities of *Aspergillus* spp. (and other filamentous fungi) are variable according to season in some geographies, corresponding to weather changes (both temperature and precipitation) [39]. Suffice it to say that exposure appears to be a complex variable that differs between transplant centers, dictated by hospital sources and also environmental variables that influence the amount of filamentous fungus in the air.

An individual’s quantitative risk for IA is complex, and dictated by host and treatment variables that impact the immune response at the level of airway conidial clearance, local pulmonary immune responses, and systemic immunity. With this in mind, host genetics, pulmonary function, type of underlying diseases and prior therapy, conditioning regimens, stem-cell sources, and numerous complications, including GVHD (and treatment), and other infections [e.g. cytomegalovirus (CMV)] serve as clinical risks for IA during either early and/or late risk periods after allogeneic HCT. Some risks are detailed in Table 86.2.

Multiple studies have attempted to determine the actual observed incidence of IA in HCT recipients. While large single-center studies have typically reported relatively high incidences of pulmonary aspergillosis, some more recent multicenter studies did not support these findings. In the TRANSNET study, the measured cumulative incidence of IA following allogeneic HCT was less than 5%, and the incidence of IA following autologous HCT was less than 1% [6]. Another multicenter observational study performed in the United States (the PATH Alliance) reported similar low rates [40]. However, the latter study produced a very important observation, namely that there is a wide variability in IA reported by different centers; in that study, the bulk of cases in either allogeneic or autologous HCT recipients were reported from just two centers. This wide variability is likely the result of differences in geography and also a large amount of diagnostic bias that results from differences in the utilization of bronchoscopy to confirm diagnosis, and variable long-term follow-up case capture. It is important to note that neither of these £prospective surveillance” studies employed active surveillance or event-driven data capture. Many questions about the epidemiology of IA remain.

Pathogenesis and immunity

These organisms are typically acquired by inhalation of conidia, which are produced by sporulating molds in the environment; the small size and hydrophobic coating of these spores facilitate efficient aerosolization deep into the airway, and immune evasion [41]. The inoculum necessary to cause disease and the time of exposure relative to disease onset are largely unknown and likely different in individual people. Patients may be colonized for some time before development of disease with subsequent immunocompromise.

Conidia that escape mucociliary defenses or uptake by airway epithelial cells or local phagocytes can germinate into hyphae, which are the tissue-invasive form of the organism. Soon after tissue invasion, the hyphae are damaged and destroyed by neutrophils. A variety of different antigens are expressed during the morphologic changes between conidia and germtubes; probably the most important appears to be morphologic transitioning of β-glucan exposure. This is exposed on the cell wall after the hydrophobic coat of conidia sloughs off, resulting in effective recognition by phagocyte dectin-1 receptors, which drives differential inflammatory responses and the nature of resultant adaptive immunity [42].

Hence the presence and/or functioning of neutrophils represents only one, rather late response to *Aspergillus* species. There are numerous interactions proximal, and coordinate with polymorphonuclear neutrophil (PMN) responses, which involve effective recognition and clearance of conidia, and they dictate the quantity and nature of both local and systemic inflammatory responses and T-cell immunity. The importance of the latter in generating effective, sustained protection has been shown in both animals and humans. For example, DCs have a pivotal role in the defense against aspergillosis in mice and humans [43]. In addition to phagocytosing both conidia and hyphae, DCs migrate to the lymph nodes and spleen, influencing T-cell reactivity [44]. Multiple animal and clinical studies have consistently demonstrated that the specific nature of the resultant T-cell reactivity influences the host's ability to resist IA, with T_H1, T_H2, and T_H17 type of responses impacting both effector activity and potentially pathologic inflammatory responses.

The importance of cellular immunity has triggered multiple animal and human studies to explore not only risks, but also the potential utility of therapies focused on immune modulation in this unique population. In one study, lymphoproliferative responses to *A. fumigatus* antigens were more robust in healthy individuals and patients with aspergillosis who were felt to have a favorable response to antifungal agents than in patients with progressive infection [45]. Some unique *A. fumigatus* antigens may have utility in either vaccine strategies or cellular immunotherapy [46,47]. Adoptive transfer of pathogen-specific, non-recipient reactive lymphocyte clones resulted in sustained high-level IFN-γ, low levels of IL-10, and reduced mortality due to IFI in 35 recipients of haploidentical HCT [48]. This is an area of increasing understanding and exploration.

Genetic variability in innate immunity impacts risks in the transplant setting. For instance, polymorphic variation in donor Toll-like receptor 4 (TLR4) DNA confers high risks for IA in HLA-matched related HCT recipients; this risk is enhanced if the donor and/or the recipient are seropositive for CMV [49]. Other studies have associated risks for IA with polymorphisms in dectin-1, TLR1, TLR6, CXCL10, and IFN-γ genes [50–53]. Although functional phenotypes are not yet defined, and polymorphic variation likely influences the development of other HCT complications (e.g. GVHD), one may envision that these observations could eventually inform targeted prevention strategies.

The hallmark of *Aspergillus* disease is angioinvasive pulmonary infection that can result in hemorrhage, infarction, and development of lung necrosis. Angioinvasion can also result in hematogenous seeding. This classic description is much more common in people who have baseline neutropenia, which permits a higher pulmonary fungal burden; another entity, which is more consistent with progressive pulmonary inflammatory responses, or localized airway disease, has also been observed both in animal models and in HCT recipients. The distinction between airway aspergillosis and angioinvasive aspergillosis may be predictable based on both underlying diagnosis and leukocyte count [54]. Some investigators have suggested that there is a unique "immune reconstitution syndrome" that can develop in non-neutropenic hosts, with implications on diagnostic test performance [55]. More studies are necessary to understand the natural history of diseases in different hosts in order to inform diagnostic and therapeutic approaches.

Clinical manifestations

As the biology of *Aspergillus* would predict, the most common sites of inoculation are the airway and paranasal sinuses; however, disease can be acquired from and involve other sites exclusively, including the GI tract and the skin.

Sino-pulmonary aspergillosis

Both allergic and invasive diseases impact the sino-pulmonary tract. Invasive disease ranges over a spectrum that extends from manifestations isolated to the airways (e.g. tracheobronchitis) to lung parenchymal invasion, with potential for extrapulmonary dissemination.

Aspergillus tracheobronchitis is an entity that has been previously reported to occur in people who have large airway exposures concurrent with inappropriate local responses, such as that occurring in HIV-infected people who smoke marijuana. More recently, it has been reported in people with hematologic malignancies [56]. The radiographic pattern of tracheobronchitis is not "typical" of what clinicians have historically considered related to *Aspergillus* species; the entity is likely under-diagnosed. These patients typically present with airway reactivity, persistent cough, and tracheobronchitis, with characteristic findings on CT scan (Figure 86.3a). Sputum samples may persistently yield colonies of mold – often more than a few colonies – but negative cultures do not rule out this diagnosis. Bronchoscopy should be considered in patients with unexplained or chronic cough, especially when symptoms are refractory to antibacterial therapy. A variety of bronchoscopic and post-mortem findings have been described, including pseudomembranes, plaques, and balls of fungus. Risks for this type of airway disease have not been well defined; anecdotally, these patients are typically not neutropenic, and have conditions that predispose to poor airway conidial clearance, such as chronic lung disease, potentially with habits that predict high airway exposures (e.g. smoking). Other infections limited to the lower airways include laryngitis and tracheitis. The invasive potential for tracheobronchitis is documented by the detection of *Aspergillus* antigen in the blood prior to initial clinical presentation, and the subsequent development of parenchymal lesions [57].

The term "aspergillosis" is typically used to refer to a large number of invasive pulmonary manifestations. When extended

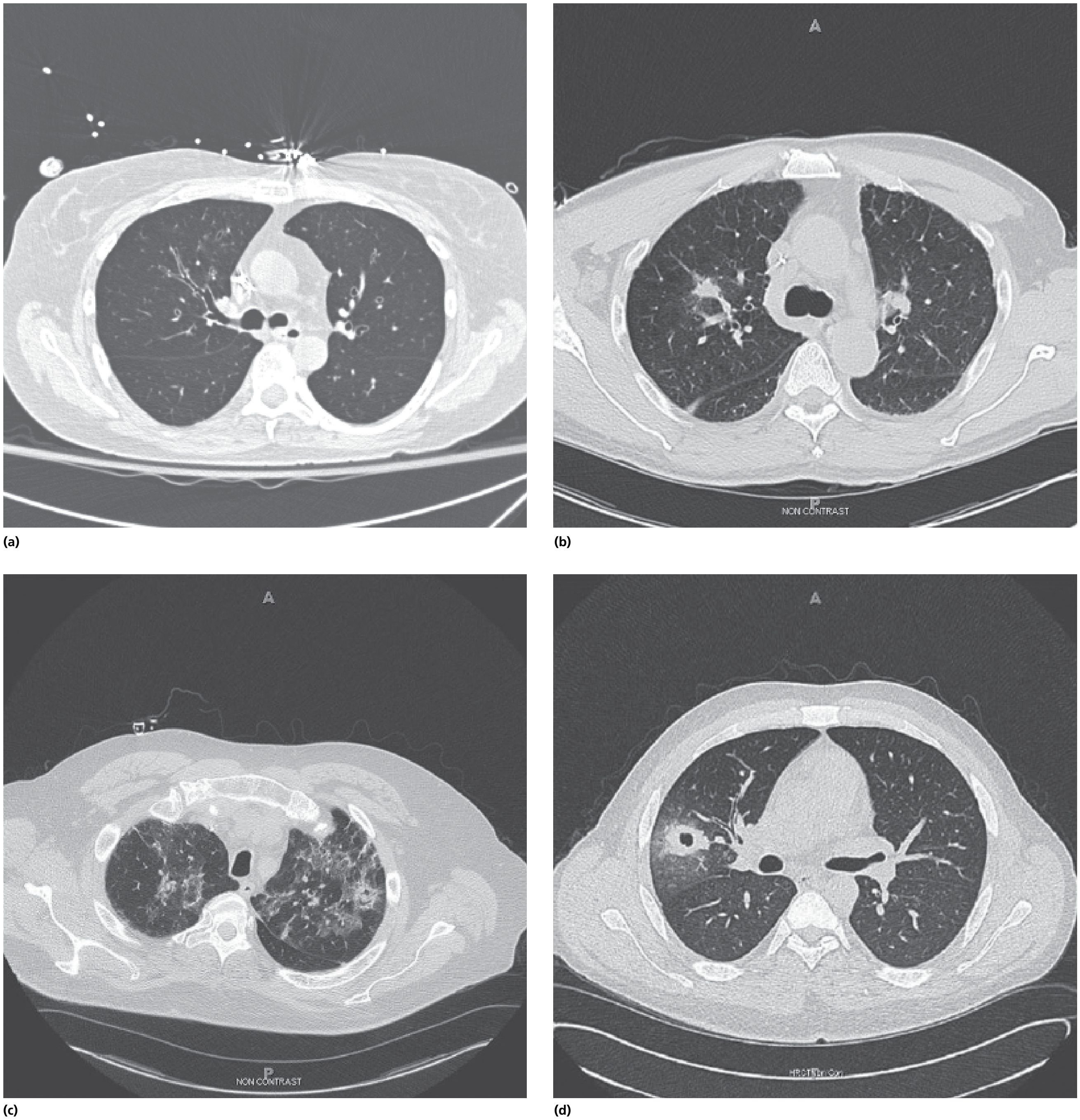

Figure 86.3 Variable radiographic appearances of documented aspergillosis in HCT recipients.(a) CT scan showing thickening and dilation of airways in an allogeneic HCT recipient with bronchoscopy-confirmed *Aspergillus* tracheobronchitis. (b) A more typical presentation of focal nodular IA. (c) Scattered nodular lesions with more ground-glass appearance and early development of a cavity. (d) A typical cavitating lesion.

beyond the airways, these organisms can be recognized to cause isolated pulmonary nodules, focal pulmonary infiltrates, or even more scattered "ground-glass" opacities (Figure 86.3b–d). Histopathologically, this disease is characterized by parenchymal hyphal invasion, which can elicit a large amount of local inflammation, hemorrhage, and eventually evolve towards necrosis. This is the genesis of radiographic findings that are frequently considered indicative of aspergillosis, with a radiographic halo produced with inflammation and hemorrhage, and cavitation developing in areas of necrosis. It is important to distinguish between an "aspergilloma" that occurs in this setting, as a result of the end-product of invasive disease, from the "aspergilloma" that can occur in a different, potentially less immunosuppressed host. In the latter, the term has been used to indicate the saprophytic overgrowth of *Aspergillus* spp. that occurs in the setting of prior lung cavities.

The presentation of IA has been historically described as most often involving fever, cough, and potentially, pleuritic chest pain. Although all of these can indicate disease, pleuritic findings appear to be more common in neutropenic patients who have some involvement of the pleura (potentially with effusion). In HCT recipients, findings may be non-specific and protean, and may not even indicate potential involvement in the lungs at all. On the other end of the spectrum are people who present with disease that has already disseminated to other organs, such as the skin or brain. As disease progresses in the lungs, it can involve adjacent structures, including the pleura, pericardium, and myocardium.

Sinusitis is usually symptomatic and not distinguishable from sinusitis due to other pathogens. Signs and symptoms suggestive of an invasive process include erosive lesions of the nasal septum, cavernous sinus thrombosis, pain with eye movement, extraocular muscle plegias, diplopia, and periorbital swelling; as this reflects late disease, with anticipated poor outcomes, a high index of suspicion is required to detect earlier fungal involvement.

Non-pulmonary aspergillosis

Aspergillus species can cause both focal infection at isolated non-pulmonary sites, and cause disease after hematogenous spread. The classic non-pulmonary site of focal infection is with cutaneous disease at sites of skin trauma, including catheter insertion. Skin lesions typically start as focal lesions, and necrosis is characteristic. If the lesion is not at a site of known trauma or there is more than one lesion, the possibility of hematogenous spread from an intravascular focus of infection including endocarditis should be considered. Dissemination from the lungs to virtually every tissue has been described, including the thyroid, kidneys, eyes, and GI tract. Classically, the worst prognosis has been observed in people who present with concurrent disease in the CNS; however, recent studies have shown that outcomes appear to be improving with voriconazole-based therapies [58].

GI involvement may result from direct invasion from the gut lumen, and can present as focal colitis. Any region of the GI tract may be involved, and a variety of findings, including diffuse infiltration, necrotizing lesions, and pseudomembrane formation, have been described. A recent retrospective study noted that a large number of patients with observed gut aspergillosis had diets that were high in spices, suggesting potential direct exposure from food products [59]. The presence of isolated GI disease should be considered in people who have persistent high galactomannan assay results in the absence of any identified pulmonary disease.

Diagnosis and therapy

Diagnosis

CT scans are important to establish an early diagnosis of IA, as they are more sensitive than chest X-ray [60]. Parenchymal lesions due to *Aspergillus* spp. may appear radiographically in multiple forms, including nodules, with or without a circumferential hemorrhage (often described as a "halo"), focal infiltrates, or lesions with necrosis (cavitation), or a combination of all of these. Radiographic findings may be highly variable following HCT, given differences in risk periods corresponding to neutropenia and/or defects in cellular immunity (Figure 86.3). In a systematic review of high-resolution CT findings in patients with pulmonary infection after HCT, the presence of large nodules and visualization of the halo sign were most suggestive of fungal infection, but these were not specific. For example, nodules that were 1 cm or more in diameter were seen in 13 (62%) of 21 patients with fungal pneumonia, but also in five (19%) of 26 patients with bacterial pneumonia, three (10%) of 30 with respiratory syncytial virus pneumonia, and three (14%) of 22 with CMV pneumonia. The halo sign was present in 10 of 21 patients with fungal pneumonia, two of 26 with bacterial pneumonia, three of 30 with respiratory syncytial virus pneumonia, and one of 22 with CMV pneumonia [61]. Also, focal infiltrates appear more common in the non-neutropenic period after HCT [62]. It has been observed that this type of "bronchopneumonic" aspergillosis may have a worse prognosis than IA compared with the classic angioinvasive presentation, although many different host-related factors also likely impact the outcomes [62].

Several studies have now documented that bronchoalveolar lavage (BAL) is both safe and assists treatment decisions. In a large review of 598 BALs performed in 501 HCT recipients at the M.D. Anderson Cancer Center, the overall yield for identification of pathogens was 55%, with a higher yield recovered from BAL performed during the first 4 days of presentation (75%). This information resulted in adjustment of antimicrobial therapy in 51% of patients, and the complication rate associated with the procedure was very low (<1%) [63]. Given the complexity of treating infectious complications in the current era of multiple drug resistance and numerous antimicrobial options, an aggressive diagnostic approach is wise.

Historical means to diagnose IA have relied on culture of the organism and identification of elements via histopathology; however, the sensitivity of culture of any respiratory sample – sputum or BAL – and the sensitivity of biopsy-based diagnosis remain very low, approximating 50% or less depending on the stage of disease. For this reason, the field has been focused on development of non-culture based methods to enhance detection of *Aspergillus* spp., using both nucleic acid and antigen detection. Numerous PCR-based tests have been developed and studied, with variable performance; some are in use on blood and/or tissues (including BAL) in different institutions.

A major advance in diagnosis has also been witnessed with more global adoption of antigen-detection strategies, especially those that detect galactomannans (GMs). GMs are cell wall components of multiple fungi, and the basis of numerous diagnostic tests, including those for IA (discussed here), and other infections (e.g. histoplasmosis). The current assay that is available in most countries is one that utilizes a rat monoclonal antibody in a double-sandwich enzyme-linked immunosorbent assay (ELISA) to detect *Aspergillus* GM. It has been studied extensively in patients with hematologic malignancies and in HCT recipients, and cleared by the FDA for use on blood and in BAL fluid. An advance in the performance of the assay was established when the cut-off to define positivity was lowered in order to achieve better sensitivity, with little compromise in specificity. A meta-analysis of 27 studies estimated that the sensitivity and specificity of the assay utilized on serum approximate 71% and 89%, respectively [64], although sensitivity is lower in people who are receiving mold-active antifungal therapy [65]. Testing for the presence of GM in BAL fluid is also very useful, particularly in people who have a high pretest probability of IA (the HCT population); studies estimated that there is a gain in sensitivity over culture alone [66,67]. This assay detects GM that is present from *Aspergillus* or related filamentous organisms, but there is some cross-reactivity caused by GM (or GM-like molecules) of other fungi. Also, false-positive assays can occur with concurrent infusion of "contaminated" infusates; several years ago, this appeared to be a particular problem with batches of piperacillin–tazobactam that were used worldwide, although this issue appears to be relatively resolved [68,69]. Higher levels of false positives in pediatric patients were historically reported, but more recent prospective studies demonstrated similar performance to that reported in adults [70]. Although not perfect, testing for GM in serum and/or BAL of people with suspected IA provides a useful adjunct to culture, and it should be performed. Results neither rule in nor rule out invasive disease, but both positive and negative test findings are informative.

Other investigators have suggested that GM and/or PCR assays can be used as screening assays to detect fungal components prior to the development of clinical signs and symptoms of disease. This approach, frequently termed "preemptive therapy," has been reported to result in earlier diagnoses, and more targeted use of antifungal drugs, and it is used in some centers, particularly in Europe [71]. Large randomized trials have not been completed to analyze clinical performance as yet, but some are under way. Preliminary results of a trial recently completed in Australia suggest that a combined screening with PCR and GM enzyme immunoassay can result in use of less antifungal drugs [72]. Optimization of these types of screening strategies is probably necessary before more widespread adoption in HCT centers.

Preventative therapy

Central to the prevention of aspergillosis is the avoidance of inhalation of spores. Given published associations, avoidance of contact with soil and plants, smoking, gardening, and maintenance of compost piles is prudent, but not demonstrated to have an impact on the rate of disease. The efficacy of wearing high-efficiency particulate air (HEPA)-filtered air masks has also not been proven in controlled studies but is a reasonable consideration. Behavioral modifications that result from wearing the mask may theoretically decrease the transmission of other infections such as those due to respiratory viruses, especially as an outpatient. Unfortunately, a small randomized trial failed to show that masks had utility [73]. In the inpatient setting, laminar airflow rooms and HEPA-filtered air may be beneficial. Although it is noteworthy that *Aspergillus* has been isolated from water, it is not clear whether avoidance of showers results in clear benefit. Evidence and guidelines for preventing infections have been reviewed by the American Society for Blood and Marrow Transplantation (ASBMT) [74].

Prophylaxis studies have been performed to evaluate multiple mold-active azole drugs (itraconazole, posaconazole, and voriconazole). The most promising results have been with the newer azole formulations posaconazole and voriconazole, which decrease the risk for invasive aspergillosis compared with fluconazole [75–77]. However, none of these studies showed improved survival, creating controversy over the utility of administration. Results of these studies are discussed in more detail here. While treatment of fever during neutropenia, or "empiric antifungal therapy," may also be considered preventive therapy for unrecognized IA, these studies are not discussed, given the complexity in interpretation of fever responses and outcomes. Readers are referred to reviews for more in-depth discussion of preventive therapies [20,78].

Itraconazole was the first mold-active azole that was studied and adopted as a prophylactic agent in this setting. Two randomized trials in allogeneic HCT recipients were performed in the United States after both the oral solution and IV forms of the drug became available. Both studies demonstrated that itraconazole administration lowered the probability of IA compared with fluconazole; however, both studies noted trends to more toxicities and lower survival in the itraconazole arm [79,80]. In one study, concurrent administration of itraconazole during cyclophosphamide-based conditioning therapy resulted in more toxicities compared with that which occurred in people who received concurrent fluconazole [80]. Detailed evaluation of metabolites suggests that this is actually the result of a relative "protective" effect of fluconazole, which selectively inhibits the P450 isoenzyme that is involved in hydroxylation of cyclophosphamide [81]. Collectively, the results of these studies suggested that prevention of IA is possible, but itraconazole is not optimal given the toxicities.

Subsequent studies evaluated the utility of the next-generation azole drugs, posaconazole and voriconazole. In a multicenter randomized trial, posaconazole decreased the incidence of IA compared with fluconazole, when administered at onset of GVHD [76]. Limitations on the use of this drug come from issues with absorption of the current formulation, which requires a slowed transit time in the small intestine, with low levels resulting in patients with severe gut GVHD [82]. A new formulation has been evaluated in phase II–III studies, with results suggesting better levels. Another multicenter study was performed in the United States, to evaluate voriconazole compared with fluconazole. This study, performed by a US consortium of bone marrow transplantation (BMT) centers (the BMT Clinical Trials Network), had a different design, with initiation of drugs pre-engraftment, and adoption of a different primary end-point, fungal-free survival (survival without an IFI) [79]. Although this study failed to show superiority of voriconazole when considering 6-month post-HCT fungal-free survival, evaluation of infection end-points again favored the mold-active drug compared with fluconazole, with a lower incidence of IA. Currently, many large centers still use fluconazole during the pre-engraftment period and then transition to either voriconazole or posaconazole with treatment of GVHD.

Other studies have been performed to evaluate echinocandins (micafungin) and lipid–amphotericin B formulations, using different routes and dosing. However, none of these studies was adequately powered to say anything definitive with regard to utility in preventing IA. The azole drugs appear to provide the most compelling potential for primary prophylaxis, given robust data and efficacy and relatively predictable toxicities, although many issues have been raised. For instance, long-term administration of voriconazole has been associated with phototoxicity that can masquerade as GVHD [83], and has been associated with skin cancers. Breakthrough fungal infections occur during administration of all azoles, sometimes associated with innate microbial resistance (e.g. zygomycetes resistance to voriconazole), and acquired resistance [84]. For instance, select institutions have reported variable but high rates of *Aspergillus* isolates that demonstrate relative resistance to azole drugs, with mutation in the target enzyme [85]. Transplant clinicians should recognize the potential for breakthrough infection associated with both microbial resistance and "failure" of effective drug delivery in chronically immunosuppressed people.

Therapy of invasive aspergillosis

Amphotericin B formulations comprised essentially the only treatment options for IA until the performance of a large randomized trial that demonstrated superiority of voriconazole compared with amphotericin B deoxycholate [86]. This study, which enrolled patients over a decade ago, showed that the 12-week survival rate after receiving voriconazole was better than after initiation of conventional amphotericin B; as predicted, a large number of patients in the conventional amphotericin arm developed infusion-related and nephrotoxicities that initiated a switch to a more tolerable formulation. One of the most notable observations was that 24% of patients with CNS aspergillosis survived – a proportion that is significantly better than what was historically observed [86]. This trend to improved survival with CNS disease has been upheld in subsequent observational studies [86].

Two large randomized trials were performed subsequently to address treatment of IA. One compared a low dose (3 mg/kg/day) of liposomal amphotericin B with a higher dose (10 mg/kg/day) [87]. The results did not show any difference in either clinical responses or survival, not supporting the high-dose regimen. At this point, the study that would be of most interest is a comparison of an azole-based (voriconazole) regimen with the liposomal amphotericin formulation. However, such a study has not been performed, so there is remaining controversy over comparative efficacy using either primary treatment option. A consensus panel convened by the IDSA suggested that voriconazole is the primary therapy of choice – at least when the diagnosis of disease due to *Aspergillus* species is confirmed [78].

The second large randomized trial performed to evaluate treatment of IA was a recently completed randomized, placebo-controlled trial that evaluated voriconazole monotherapy in comparison with the combination of voriconazole with anidulafungin, an echinocandin. Performance was supported by in vitro data, animal models, and case series that suggested potentially improved outcomes using the combination of drugs. Results have been presented in abstract form [88]. The study was different from earlier trials in that it only enrolled patients with hematological malignancies and/or HCT, and evaluated a primary end-point of 6-week survival, rather than the composite variable previously utilized to define success. The modified intent-to-treat (mITT) population included 277 patients with a diagnosis of proven or probable IA. Most patients (218/277, 78.7%) had probable IA based on positive GM antigen with concurrent radiographic findings. In the mITT, all-cause mortality at 6 weeks was 26/135 (19.3%) for combination and 39/142 (27.5%) for monotherapy [$p = 0.09$, 95% confidence interval (CI) -19.0 to 1.5]. In patients with probable IA based on GM antigen, mortality at 6 weeks was 17/108 (15.7%) for combination and 30/110 (27.3%) for monotherapy ($p < 0.05$, 95% CI -22.7 to –0.4). Safety parameters showed no significant differences between treatment groups. Based on these data, the relative enthusiasm for combination therapy has increased; however, interpretation of the results of this study, in defining whether primary combination therapy is of benefit to everyone, is a matter of active debate.

Multiple issues need to be discussed with regard to the use of voriconazole. First, dosing of the drug is a primary issue; the oral dose of 200 mg twice daily, appears insufficient for a large proportion of patients. Many have gone to weight-based dosing of both the IV and the oral formulation, and there is likely to be a role for therapeutic monitoring of drug levels to guide therapy [89]. Recently, the focus has been on other toxicities of the drug, especially the tendency towards photosensitivity and development of skin cancers. A variety of neurologic disturbances have been described, including altered or enhanced visual perception, blurred vision, changes in color vision, and photophobia. They may persist throughout the course of therapy; however, no long-term visual sequelae have been noted in animals or in clinical trials. Two randomized trials comparing other agents, posaconazole and isavuconazole, are in various stages of enrollment.

Utility of azole drugs as primary therapy for IA appears to be threatened in some geographic locations by observations of increased issues with microbial resistance. As mentioned previously, several members within the *Aspergillus fumigatus* group of organisms (e.g. *A. lentulus*) demonstrate relatively high in vitro susceptibilities to multiple drugs, and *A. fumigatus* itself has been shown to acquire mutations that confer relative resistance. The latter observation has been particularly frequent in geographic locales in which large amounts of azoles are used in agricultural practices, such as in The Netherlands, potentially explaining the variability in the phenomenon [85]. Other species, such as *A. ustus*, demonstrate variable and low susceptibility to both azoles and amphotericin B, and *A. terreus* appears relatively resistant to amphotericin specifically, with better outcomes observed with voriconazole. While there is much to learn, one needs to emphasize the relative importance of establishing a microbiologic diagnosis to guide treatment decisions.

Other issues that arise when considering treatment of IA include the utility of immune modulating therapy and the need for surgical resection of lesions. A number of in vitro studies have confirmed the salutatory effect of macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), and granulocyte–macrophage colony-stimulating factor (GM-CSF) on cellular response to fungus and the shortening of the neutropenic period following chemotherapy and transplantation of stem cells. GM-CSF has been shown to enhance the killing of *Aspergillus* hyphae and *Candida* spp., and also to prevent the suppression of neutrophil killing of *A. fumigatus* hyphae resulting from steroid administration [90]. Despite these findings, there has yet to be a definitive study documenting the reduction in incidence of or mortality due to IFIs. Changes in the graft composition have shortened the time to neutrophil engraftment, which has resulted in a significant reduction in the incidence of IFIs during the early post-transplant period. Other studies have evaluated the potential safety and efficacy of granulocyte transfusions, with small case series resulting in equivocal data [91,92]. Numerous other approaches are under investigation, including the use of "educated" T cells, pulsed DCs, and vaccines, but none has led to widespread clinical use to date.

The role of surgery as an adjunct for treatment of pulmonary IA is somewhat controversial. Small series have been published describing survival following both open and thoracoscopic approaches in the excision following HCT, even in the setting of GVHD, but success is not universal. Caution is necessary when lesions are adjacent to the pleura, as complicated empyemas can result from surgical disruption. Current recommendations are to consider resection in patients who are deemed acceptable surgical candidates with predominant, large lesions that are abutting vascular structures or are presenting complications with airway management, such as in the setting of contiguous necrosis with the airway or with hemorrhage. Surgical debridement is almost invariably necessary for the successful treatment of fungal sinusitis, especially with involvement with local structures.

Less common fungi

Pneumocystis jirovecii

Pneumocystis is another fungus that has attracted recent attention. Although this organism was once considered a protozoan, it was reclassified as a fungus based on genetic analyses. *Pneumocystis* causes disease only in mammals, and is species specific; whereas historical convention was to call the form *P. carinii*, the species that causes disease in humans is now appreciated to be *P. jirovecii* [93]. The natural history of disease is also becoming increasingly revealed. Most people appear to become exposed (and infected) with *P. jirovecii* early in life, and the organism can exist in the host as an airway "colonizer" for a long time. Disease may be due to either reactivation or de novo exposure, and genetic analyses of isolates recovered during outbreaks of disease in immunosuppressed hosts suggest the potential of human-to-human transmission [93].

Disease is usually limited to the lungs, although dissemination has been noted in multiple immunosuppressed hosts. The clinical manifestations have been best described in people with HIV infection; comparatively, people who have been transplanted (or are immune suppressed for reasons other than HIV) have relatively more heterogeneous manifestations, with focal in addition to diffuse disease recognized. Fungal burden in the lungs can be lower than in HIV-infected hosts, reducing the yield of diagnostic tests. An important contrast in this population is that the yields of antigen-based assays on BAL are lower than in the HIV-infected cohorts initially studied; recently, studies have suggested that serum 1,3-β-D-glucan assays have good diagnostic accuracy for *Pneumocystis* pneumonia (PCP) in many different types of immunosuppressed patients [94]. Effective prevention strategies are available, and the most effective option is trimethoprim–sulfamethoxazole (TM–SLF), which can prevent PCP with daily administration, or even staggered administration two or three times weekly [95,96]. Alternatives to TM–SLF include atovaquone, dapsone, and aerosolized pentamidine, although both pentamidine and dapsone are less effective than TM–SLF, and toxicities associated with dapsone (e.g. methemoglobulinemia and anemia) are significant [97,98]. Overall, TM–SLF is the mainstay for prevention, although *P. jirovecii* isolates with resistance to the drug due to mutations in the target enzyme gene dihydropteroate synthase have been appreciated as a cause of disease in different populations studied [99,100]. Finally, it is also important to emphasize the other "secondary" benefits associated with TM–SLF prophylaxis, as this drug may effectively prevent toxoplasmosis, pulmonary *Nocardia* infections, and infection with other susceptible organisms, such as pneumococcus [101–103]. Readers are referred to reviews for more detailed discussions on the pathogenesis, diagnosis, and treatment of PCP [96,104].

Other fungal infections

Reports of serious invasive infections caused by less commonly encountered fungi continue with apparently increasing frequency. These fungi are often referred to collectively as "emerging fungal infections," and the most commonly reported non-*Aspergillus* molds include Zygomycetes, *Fusarium* spp., and *Scedosporium* spp. [66–68]. Little is known about the epidemiology and treatment of infections due to these fungi following HCT. As these fungi often exhibit intrinsic resistance to many antifungal agents, this trend underscores the need for a detailed history of prior antifungal therapy, definitive identification of the pathogen, and appropriate use of antifungal susceptibility testing.

Zygomycetes, also referred to as the "agents of mucormycosis," are filamentous fungi that have attracted increasing attention because several epidemiologic studies performed in the early 2000s reported that they are being diagnosed at greater frequency as a cause of pulmonary disease [2,105,106]. Of particular concern was the observation that these organisms can cause "breakthrough" infection in people receiving voriconazole, either for prophylaxis or for treatment of invasive aspergillosis [107,108]. Disease and risks have been compared with aspergillosis in case–control studies; important findings include the observations that these organisms are more common in people with underlying diabetes, malnutrition, and prior voriconazole exposure [109]. These infections tend to occur later in allogeneic HCT recipients, in large part associated with complications of GVHD [6,40]. As infection can cause disease in both the sinuses and lungs, concurrent involvement should elicit suspicion for these organisms and direct antifungal therapy with an active agent [109]. As these organisms are particularly common in food and other ingested products (such as herbal medicines), disease localized to the GI tract has been reported fairly frequently. Diagnosis currently requires culture and/or histopathology, as there are no adjunctive diagnostic assays that are able to detect antigens in these organisms reliably (the GM and glucan assays do not detect these organisms). Therapy necessitates an amphotericin formulation, although some azoles, particularly posaconazole, have in vitro activity and have resulted in cure. Also, aggressive surgical debridement is a mainstay of successful therapy of invasive mucormycosis, as these organisms persist well in necrosis, are difficult to kill with antifungals, and cause a good deal of locally invasive disease. Readers are referred to reviews for further discussions of epidemiology, diagnosis, and therapy [110–112].

Fusarium species are the most common mold to be cultured from the blood, and hematogenous spread may be manifested by a stuttering appearance of skin lesions which are frequently erythematous and tender. As this organism can form "adventitial forms," or effectively sporulate, in vivo, disseminated disease is common [113]. Intrinsic resistance to multiple antifungal agents is notable, although successful treatment has been described most frequently with the use of combinations of drugs (e.g. voriconazole with liposomal amphotericin B). Several reviews on epidemiology, diagnosis, and therapy have been published [114].

Infections with *Scedosporium* spp. (*S. apiospermum* and *S. prolificans*) may mimic aspergillosis in their predilection for sinopulmonary disease; however, clues to these organisms include a higher risk of embolic skin lesions, ophthalmologic infections, and CNS disease. Like *Fusarium* species, infection is typically associated with higher fungal burdens and disseminated disease. These infections appear particularly common in certain parts of the world, such as Spain and the southeastern United States; it is likely that they are inherently "endemic" to certain regions based on environmental growth conditions. Outcomes are particularly poor with *S. prolificans*, which exhibits considerable resistance to all antifungal options. Given a substantial frequency of resistance, multiple antifungal drugs are usually recommended. Readers are referred to reviews for further discussions of epidemiology, diagnosis, and therapy [115,116].

There are many other fungi that cause disease, including multiple notable organisms distributed worldwide (*Cryptococcus neoformans*), and others that are distributed with classic "endemic" distributions in parts of North America and other regions (e.g. *Histoplasma* spp., *Coccidioides* spp., *Cryptococcus gattii*). However, for the most part, these infections are rare in HCT recipients, owing either to the type or duration of immune suppression or to the use of azole-based prophylaxis. However, it is useful to consider them among differential diagnoses, especially in endemic regions; disease can be associated with either latency with reactivation, or de novo exposure, complicating disease patterns and epidemiology.

Conclusion

Fungal pathogens are numerous and distributed worldwide, causing disease in both immune-competent and immune-suppressed people and animals. The relative "emergence" of these organisms occurred coincident with the increasing use of medical transplantation (both organ and stem cells) and the appearance of HIV infection, pointing to the relative importance of these organisms as "opportunists." Much has been learned about the epidemiology of disease in HCT recipients, especially those with longer-term cellular immunosuppression associated with GVHD; although historical reports focused almost exclusively on the role of neutrophils in combating infection, our understanding of acquired immunity and innate defenses now outlines a much more complex picture of effective host defenses and the clinical risks that lead to invasive disease. Fortunately, there have been numerous advances in diagnostics and therapies, with recent outcomes studies observing improved transplant outcomes associated with more effective preventative approaches and treatments of documented invasive disease. The expanding array of antifungal agents with differential activities and toxicities underscores the importance of diagnostics and opens up the potential for future advances. Most importantly, this chapter highlights that this field continues to be dynamic, expanding, and ever more complex, with new basic and clinical insights reported with relative frequency.

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CHAPTER 87

Cytomegalovirus Infection

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Introduction

Human cytomegalovirus (CMV) has been a problem that has limited the success of hematopoietic cell transplantation (HCT) since the earliest days of the procedure [1], but, with the development of antiviral agents and improved immunosuppressive agents, CMV infection can be controlled in most patients [2]. This chapter describes the clinical aspects of CMV infection and the methods and rationale for the prevention of morbidity due to this virus. The 30-plus years of clinical studies that have led to our current methods have been reviewed in earlier editions of this book. This chapter focuses on the practical aspects of prevention and management of this infection that are important for the student, the transplant physician, and others interested in medical care of the HCT patient.

Clinical aspects of CMV infection

Viral latency

CMV is known taxonomically as human herpesvirus 5 and classified as a beta herpesvirus. CMV is a DNA virus with typical herpes virion structure (see Figure 87.1) with a genome that encodes nearly 200 proteins [3]. CMV infection establishes a latency in cells primarily of the myeloid lineage that accounts for life-long infection. The complexity of its molecular biology and functional genomics is beyond the scope of this chapter, and the reader is referred elsewhere for in-depth reviews [4].

CMV epidemiology

The epidemiological description of CMV infection in the HCT population is well described and has established that CMV infection can originate from both endogenous and exogenous sources. As shown in Table 87.1, the laboratory documentation of infection involves the detection of CMV in blood by culture ("viremia"), by detection of CMVp65 in leukocytes ("antigenemia"), by DNA polymerase chain reaction (PCR) assay ("DNAemia"), or by RNA PCR assay ("RNAemia"). Prior CMV infection in either the donor or the recipient creates the major risk factor for CMV infection in the HCT recipient, and with curtailment of blood-borne CMV exposure by the use of filtered blood products, there is virtually no CMV infection in the CMV seronegative recipient (R^-) of a seronegative donor (D^-) product. The incidence of CMV infection in allogeneic D^-R^- HCT first dropped from ~20% to nearly 0% with the introduction of CMV seronegative blood support, and currently, with filtration, it is not necessary to use selected CMV seronegative blood products for the HCT recipient [5].

In CMV seropositive recipients of either autologous or syngeneic HCT, the CMV infection rate can reach levels of up to 45%, yet CMV disease rarely occurs in these patients [6,7]. In the CMV seropositive allogeneic recipient, either D^+R^+ or D^-R^+, the incidence of CMV infection ranges from 20 to 75%, and it is in this population that the risk for CMV-associated disease occurs. The incidence of all CMV infection diagnosed at City of Hope over the past decade provides a view of CMV incidence by HCT recipient type (see Table 87.2). The lowest incidence is in autologous HCT with increasing incidence in matched related donor HCT and unrelated matched donor HCT, and highest in cord blood donor HCT. Although variation in CMV incidence is institution specific, this hierarchy of infection risk with increasing potential for HLA mismatch is the general rule.

The pathogenesis of CMV disease in the setting of HCT involves two main factors, reactivation of CMV infection and failure to control this infection, and the events that alter either of these will influence the rate of disease. The reactivation of CMV is a constant factor in autologous, syngeneic, and allogeneic HCT, suggesting that the regeneration of the marrow and reconstitution of blood elements create a milieu for CMV reactivation from a less-active state of virus dormancy, called "latency." CMV does not integrate into infected cells, but does become latent in non-dividing cells. Reconstitution of the blood elements post-HCT appears to provide the stimulation for replication of this DNA virus. Other conditioning regimen-specific factors that activate DNA repair, such as total body irradiation (TBI), could play a role in inducing CMV reactivation. However, the main element in disease pathogenesis is the initiation of control of infection, and this requires both innate and acquired immune cellular functions. Undoubtedly, variations in reconstitution of CMV immunity explain the differences in CMV disease rates among the HCT groups. As discussed later (see Cellular immunity to CMV), risk of CMV reactivation is linked to natural killer (NK) cell function and to CMV-specific T-cell immunity. With regard to T-cell reconstitution after HCT, both a homeostatic expansion of adoptively transferred T cells from the graft and T-cell production from progenitor stem cells are important. T cells transferred from the donor can provide protection from CMV [8]. However, the function of any adoptively transferred T cells is affected by inherent iatrogenic immunosuppressive treatments used in the allogeneic HCT method, such as T-cell depletion for prevention of graft-versus-host disease (GVHD) in mismatched or haploidentical HCT and corticosteroid therapy of

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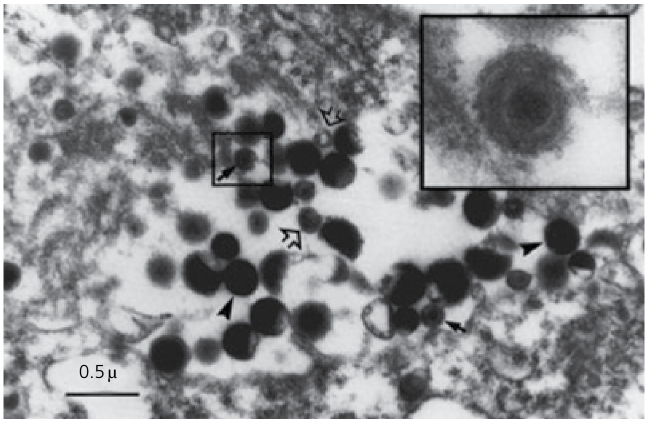


Figure 87.1 Electron micrograph of cytomegalovirus virions and defective particles. CMV-infected human foreskin fibroblasts were subjected to glutaraldehyde and osmium tetroxide fixation and electron microscopy, revealing intact virions (solid arrows), defective virions (open arrows), and dense bodies (arrowheads). Magnification ×38,750; inset ×182,500. Source: reproduced with permission of John Hardy, City of Hope National Medical Center.

Table 87.1 CMV infection in blood

Event	Definition	Median days to first positive assay post-allo-HCT (range)	Median days until clearance of virus on antiviral therapy (range)
Viremia	Tissue – isolation of CMV from blood [89]	51 (37–93) [90]	0 (0–7) [90]
Antigenemia	Detection of CMV pp65 in blood leukocytes [90]	45 (27–75) [15]	13 (7–41) [90]
DNAemia	Detection of CMV DNA in plasma, whole blood, or leukocytes [15]	32 (18–46) [15,90]	12 (7–41) [90]
RNAemia	Detection of CMV RNA in plasma, whole blood, or leukocytes [24]	21 (4–82) [24]	19 (2–32) [24]

Table 87.2 Incidence of CMV infection in HCT at City of Hope[a]

Year	Incidence % (*n*)			
	Auto	MRD	URD	UCB
2004	0.0 (203)	25.7 (101)	37.3 (67)	27.3 (11)
2005	0.4 (245)	13.4 (97)	34.6 (78)	29.4 (17)
2006	0.7 (300)	12.8 (109)	28.1 (89)	42.9 (7)
2007	0.4 (264)	16.8 (113)	28.0 (118)	50.0 (12)
2008	0.4 (270)	14.5 (110)	19.2 (125)	31.6 (19)
2009	1.5 (264)	20.1 (134)	26.9 (167)	38.5 (13)
2010	1.6 (246)	20.3 (123)	28.6 (126)	69.2 (13)
2011	0.0 (254)	14.3 (105)	22.4 (134)	45.5 (11)
2012	0.3 (294)	15.6 (135)	26.6 (139)	50.0 (18)
2013[b]	0.4 (251)	11.2 (116)	25.2 (115)	59.1 (22)

Auto, autologous HCT; MRD, matched related donor HCT; URD, unrelated donor HCT; UCB, umbilical cord blood donor HCT.

[a] Percentage of HCT recipients with CMV infection in blood as determined by shell vial culture or DNA PCR in monitored blood specimens post-HCT or in tissue as determined by histopathology, n = number of HCT recipients.

[b] Data for January–October 2013.

Source: Data kindly provided by Dr. B. Tegtmeier, City of Hope Pathology Department and Quality, Risk and Resource Management.

active GVHD. Thus, T-cell depletion produces a significant risk for CMV disease, and corticosteroid therapy for GVHD significantly influences disease [2]. Even subtle effects on T-cell function can influence CMV infection and diseases, as seen with the lower infection and disease rates in HCT recipients of bone marrow versus peripheral blood-derived stem-cell products, a phenomenon thought to be due to a differential but transient delay in CMV-specific immune reconstitution [9]. Even among autologous HCT recipients, there is more risk for CMV disease if graft manipulation results in T-cell depletion. This role of T-cell protection is best demonstrated in umbilical cord blood (UCB) HCT in which only naïve T cells are transferred in the graft and immune reconstitution must rely solely on the emergence of T cells that undergo receptor rearrangement by thymopoiesis [10]. In UCB recipients, there is delayed clearance of CMV viremia until reconstitution of thymopoiesis occurs, as shown by the presence of T-cell receptor excision circles, Thus, the risk of CMV disease after HCT is a function primarily of protective cellular immunity and, as this varies with HCT method, the epidemiology of CMV disease will vary.

Historically, CMV disease presented either as pneumonitis or gastroenteritis during the 2–3 months post-HCT. With the use of preemptive antiviral treatment strategies, the natural history of CMV disease was changed to an occurrence later in the first 6 months after HCT [11]. The nature of the CMV infection can be primary or secondary, with primary infection seen mostly in the D^+R^- setting, since infection can be prevented in the D^-R^- patient treated with filtered blood products. CMV infection occurs after HCT when donor, recipient, or both have prior CMV infection (termed D^-R^+, D^+R^-, or D^+R^+), and such infections result from reactivation of CMV from latency. CMV can be typed by molecular techniques, and in reactivation infection, the CMV strain would be the same as in the primary infection. However, it is possible that reinfection could occur with a new strain of CMV, and in this situation, the virus would likely be from the seropositive donor. If CMV infection occurs after cessation of antiviral treatment, this is termed "recurrent" CMV infection.

Prevention of CMV infection after HCT

From a practical standpoint, the central risks that define how an HCT recipient should be managed are those that have predictive value for development of complicated CMV infection in the setting of inadequate immune function. When considered in this way, the important risk factors for the development of severe CMV disease are patient age, pre-transplantation CMV antibody seropositivity of donor or recipient, recipient–donor HLA-mismatch status, occurrence of acute GVHD, and delayed recovery of immunologic function post-HCT [2,5].

There are two general approaches to the prevention of CMV disease using either ganciclovir or foscarnet: (1) treatment after early detection of blood-borne CMV infection prior to onset of disease, and (2) treatment of all at-risk patients for the defined period of risk. The first approach is a "preemptive" strategy and was first shown to be effective in the HCT setting where the period of risk is well defined [12,13]. With this method, the requirement for accurate detection of CMV infection is the limiting feature, but the benefit is that fewer patients are exposed to potentially toxic antiviral chemotherapy. The second approach, termed "general prophylaxis," is used when selected patients are at particularly high risk for CMV infection, such as recipients of

UCB HCT, and all such patients are treated with an antiviral agent for the period of risk. For both strategies, the risk of CMV disease and the available virology support capabilities govern the management. There is no recommended use of intravenous immune globulin for CMV prevention in the HCT recipient [5].

Preemptive treatment strategy for prevention of CMV after HCT

Patients should be monitored for CMV infection using a test of CMV DNAemia, although other methods for determining the presence of CMV by antigenemia or RNAemia can be used [5,14]. Viremia is too insensitive and is no longer used for triggering preemptive treatment. Surveillance is started based on medical history, such that patients with a history of CMV infection in the past 6 months should be monitored prior to the conditioning therapy and all others should begin at approximately 10 days post-HCT and continued to approximately day 100. Documented CMV blood infection is treated starting with either ganciclovir, valganciclovir, or foscarnet (*note:* foscarnet is not approved in the United States for this use). Because of the variation is sensitivity of the CMV assays, there is no recommended CMV level that can uniformly trigger preemptive treatment [2]. Local institutional guidance for levels of DNAemia can be established, since it has been shown that risk groups can be defined and treatment can vary based on the level of DNAemia [15]. For example, patients treated with anti-T-cell therapies or with ≥1 mg/kg prednisone equivalent should be treated at lower thresholds of DNAemia than patients with minimal risks for CMV disease.

As shown in Table 87.3, preemptive therapy is initiated with an induction dose level of drug used for a minimum of 2 weeks, and this is followed by a maintenance period with reduced dose that can vary in duration from an additional 2 weeks, necessary to bring CMV to an undetectable level, or continued through the period of maximum immunosuppression to day 100 [2,5]. CMV infection will clear in most patients within 2 weeks, but for those with stable or increasing CMV levels, the induction period should continue. Maintenance therapy begins with clearance of CMV and consists of ganciclovir or valganciclovir as shown in Table 87.3 and given for 5–7 days per week for an additional period based on the patient's risk factors. For example, if the CMV blood levels are negative ×2, and the subject is not receiving corticosteroids for GVHD treatment, then maintenance can stop. For patients at higher risk for CMV disease, such as those on immunosuppressive medication for acute or chronic GVHD, treatment should continue for up to day 100 post-HCT or even longer depending on risk status. Monitoring for CMV should continue for at least 1 month, and longer for the patient with risk for late disease. The highest risk group meets all of these criteria: (1) matched HCT recipient with continued GVHD or non-matched HCT recipients irrespective of GVHD status, (2) recurrent episode of CMV infection after preemptive therapy, and (3) lymphopenia at day 100 post-HCT or D^-R^+ CMV serostatus [16], but recipients of haploidentical, UCB, or T-cell-depleted HCTs should be considered for prolonged monitoring [2,5]. Recurrent infection requires treatment resumption using the induction/maintenance regimen as before until the virus clears.

General prophylactic treatment strategies for prevention of CMV after HCT

Not all HCT recipients will do well with preemptive anti-CMV management, and failure of preemptive therapy occurs in those at the highest risk for CMV infection [5]. These high-risk groups include haploidentical, UCB, or T-cell-depleted HCT recipients. For these patients, the alternative approach for prevention of CMV is to treat everyone with an anti-CMV agent during the period of risk, namely from the onset of HCT until engraftment is secure and immunosuppressive medications are minimal. Ganclovir, high-dose acyclovir, and valacyclovir have been shown in randomized studies to be effective in CMV prophylaxis

High-dose acyclovir or valacyclovir is usually used during the period prior to stable engraftment, and then ganciclovir is used [2] (see Table 87.3 for dosing recommendations). In a blinded, controlled evaluation of high-dose acyclovir, recipients of matched related or unrelated HCT were randomly assigned to receive either (i) high-dose acyclovir for 6 months (500 mg/m^2 intravenously three times daily) from day −5 to day +30 post-HCT and then oral acyclovir (800 mg four times daily) or (ii) the same high-dose acyclovir for 1 month, or (iii) a standard herpes simplex prevention regimen for 30 days (400 mg orally four times daily) [17]. The use of high-dose acyclovir for 30 days or for 6 months were both associated with reduced CMV infection compared with standard-dose acyclovir.

When ganciclovir is used for general prophylaxis, CMV infection can be significantly reduced, but there is no difference between control and treated groups in terms of mortality. This lack of effect on mortality is different from the effect observed with preemptive use of ganciclovir [13], and this is likely due to the side effects of ganciclovir, which produces neutropenia with increased risk of infection [18]. Because of the toxicity of these agents and their expense, general use of ganciclovir in all CMV-seropositive persons is currently not recommended [5].

A risk-adapted strategy has been described in which patients with GVHD requiring increased corticosteroid therapy were given a 2-week course of ganciclovir. Using this approach, there was no CMV disease and no neutropenia was observed [19]. This has been applied to other HCT populations at high risk for CMV infection, including in vitro or in vivo T-cell depletion, matched-unrelated or haploidentical HCT, or UCB transplantation. Similarly, the risk for CMV disease can increase during the course of any allogeneic HCT owing to significantly increased immunosuppression, and here the method of risk-adapted general prophylaxis should be applied.

The risk for CMV in UCB is among the highest rates of HCT recipients (see Table 87.2) with a corresponding increase in CMV disease [20,21]. For this reason, a prophylactic strategy usually includes high-dose acyclovir or valacyclovir followed by ganciclovir/valganciclovir at engraftment or at the time of first CMV infection (see Table 87.3 for dose recommendations). A recent intensive prophylactic program was reported in which ganciclovir was administered before transplantation from day −8 to −2 pre-UCB, followed by high-dose acyclovir post-transplant with biweekly monitoring for CMV with PCR-based preemptive therapy [22]. This method, although not affecting survival, resulted in lower CMV DNAemia, less use of preemptive ganciclovir, and less CMV disease [22]. Despite the prophylactic method chosen for use in the UCB recipient, the incidence of CMV infection can be expected to be ~50–60% [21,22].

Specific issues in CMV infection prevention

When should preventive strategies be used for autologous HCT recipients?

All CMV-seropositive recipients of HCT are at risk for CMV reactivation, and in the autologous HCT population, the rate of reactivation ranges from 25 to 45% with CMV disease occurring in 2–10% [6]. However the risk for disease in this group is low and is related to conditioning with TBI, use of CD34$^+$-selected T-cell-depleted peripheral blood progenitor cells, and prior use of alemtuzumab, fludarabine, or 2-chlorodeoxyadenosine [2]. Autologous HCT recipients with these risk factors should be managed with preemptive monitoring and early treatment with ganciclovir [5].

Table 87.3 Use of Anti-Cytomegalovirus Antiviral Agents

Drug	Mode of action	Dose	Duration	Comments
Ganciclovir	A synthetic nucleoside analogue of 2′-deoxyguanosine that inhibits CMV DNA polymerase	Induction regimen[a]: 5 mg/kg 2 × daily IV;	14 days or until DNAemia clears	Associated with neutropenia and anemia; interacts with probenecid and mycophenolate mofetil
		Maintenance therapy: 5 mg/kg once daily	Variable based on immune status	
Valganciclovir	Orally available valine ester of ganciclovir; same mode of action	Induction regimen[a,b]: 900 mg twice daily PO;	14 days or until DNAemia clears	Same as ganciclovir
		Maintenance therapy: 900 mg once daily PO	Variable based on immune status	
Foscarnet	An anion pyrophosphate inhibitor of CMV DNA polymerase	Induction regimen[a]: 90 mg/kg twice daily IV;	14 days or until DNAemia clears	Associated with nephrotoxicity and altered Ca^{2+}, Mg^{2+}, K^{+}, and phosphate levels
		Maintenance therapy: 90 mg/kg once daily IV	Variable based on immune status	
Cidofovir	A synthetic nucleoside analog of deoxycytidine that inhibits CMV DNA polymerase	Induction regimen[a]: 5 mg/kg once weekly IV;	14 days or until DNAemia clears	Associated with nephrotoxicity
		Maintenance therapy: 5 mg/kg once in 2 weeks IV	Variable based on immune status	
Acyclovir[e]	A synthetic nucleoside analog of guanosine that inhibits virus DNA replication	High-dose regimen[c]: 500 mg/m^2 3× daily IV or 800 mg 4× daily PO, or 600 mg/m^2 4× daily	Variable based on immune status	Used only for prophylaxis
Valacyclovir[e]	Orally available valine ester of acyclovir; same mode of action	High-dose regimen[d]: 0.5–2 g 3× daily PO	Variable based on immune status	Used only for prophylaxis

[a] The induction dose is intended to clear infection from blood in preemptive management and from tissue during treatment of CMV disease. For CMV pneumonia, add IVIg 0.5 g/kg every other day for 10 doses and then weekly until pneumonia resolves.

[b] Pediatric dose of valganciclovir: induction regimen 15–18 mg/kg twice daily PO; maintenance therapy 15–18 mg/kg once daily PO.

[c] Pediatric dose of acyclovir: 500 mg/m^2 3× daily IV or 18 mg/kg 5× daily PO.

[d] Pediatric dose of valacyclovir: for weight 20–40 kg, 500 mg 3× daily; weight <20 kg, use IV acyclovir.

[e] Not approved for prevention of CMV infection. When used, routine CMV surveillance is required with duration to 80–100 days post-HCT or until CMV DNAemia occurs.

What is the optimal assay for detecting CMV?

The preemptive management strategy places a burden on the transplant team to monitor and detect CMV infection before it progresses to overt disease, and clearly the window of opportunity for such early treatment varies with the sensitivity of the assay. As noted in Table 87.1, there are four general types of assays for the detection of CMV: culture-based assays, antigen assays, and DNA or RNA assays. The tissue culture assay for CMV, called the "shell vial" assay, is rarely used today because it becomes positive later after HCT [5]. Using DNA/RNA-based methods, detection of CMV in blood can be made at a median time of approximately 35 days post-HCT [15,23,24]. Hence the DNA/RNA-based assays widen the opportunity for early treatment by 1 week. Although this seems small, there is evidence that this earlier treatment of CMV infection was sufficient to reduce CMV disease and overall mortality significantly [23]. Also important, the quantitative aspects of these tests allow them to be useful for monitoring the response to treatment.

When should ganciclovir and foscarnet be used together?

There has been no evaluation of dual ganciclovir and foscarnet therapy for CMV disease or for CMV treatment failure. However, it has been shown that for recipients at high risk for CMV disease, foscarnet and ganciclovir could be used in combination as preemptive therapy [25]. In this method, during maintenance therapy, these drugs were used on alternate days. Because of the potential for induction of resistance, however, the alternate-day use of an anti-CMV agent cannot be recommended.

When should cidofovir be used in the HCT recipient?

Cidofovir has been used for the preemptive management of the HCT patient [26]. As shown in Table 87.3, the dosage for cidofovir is 5 mg/kg per dose in two doses give 1 week apart, followed by maintenance therapy provided every other week. The drug is given with probenecid and adequate preinfusion hydration. In the United States, cidofovir is not approved for the prevention of CMV infection in the HCT recipient. It is nephrotoxic, and its use in the HCT setting remains a third-line approach when preemptive use of ganciclovir or foscarnet has failed.

CMV-associated diseases after HCT

Diagnosis of CMV-associated infection and disease

The clinical course of CMV infection involves a spectrum from earliest detected infection to specific signs and symptoms as the virus reactivates from latency and expands and spreads to involve specific organs. Today, a quantitative DNA PCR or CMV antigenemia assay is most commonly used clinically; positive results occur at expected times post-HCT, with anticipated clearance during antiviral therapy (see Table 87.1).

The diagnosis of CMV-associated end-organ disease has requirements that have been described [27]. Since CMV is a ubiquitous virus based on its presence in blood elements, it is important to use these definitions. For each end-organ infection, it is essential that there be a combination of signs/symptoms of disease and laboratory detection of CMV in tissue material. The signs/symptoms would be related to lung, intestine, liver, brain, retina, and so on, and the laboratory detection would be virus isolation, tissue biopsy analysis by histopathology, immunohistochemical staining, or in situ hybridization. CMV antibody and DNA PCR are not appropriate for confirmation of end-organ disease because antibody production is not reliable post-HCT during periods of immunosuppression and because DNAemia can contaminate a tissue specimen and cause a false-positive test [27].

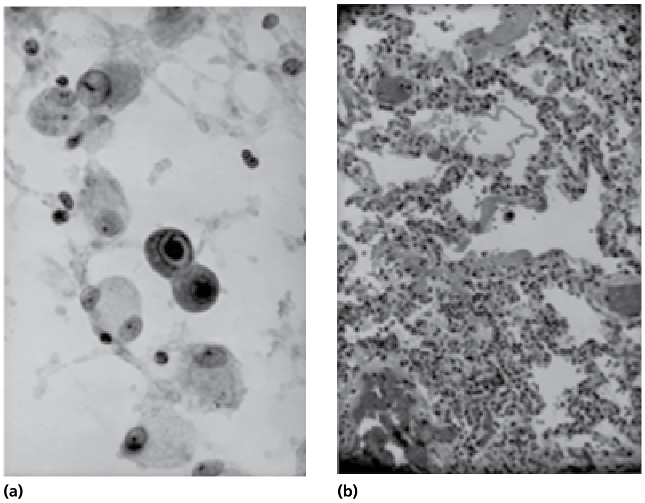
CMV-associated pneumonitis

CMV-associated pneumonia is defined as a progressive interstitial pulmonary process, as evidenced by hypoxia and by lung imaging, with simultaneous evidence of CMV infection in the lung and without evidence for other causes of pneumonitis. Prior to the development of antiviral methods for the prevention of CMV infection, CMV-associated interstitial pneumonia (CMV-IP) occurred in 15–30% of HCT allograft recipients. Today, with the availability of methods to prevent CMV-IP, the disease is rare and usually appears in late-stage patients with poor immune reconstitution or chronic GVHD [15,16].

Bronchoalveolar lavage (BAL) is the preferred method of diagnosis of CMV-IP, and the specimen should be analyzed for infectious virus and for cytological evidence of CMV. As shown in Figure 87.2b, inclusion bodies typical for CMV can often be seen in CMV-positive BAL samples. No matter when CMV-IP occurs after HCT, hypoxia is the major physiological abnormality observed and, as illustrated in Figure 87.2a, the histopathology of CMV-IP involves thickening of the interalveolar membranes with cellular infiltrates and edema. The radiologic abnormalities suggestive of interstitial pneumonitis can be seen on X-ray films even without more advanced imaging (Figure 87.3). However, computed tomography (CT) imaging will demonstrate earlier findings with heterogeneous mixed areas of ground-glass opacity, small centrilobular nodules, and consolidation. The differential diagnosis includes radiation-induced pulmonary disease, cytotoxic chemotherapy-induced pulmonary damage, pulmonary hemorrhage, pulmonary edema, metastatic neoplasia, and other infections of fungal, viral, or bacterial etiology.

CMV-associated enteritis and other organ-specific diseases

CMV enteritis is defined as an enteropathic syndrome with pain, nausea and vomiting, diarrhea, and evidence of CMV infection at the site of an inflammatory or ulcerative mucosal lesion. Gastrointestinal syndromes associated with CMV are the most common CMV-related medical problems in the allogeneic HCT



(a) (b)

Figure 87.2 Histopathology of cytomegalovirus interstitial pneumonia (CMV). (a) Photomicrograph of thin section of lung from a patient with CMV-IP stained with hematoxylin and eosin. Note the cell in alveolar space containing Cowdry type A intranuclear inclusion typical of CMV. Magnification ×640. (b) Photomicrograph of Papanicolaou-stained bronchoalveolar lavage specimen from a patient with CMV-IP. Note similar CMV-induced "owl's eye" intranuclear inclusions. Magnification ×1000.

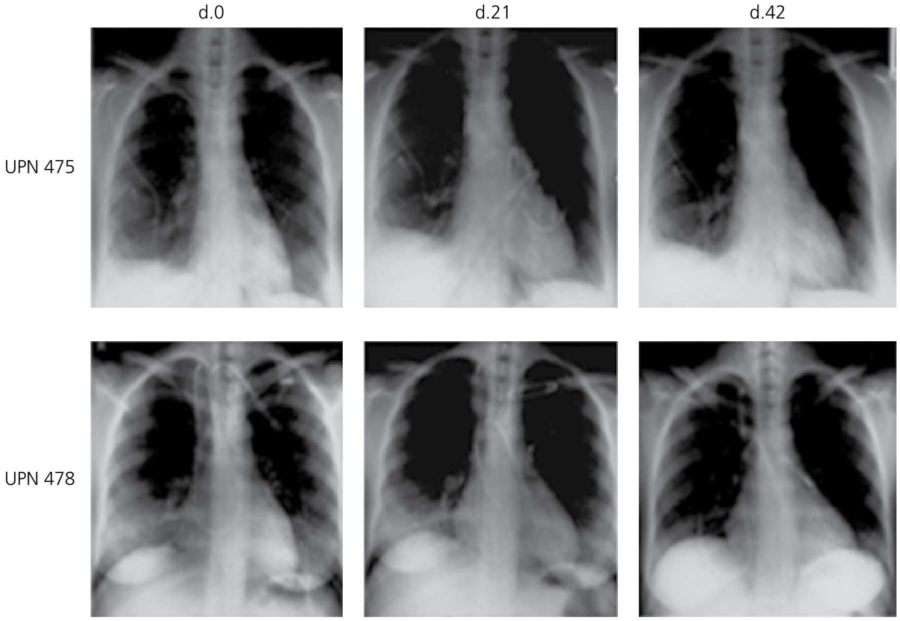


Figure 87.3 Clinical course of treated cytomegalovirus (CMV)-interstitial pneumonia. This is a series of X-ray films from two patients (UPN475 and UPN478) treated with ganciclovir as described in Table 87.3; radiographs are from days 0, 21, and 42 of treatment. Note the resolution of the right lower lobe pneumonitis in both patients.

recipient but are not associated with altered survival outcome [15]. Ulcerations associated with CMV infection can be identified in the esophagus, the stomach, the small bowel, and the large intestine. The diagnosis is made by the association of CMV infection with mucosal pathology and appropriate symptoms [27]. The median onset of CMV enteritis is 91 days post-HCT (range 17–527 days) and a 2-year overall survival rate in this population of 35% [28]. CMV enteritis must be distinguished from gastrointestinal GVHD by appropriate endoscopic and histopathology.

Other CMV-associated syndromes

Since the majority of at-risk HCT recipients will reactivate CMV infection, the accurate association of other syndromes with this infection can be difficult. As noted already, organ-specific diagnosis of CMV infection must be based on tissue-based evidence of CMV infection [27]. In addition to mononucleosis-like syndromes with fever, arthralgia, and malaise, both hepatitis and suppressed marrow function including neutropenia and thrombocytopenia have been associated with acute CMV infections. The course of reactivated CMV infection without organ-specific signs is not well described in the allogeneic HCT recipient, but febrile episodes can be a significant part of this infection. In general, other CMV-associated organ-related syndromes, such as hepatitis and even encephalitis, are better defined as syndromes with specific organ dysfunction and concomitant presence of active CMV infection.

Treatment of CMV-associated diseases

Therapy of CMV-associated interstitial pneumonia (CMV-IP)

The treatment of CMV-IP has had mixed success in immunocompromised persons, probably due to variations in patient groups, viz., different hematologic diagnoses, different levels of immunosuppression post-HCT, and varying contributions from host factors, known and unknown, that modify protection from CMV infection. Historically, the outcome for the untreated disease was very poor until the availability of ganciclovir, but even when ganciclovir was used alone, at doses ranging from 7.5 to 15.0 mg/kg/day, outcome was uniformly poor [29]. Thus, unlike the initial experience in the use of ganciclovir for treatment of CMV diseases in AIDS patients, in which prompt clinical response was the rule, ganciclovir alone was not able to improve the outcome of CMV-IP in the HCT recipient. It was not until the combined use of ganciclovir with CMV antibody-enriched intravenous immunoglobulin (CMV-IVIg) that improved outcome of CMV-IP was observed . This method used ganciclovir at 10 mg/kg daily and IVIg 500 mg/kg every other day for 21 days, followed by ganciclovir at 5 mg/kg daily 5 days per week and IVIg at 500 mg/kg weekly until day 180 after HCT [30]. Ganciclovir plus IVIg continues to be the recommended treatment for CMV-IP (see Table 87.3), yet it still meets with only varying success, probably owing to variations in patient-related factors. For example, patients who do not receive TBI compared with those who do have significantly improved outcome and, in general, patients with multiple organ dysfunction or respiratory failure are less likely to respond to antiviral therapy [31].

During treatment, a repeat BAL is suggested after the initial 21 days of treatment if there is any question regarding response to the initial antiviral therapy. The duration of maintenance therapy varies but is usually continued for the duration of major immunosuppressive treatment. Fever and hypoxia usually resolve within the first week of treatment, but X-ray changes can persist for many weeks. The patient with a slow response to treatment or having deteriorating pulmonary function on treatment prompts the consideration of ganciclovir-resistant CMV, or the presence of secondary infections , and both of these questions can be addressed by repeat BAL. CMV infection is usually eliminated from the lavage specimen by 21 days after the start of treatment, and any deterioration of pulmonary function with continued CMV lung infection should prompt a work-up for antiviral drug resistance (see below).

If ganciclovir resistance occurs, or if ganciclovir cannot be used because of marrow toxicity, then foscarnet is indicated. As shown in Table 87.3, foscarnet is given at a dose of 90 mg/kg twice daily with saline infusion over 2 hours during the initial induction period. Alternatively, a regimen of 60 mg/kg three times daily can be used. Maintenance therapy follows this at a dose of 90 mg/kg per day for an indeterminate period, as determined by the immunosuppression of the patient and the response to therapy. Foscarnet is nephrotoxic, and it is recommended that the calculation of the dosage be determined by creatinine clearance, with appropriate attention to hydration and electrolyte balance. There has been no systematic evaluation of the efficacy of foscarnet plus immunoglobulin for the treatment of CMV-IP.

Therapy for CMV-associated enteritis and other syndromes

A controlled study using ganciclovir has shown that despite antiviral effects, there was no significant improvement of CMV gastroenteritis [32]. Nevertheless, because of its antiviral effect, it is common practice to use ganciclovir without IVIg for CMV enteritis according to the dosing schedule shown for CMV-IP (see Table 87.3). For other CMV-associated syndromes such as hepatitis, retinitis, and CMV-associated marrow suppression, the same regimen as shown in Table 87.3 is used.

Complications of CMV therapy

The major complications associated with ganciclovir treatment are neutropenia and serum creatinine elevation. Analysis of patients with ganciclovir-associated neutropenia has indicated that risk factors include elevated bilirubin level (≥6 mg/dL) during the first 20 days after HCT, a serum creatinine level of ≥2 mg per dL after day 21 post-transplantation, a low marrow cellularity between days 21 and 28 post-HCT, or a low white blood cell (WBC) count at time of ganciclovir initiation [18,33]. Of importance, this treatment-related neutropenia is an independent risk factor for poor overall survival [relative risk (RR) 3.8, $p = 0.001$], for poor event-free survival (RR 2.1, $p < 0.0001$), and for relapse (RR, 1.7, $p = 0.03$) [18]. Therefore, in the event of ganciclovir-induced neutropenia unresponsive to granulocyte colony-stimulating factor (G-CSF) or granulocyte–macrophage colony-stimulating factor (GM-CSF), ganciclovir/valganciclovir therapy should be switched to foscarnet. To minimize this problem, a stopping rule for ganciclovir use is invoked when the absolute neutrophil count is lower than 1000/μL on two consecutive days.

Antiviral drug resistance

Breakthrough CMV infection is not uncommon while on seemingly adequate antiviral therapy and is not an indication of treatment failure [34]. Studies have shown that approximately 15–25% of patients treated preemptively with ganciclovir or foscarnet will require later retreatment for recurrence [23]. As noted, ganciclovir resistance is uncommon , and rising CMV DNA levels or the presence of recurrent CMV infection are the best guide for suspecting true antiviral drug resistance. The signature mutation for ganciclovir resistance occurs in the CMV gene UL97 that encodes a phosphotransferase enzyme necessary for activating ganciclovir to its active phosphorylated form or in the CMV DNA polymerase gene UL54 [35]. In a recent study of approximately 150 CMV infections in transplant recipients in Copenhagen, none had a genetic mutation suggestive of ganciclovir resistance at first detection of CMV, but at recurrence, 37% had such a mutation [36]. Hence early CMV infection, even when clearance of plasma DNAemia is delayed, is not an indication for ganciclovir resistance, but failure to resolve DNAemia during recurrent infection suggests drug resistance. Confirmation of drug resistance is done by phenotypic or genetic analysis of CMV-positive blood [35]. Forcarnet resistance has rarely been observed in HCT but when seen is due solely to mutations in UL54 [37], and dual resistance of CMV to both forcarnet and ganciclovir has occurred. Therapeutic decisions usually have to be made in the absence of definitive evidence of drug resistance, and foscarnet should be substituted in place of ganciclovir in the following situations: (i) whenever there is marrow toxicity and the CMV assays remain persistently positive or show rising CMV DNAemia, (ii) when persistent or recurrent infection is present, especially at the time of increased treatment of GVHD with corticosteroid or other secondary anti-GVHD drugs, and (iii) when there are changing clinical signs suggestive of CMV disease.

Use of cidofovir in the setting of CMV treatment failure has been reported [26] and remains an alternative therapy. However, because of the potential for cross-resistance with ganciclovir, genetic testing should be carried out in association with such use. Although not yet available commercially, an orally available prodrug of cidofovir, brincidofovir, may become useful in the management of drug-resistant CMV (see below).

Cellular Immunity to CMV

CMV reactivation is initially controlled by the innate immune system and extension of infection to organ-specific disease is prevented by the adaptive immune system (see review [38]).

Innate immunity

The importance of NK cell protection from CMV infection was first suggested by the occurrence of repeat CMV infection in patients with NK cell deficiency [39]. CMV infection and the innate immune system are mutually involved in a remarkable system of maturation and control. The NK cell receptor system is complex and ranges from killer immunoglobulin-like receptors (KIRs) that bind to HLA molecules, to immunoglobulin binding CD16, to CD94:NKG2 receptors that bind lectins [40], and other pathogen recognition receptors (PRRs) on NK cells that recognize CMV [41,42]. These components of the "resistome" have been shown to be particularly important in the control of murine CMV [43], and there is expanding but incomplete understanding of how this complex innate immune system is modulated by and protects from human CMV [38,42]. When CMV first binds and enters a cell, there is initiation of events that upregulate the expression of nuclear factor kappa B (NFκB) and interferon regulatory factor 3 leading to expression of inflammatory cytokines. TLR2, which senses glycopeptides and lipopeptides, can recognize CMV surface glycoproteins gB and gH, and it is responsible in part for this cytokine upregulation [44]. TLR2 and TLR3 have been shown to be expressed on NK cells, and CMV activation of NK cells in vitro can be partially blocked by anti-TLR2. It is thought that, in association with cytokines such as IL-12 or type I interferons, activation by TLR agonists leads to enhanced NK effector function. TLR2 polymorphism R753Q has been associated with increased CMV infection and disease after HCT [45]. TLR3 polymorphism L412F appears more frequently among patients with chronic mucocutaneous candidiasis who have increased CMV infection, and among allo-HCT patients, polymorphisms in TLR9 have been associated with increased CMV infection [46,47]. In the murine model of CMV, multipleTLR genetic variants have been associated with loss of protection [43], and it is possible that as we gain a better understanding of the role of TLRs in allo-HCT, TLR polymorphisms will be linked as risk factors for CMV infection and disease.

Early post-HCT, NK cells are the major donor lymphoid cells to appear, and these NK cells express predominantly an inhibitory receptor CD94-NKG2A, which is associated with low cytotoxic function and little expression of KIRs [48]. At this point, control of CMV infection appears to be under the aegis of three immune mechanisms: first, the priming of cytokine release by PRRs as noted above; second, the maturation or education of the NK cells to a more functional cell; and third, the enhancement of adaptive immunity by these innate processes. The proportion of KIR$^+$ NK cells normalizes by cell differentiation, and the function of these KIR$^+$ cells is linked to corresponding HLA ligands [49]. CMV infection appears to play a role in this maturation, as seen by the expansion of NK cells positive for CD94-NKG2C, an activating receptor (see review [42]). The CD94-NKG2A phenotype is altered in association with CMV infection, by unknown processes, to the more mature NK phenotype, which then persists as NKG2C$^+$ NK cells that not only are more functional as measured by granzyme B expression and CD107a, interferon-gamma (INF-γ), and tumor necrosis factor-alpha (TNF-α) release, but also express KIRs specific of HLA class I [50]. This event is recapitulated in HCT in CMV-positive recipients following reactivation of CMV infection [51]. This is analogous to the persistence of Ly49H$^+$ murine NK cells, which expand in response to murine CMV and protect from reinfection [52]. hence it is hypothesized that under the influence of CMV, NK cells mature and develop a heightened function with "memory" NK cells [53]. In the mouse model of CMV, adoptive transfer of such cells provides protection from infection [53]. It remains to be determined in human CMV whether NK cells can recognize viral polypeptides in a similar manner.

NK cell KIRs consist of surface receptors termed activating (aKIRs) and inhibitory (iKIRs) receptors (see review [54]). The iKIRs encode receptors that recognize HLA class I molecules and block further interaction with "self," and this provides a mechanism for NK cell tolerance [40,41]. In healthy individuals, this system of aKIRs and iKIRs regulates cytotoxic functions important in both tumor recognition and viral infection. When the KIR and its ligand are present, the NK cells are "licensed" to function in providing either an inhibitory signal to extinguish cytotoxicity or an activator signal to accelerate cytotoxicity. This is of particular interest in allogeneic HCT where there is the potential for the newly engrafted donor cells to have a KIR–ligand mismatch when a ligand is absent from the genotype, a so-called "unlicensed" state. This can influence the outcome of allogeneic HCT in conflicting ways by affecting GVHD, leukemic relapse, and even survival [40,55]. Clinical benefits are seen when donor NK cells encountering recipient target cells that lack the HLA class I ligand mediate an antileukemic effect [56]. It remains to be determined how this can best be exploited in donor selection pre-HCT.

With regard to CMV infection, KIR haplotypes have been associated with a significant reduction in CMV reactivation [57]. The presence of additional activating KIR genes or identity between donor and recipient aKIR genotypes has also been associated not only with decreased CMV reactivation, but also with improved survival [58]. A donor aKIR profile predictive of low risk of CMV reactivation has been described [59]; however, similarly to the anti-leukemia effects noted above, it appears that the absence of ligand is most important for control of early infection. In the murine model of CMV, it is the unlicensed NK cells that are protective, even when used adoptively [60]. Similarly, it has been shown that in the setting of allogeneic HCT, the risk of CMV infection is associated with donor KIR genes only when the specific HLA-C ligand is absent from the shared donor/recipient genotype [61]. Thus, unlicensed iKIRs appear to suppress protective responses and increase the risk of CMV infection and unlicensed aKIRs significantly reduce the risk of infection. Consistent with the NK disarming model of Raulet and Vance [62], in the first 100 days post-HCT the HLA ligand appears to serve as a gatekeeper of inherent NK cell function such that, with ligand, KIR-mediated activity is held in check and, without ligand, KIR activity is unrestricted. The consequences of this for the management of HCT patients remain to be determined.

T-cell immunity

T-cell immunity to CMV involves a complex relationship between virus and host with multiple antigens recognized by the host and several immune-escape mechanisms encoded by the virus (see review [38]). As noted over three decades ago, cytotoxic T-lymphocyte (CTL) function is most critical for protection from severe disease and mortality in the setting of HCT [63], and HLA-restricted CTL function was associated with survival from CMV-IP [64]. Subsequently, using new methods of quantifying CMV-specific cellular mediated immunity (CMI), studies demonstrated that patients lacking CD4-specific CMV recognition were at greater risk for CMV complications [65]. Recipients with D^+R^+ CMV serostatus have better multifunctional recovery of CMV-specific $CD8^+$ T-cell immunity and require less anti-CMV therapy [8]. Immunologic recovery of CMV-specific cellular immunity by 3 months post-HCT is a critical factor in protection from CMV infection, and the factors that indicate that a recipient is at high risk for CMV due to impaired cellular immunity include the use of high-dose corticosteroids, defined as >1 mg/kg per day, $CD4^+$ cell count $<100 \times 10^9$/L, and $CD8^+$ cell count $<50 \times 10^9$/L [2].

The two predominant immunologic targets of CMV-specific CMI are CMV pp65 and CMV-IE (see review [66]), yet there is a wide range of responses to CMV proteins [67,68]. Analysis of T-cell receptor (TCR) usage among such peptide-specific responses permits quantitative measure of CMI, and applying this technology to HCT recipients during the early expansion of $CD8^+$ lymphocytes, those patients developing at least 10^7 CMV-specific $CD8^+$ cells/μL appeared to be protected from CMV disease. Failure of immune reconstitution to this level within the first 65 days post-HCT is a risk factor for CMV complications [69]. Patient groups could be dichotomized into those with rapid immune recovery (≥7 CMV-specific $CD8^+$ T cells/μL) and those with delayed recovery at 65 days (<7 CMV-specific $CD8^+$ T cells/μL), with the latter group at risk for recurrent infection and disease [69].

Future developments relating to CMV infection in HCT

CMV infection and protection from leukemic relapse

The interaction of CMV and the immune system provides a hypothesis that infection might induce activation of innate processes that are protective in non-specific ways. The observation that CMV can activate NK functions, which then remain for long periods [42], supports such speculation. In this regard, it was shown almost 30 years ago that HCT recipients with CMV reactivation had less leukemia relapse that non-reactivators [70]. Epidemiologic studies have varied in regard to CMV seropositivity and risk of relapse, with some showing that seronegative transplants were at greater risk [70,71]. However, a large population analysis recently showed no difference in relapse-free survival at 2 years post-HCT based on CMV serologic status [72].

Recently, however, there have been several reports that CMV reactivation is associated with protection from relapse during the first year post-HCT [73–75]. Of note, there is differing duration of the putative protective effect of CMV infection versus no CMV. Elmaagacli et al. studied 266 acute myeloid leukemia (AML) patients and reported a significant reduction in relapse (9% vs. 42%, respectively) at 10 years' follow-up [73]. Green et al. studied 2566 patients, including AML, acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), lymphoma, and myelodysplastic syndrome (MDS), and reported a protective effect only in AML and only to day 100 post-HCT [74]. In the report of Manjappa et al., 264 AML patients were studied and CMV reactivation was associated with decreased relapse that was sustained for the overall group and for those with myeloablative (MA) conditioning [75]. Those with reduced intensity conditioning with antithymocyte globulin (ATG) had more CMV reactivation than did the MA group and a relapse rate that was similar to those with no CMV infection. In addition, similarly to the large sero-status study of Schmidt-Hieber et al. [72], an increased non-relapse mortality in the CMV-infected group was observed in two of these studies [74,75]. These studies were retrospective, and prospective studies are warranted to confirm a CMV-based protective effect on relapse. More importantly, if this is a function of the conditioning regimen, it is possible to design studies to determine what aspects of conditioning influence this protection. It is interesting to hypothesize that the known effects of CMV on activation of innate immunity could play a role here, but it will be equally important to determine the removal of the effect of CMV on non-relapse mortality. Ideally, it might eventually be possible to maintain an activation of the NK system similar to that provided by CMV but without the deleterious aspects of CMV in the HCT recipient.

Improved antiviral agents for CMV

The current US Food and Drug Administration (FDA)-approved CMV-specific antiviral agents have improved management of the allogeneic HCT recipient and better post-transplant GVHD prophylaxis appears to have enhanced this improvement [76]. However, the available antiviral treatments are less than optimal because of their toxicity and because most are not orally bioavailable. Maribavir is an orally bioavailable drug that acts by blocking the nuclear egress of CMV virions by inhibiting CMV UL97-mediated phosphorylation of nuclear lamin A/C and possibly US27. In a large phase III trial, 681 patients were recruited from 90 centers in Canada, the United States, and Europe, and maribavir failed to show efficacy when used prophylactically to prevent CMV disease. The results illustrated the importance of understanding CMV risk factors in the design of antiviral studies on HCT recipients [77]. Two new agents, brincidofovir (also known as CMX001) and letermovir (also known as MK-8228), are promising oral agents that, if safe and effective for prophylaxis of CMV, could change the management of CMV.

Brincidofovir is an orally bioavailable prodrug of cidofovir that was developed as a lipid conjugate of the synthetic nucleotide analog cidofovir with 400-fold greater in vitro potency against

CMV. It is absorbed in the small intestine where it is converted intracellularly to the active form, but, unlike cidofovir, it is not concentrated in the renal tubules and lacks the same renal toxicity. In a phase I dose-escalation study of the drug used prophylactically in 230 allogeneic HCT recipients, brincidofovir at a dose of 100 mg twice weekly significantly reduced the incidence of CMV infection compared with placebo (37% vs. 10%, respectively) [78]. Myelosuppression and nephrotoxicity were not observed, but diarrhea and other abdominal symptoms were dose limiting at 200 mg twice weekly. A phase III evaluation of brincidofovir is in progress (Trial NCT01769170).

Letermovir is an orally bioavailable agent that acts to inhibit CMV by a novel mechanism targeting CMV UL56/UL89, an enzyme important in the cleavage of virus DNA into unit-length genome size that permits packaging into new virions. Because of the unique mechanism of action, no cross-resistance has been seen with existing anti-CMV agents active against CMV UL54 DNA polymerase, namely ganciclovir, foscarnet, and cidofovir. Letermovir was studied as preemptive treatment in 27 kidney transplant recipients found to have CMV DNAemia; nine patients each received either 40 mg twice daily, 80 mg once daily, or standard treatment (SOC) [79]. The drug was safe, with no serious adverse events, had plasma trough levels consistently above concentrations needed for in vitro CMV inhibition, and CMV DNA clearance from blood not different from the SOC group.

CMV vaccines

A vaccine that could establish adequate T-cell immunity for protection from CMV infection has been a long-standing goal. Vaccines have targeted antibody production using CMV gB glycoprotein plus adjuvant [80] or T-cell immunity using CMV DNA or polypeptides [81,82]. Induction of anti-gB antibody in solid organ transplant patients was associated with shorter duration of DNAemia and fewer days of ganciclovir treatment [80]. In a phase II placebo-controlled trial of a CMV DNA vaccine (ASP0113, formerly TransVax, Vical/Astellas Pharma) encoding CMV pp65 and CMV-gB proteins in a poloxamer-based formulation involving 74 allogeneic HCT recipients, the vaccine reduced the occurrence, recurrence, and duration of CMV DNAemia [81]. However, the rates of clinically significant DNAemia requiring ganciclovir therapy were not different from placebo. ASP0113 is now in phase III development (Trial NCT01877655). Finally, a vaccine in which a CD8-specific CMV pp65 peptide epitope is fused to a helper cell epitope and used with a TLR9 agonist has been shown to be safe and immunogenic in volunteers [82] and is now being evaluated in allogeneic HCT recipients (Trial NCT01588015).

T-cell Immunotherapy for CMV infection

The first adoptive transfer of CMV-specific T cells was carried out more than 20 years ago [83] and, despite a positive outcome, this therapeutic method required limiting dilution cloning and was technically challenging to manufacture. In a study of 14 recipients, however, treated at 30–40 days post-allogeneic HCT with these ex vivo expanded CMV-specific donor $CD8^+$ T cells, cytotoxic anti-CMV immunity was enhanced and persisted for at least 12 weeks with no CMV viremia but did required CD4 help for effect [84]. To improve this method, short-term bulk culture of CMV-specific $CD8^+$ T cells, primed with CMV antigen-exposed dendritic cells, was used, and 16 patients were treated with 1×10^5 lymphocytes/kg at the time of CMV DNAemia while preemptive ganciclovir was withheld unless DNAemia increased [85]. Eight patients cleared the DNAemia without further treatment and eight required ganciclovir; two of these had recurrent infection when treatment was stopped [85]. To simplify the method further, CMV-specific CD8 cells were selected from donor blood by magnetic beads and directly injected into HCT recipients at the time of CMV DNAemia [86]. At a median dose of 8.6×10^3/kg, CMV-specific CD8 cells became detectable with 10 days and CMV DNAemia was cleared in eight of nine treated patients [86]. Although simpler in design, the non-availability of clinical-grade CMV-specific magnetic beads has curtailed the development of this method. To overcome this problem, a two-step purification method was developed in which donor cells were briefly primed in vitro with CMV pp65 protein and then IFN-γ-producing cells were isolated by magnetic beads [87]. In 18 treated patients with refractory CMV infection, 15 cleared or considerably reduced the persistent CMV DNAemia, and only one of 13 patients having post-infusion expansion of these T cells died of CMV disease [87]. Three CMV-related deaths occurred in patients in whom there was no expansion of infused T cells. Finally, using $CD8^+$ T cells expanded in vitro after exposure to Epstein–Barr virus (EBV)-expressing B cells previously infected with an adenovirus (AdV) modified to express CMV pp65, this "tri-virus" T-cell product had immune recognition of EBV, CMV, and AdV [88]. In 10 CMV-infected patients treated with the polyvalent T-cell product, all developed increased CMV-specific T-cell immunity as measured by ELISPOT assay, with clinical improvement in most [88]. The tri-virus cell product is currently being evaluated in an expanded access trial (NCT01945619) at Baylor College of Medicine, and a phase II trial using CMV pp65-primed T-cells for CMV disease is active at Memorial Sloan Kettering Cancer Center (Trial NCT01646645).

Conclusion

The availability of antiviral agents has greatly altered the management of the allogeneic HCT recipient, yet control of CMV remains less than optimal because of the toxicity of available agents. Even when used successfully to prevent early disease, the patient often remains at risk for late-onset disease. An immune deficiency underlies prolonged susceptibility to CMV in this population, and this continued inability of the host to develop protective immunity in some patients remains the ultimate challenge for improved treatment. It is possible that antiviral agents alone will never completely solve the problem of CMV infection after HCT. It remains to be determined whether less toxic antiviral agents, vaccines, or adoptive immunotherapy will provide a more effective method for preventing CMV disease after HCT.

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CHAPTER 88

Herpes Simplex Virus Infections

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Introduction

Herpes simplex virus (HSV) type 1 (HSV-1) and type 2 (HSV-2) are members of family *Herpesviridae* and subfamily *Alphaherpesvirinae*. HSV possesses the unique characteristics of latency and reactivation in response to various stimuli [1]. Although fatal infections are uncommon, HSV infection can lead to significant morbidity in the hematopoietic cell transplant (HCT) recipient. In the pre-antiviral prophylaxis era, HSV reactivation occurred commonly (70–80%) in the seropositive HCT recipient in the early post-HCT phase prior to engraftment [2,3]. The reduction in HSV infection is primarily the result of universal prophylaxis with an antiviral agent (acyclovir) following HCT [4,5]. This has become the standard of care across transplant centers. Although the problem of HSV infection has diminished substantially in the early pre-engraftment period, the major problems now are related to the delayed HSV onset after discontinuation of antiviral prophylaxis and antiviral resistance.

Virology

HSV-1 and HSV-2 belong to the genus *Simplexvirus* of the subfamily *Alphaherpesvirinae*. The morphologic appearances of all herpesviruses are similar and are comprised of four structural components: (1) an electron-dense core containing linear double-stranded viral DNA; (2) an icosadeltahedral capsid containing 162 capsomeres and measuring 100–110 nm in diameter; (3) an amorphous layer (tegument) between the capsid and envelope composed of proteins that have important role in the early viral replication on entry into the host cell; and (4) a lipid envelope with glycoprotein spikes [6].

Primary infection occurs when HSV enters the host cell via damaged skin or mucus membrane. This occurs by attachment of the virus to cell surface receptors mediated by various viral glycoproteins (e.g. C, B, and D). This leads to fusion of the viral envelope with the host cell plasma membrane resulting in entry of the nucleocapsid with the tegument in to the cytoplasm. Nucleocapsid releases the viral DNA into the nucleus via nuclear pores. Viral replication then proceeds in the nucleus with transcription, DNA synthesis, capsid assembly, DNA packaging, and formation of the envelope. The process of viral replication is regulated by stepwise expression of viral genes: alpha (α), beta (β), and gamma (γ). The gene products of α gene are regulatory proteins that shut off host protein synthesis and activate expression of β and γ genes. The β gene encodes enzymes involved in nucleic acid metabolism (e.g. thymidine kinase) and DNA synthesis (e.g. DNA polymerase). The proteins expressed by the β gene serve to induce γ gene expression, resulting in the synthesis of structural proteins and nucleocapsid [6]. The end result of primary lytic infection is cell death.

Pathogenesis

HSV is neurotropic and establishes latent infection, two unique properties of *Alphaherpesvirinae* that are central to the pathogenesis and pathology of HSV infection and disease. HSV infection occurs after close/intimate contact of a seronegative individual with someone having subclinical viral shedding or with active disease. The virus enters from mucosal surface or abraded skin, replicates at the site of primary infection [7], and undergoes retrograde transport to the dorsal nerve root ganglia where it multiplies in a small number of sensory neurons, resulting in lytic infection. HSV-1 predominantly leads to oropharyngeal infection and HSV-2 to genital infections. However, either virus can infect the oropharyngeal or genital mucosa. Thereafter, the virus may spread to the mucosal surface or skin with its characteristic vesicular lesions through centrifugal migration via peripheral sensory nerves. After primary oropharyngeal infection, latency is established in the trigeminal nerve root ganglion, and sacral nerve root ganglion in cases of primary genital infection. Other dorsal root ganglia, such as vagal, geniculate, and superior cervical ganglia, may harbor the virus [6]. In the infected neurons, viral genome exists in an episomal state for the life of the individual. HSV has been retrieved from involved ganglion explanted from cadavers [8]. However, during the latent state, infectious virus can no longer be recovered from the ganglia, although viral DNA can be recovered from some ganglion cells. Although a number of mechanisms have been proposed, the mechanism(s) whereby latency is established and maintained is not well understood [6]. It is likely that the latent infection of the neuron involves intrinsic non-permissive properties of sensory neurons and viral functions, and maintenance likely involves the CD8 T cells [6,9,10]. Latency permits the virus to survive in a sequestered state not vulnerable to the inhibitory effects of antiviral agents or the host's immune system.

Reactivation of HSV from its latent state is a characteristic feature of this infection. A number of stimuli have been closely associated with reactivation: physical or emotional stress, fever, exposure to ultraviolet light, tissue damage, and cell-mediated immune suppression. Many of these risk factors are present in the recipients of HCT in the early and late phases. It is likely that the tissue damage with associated changes in cytokine profile and the repair process may influence reactivation along with immune suppression [11]. Reactivation may result in asymptomatic shedding or clinical

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disease. This phenomenon occurred in 82% of seropositive HCT recipients prior to the advent of antiviral prophylaxis [2]. Since the introduction of antiviral prophylaxis, reactivation has declined significantly [3]. In HCT recipients, virtually all HSV infections are related to reactivation of latent virus. The most commonly affected sites include the oropharyngeal mucosa and genital tract. Disseminated disease with visceral organ involvement has been observed in the severely immunosuppressed HCT recipient. The major factor in the pathogenesis of HSV disease in HCT appears to be impaired cell-mediated immune response.

Immunology

General aspects

Innate and adaptive immune responses play an important role in the control of viral infection [12]. Innate immune responses are the first line of defense and have direct antiviral effects. They involve Toll-like receptors (TLRs) (and in HSV infection involve TLR2 and TLR9), natural killer (NK) cells, macrophages, and dendritic cells. TLRs are expressed on various cells including dendritic cells, and mediate interferon production [12]. Increased shedding and reactivation of HSV has been associated with TLR2 polymorphisms [13] and a recent study suggested that polymorphisms in TLR3 may confer resistance to HSV-2 infection [14]. Severe HSV disease has been described in two patients with myelodysplasia who had undetectable NK cells and plasmacytoid dendritic cells in the blood can also suggest the role of innate and acquired immunity in controlling HSV infection in an immunocompromised host [15].

However, it is the adaptive immune response, primarily the cell-mediated immunity (CD4 T cells, CD8 T cells), that is crucial in controlling HSV infection (especially primary infection) [6,16]. This is also supported by the observation that patients with agammaglobulinemia handle HSV infection adequately, and the presence of neutralizing antibody does not prevent HSV reactivation. In contrast, patients with T-cell immune deficiencies (transplant recipients, those infected with human immunodeficiency virus, and congenitally T-cell-deficient patients) tend to have more widespread, invasive, and disseminated disease [17]. In animal models, it appears that T cells play a major role in preventing dissemination of HSV [18], although antibodies can reduce the viral titers. Cytotoxic T-lymphocyte (CTL) response correlates with viral clearance in recurrent HSV infection and is mediated by CD8 T cells with the support of NK cells and CD4 cells [17]. Cytokines also play a role in the immune response to HSV infection, including latency [19].

Immune evasion

HSV can evade and modulate the multipronged host immune response, which in turn enables it to establish latency that subsequently leads to episodes of recurrent infection. It can directly inhibit dendritic cell function, downregulate the expression of major histocompatibilty complex (MHC) I and II molecules, subvert apoptosis of virally infected cells, evade CTLs, avoid antibody-mediated virus neutralization, block antibody-dependent cytotoxicity, and induce the production of immunosuppressive cytokines such as interleukin-10 (IL-10) [6,20].

Immunology in HCT

Almost all of the components of both the innate and adaptive immune system are deficient after the HCT conditioning regimen has been administered [21]. It is probably the combination of mucosal damage, induced by cytotoxic agents of the conditioning regimen, and global but primarily T-cell-mediated immune deficiency that is responsible for the high reactivation rate seen after HCT. Meyers et al. evaluated HSV-specific lymphocyte stimulation responses in patients undergoing HCT in the absence of antiviral prophylaxis [2]. This study identified that the specific lymphocyte transformation responses remained low for the first 40 days following HCT and then returned to pre-HCT levels. The return of the HSV-specific lymphocyte response was independent of the immune status of the donor, acute graft-versus-host disease (GVHD), underlying hematologic disorder, or use of T-cell-depleting agents. However, the immune response correlated with the development of HSV reactivation. Hence it appears that the reconstitution of the HSV-specific immune response requires exposure to antigen in the form of reactivated HSV infection, and passively transferred donor lymphocytes are insufficient to establish immune reactivity in the HCT recipient.

Delayed restoration of HSV-specific lymphocyte responses has been observed when early-onset HSV infections (after HCT) were treated with acyclovir [11]. This delay in response was manifested by frequent HSV recurrences interspersed by shorter intervals between HSV episodes. Similarly, delayed reconstitution was encountered among HSV seropositive patients receiving acyclovir prophylaxis [5]. Not only was reconstitution delayed, but also a significantly lower lymphocyte response was observed in patients receiving acyclovir compared with those on placebo within the first 7 weeks of HCT. This response slowly improved until it matched the placebo group at 3 months after HCT. A more recent study in a group of cord blood (CB) transplantation recipients receiving antiviral prophylaxis demonstrated that HSV-specific T-lymphocyte responses may develop in the first 29–100 days after CB-HCT. The authors concluded that naïve T lymphocytes could contribute to the generation of antigen-specific T-lymphocyte immune reconstitution early after HCT [22]. This is in contrast to earlier studies which suggested that antigen exposure was a prerequisite for early immune reconstitution [5,11]. However, the significance of later versus earlier immune reconstitution is unknown.

Epidemiology

HSV-1 and HSV-2 have a worldwide distribution, and there are no animal vectors or reservoirs. HSV-1 infection predominantly leads to orolabial infection, is more prevalent, and is acquired earlier in life. HSV-2 leads to genital herpes and is transmitted sexually. However, recent epidemiologic investigations suggest increasing rates of genital infection caused by HSV-1 [23].

In recipients of HCT, HSV disease is mostly due to reactivation and the serologic status of the recipient determines the risk for disease, and therefore the need for prophylaxis. The likelihood of any one individual being HSV-1 or HSV-2 seropositive is dependent on a number of factors, including age, gender, race, geographic region, socioeconomic status, and sexual activity. However, once the transplantation is initiated, these factors lose importance and reactivation/disease is a function of the cytotoxic conditioning regimen and immune dysfunction.

Primary HSV-1 infections usually occur in young children under the age of 5 years and are usually asymptomatic. The seroprevalence of HSV-1 slowly increases with age until a 90% rate is achieved by the fifth decade of life [24]. In the United States, African Americans acquire HSV-1 earlier in life. A national survey conducted by the Centers for Disease Control [US National Health and Nutrition Examination Surveys (NHANES)] from 1999 to 2004 estimated the overall prevalence of HSV-1 in US adults at 57.7%, compared with 62% in the period 1988–1994, suggesting a decrease in seroprevalence. In this survey, a higher percentage of individuals with HSV-1 were diagnosed with genital herpes [25]. More recently, this trend of increased genital HSV-1 infection was confirmed in a large clinical trial [23]. The prevalence of HSV-1 antibody inversely correlates with economic status, with a higher prevalence in the lower socioeconomic groups [24,26].

HSV-2 is usually acquired later in life with antibodies beginning to appear in puberty, associated with the initiation of sexual activity. The HSV-2 seroprevalence amongst US adults is approximately 17%, and there has been a downward trend [25]. HSV-2 seroprevalence is higher in women [23]. There is considerable variability of HSV-2 seroprevalence based on ethnicity, with 25% in white women, 20% in white men, 80% in African American women, and 60% in African American men.

In recipients of HCT, 82% of the seropositive patients had HSV reactivation prior to the institution of routine antiviral prophylaxis [2]. The median time to the onset of infection was during the third week after HCT. Approximately 90% of the infections involved orolabial mucus membranes, while 6% had genital infection. Primary HSV infections occurred infrequently and were observed in only 1.5–2% of HCT recipients [2,27]. The incidence of HSV infection has decreased since the introduction of antiviral prophylaxis and onset shifted to a later time period. The incidence of reactivated HSV infection has been reported at 0 and 21% in HCT recipients on antiviral prophylaxis in two separate studies, compared with 70 and 68% in the placebo groups [3,5]. The phenomenon of viral shedding despite antiviral prophylaxis is well described and may contribute to disease development. HSV reactivation tends to occur after cessation of prophylaxis, and in one study was reported to occur at a median of 25 days in 70% [3]. Another study reported reactivation at median of 8 weeks in 38% of the seropositive HCT patients [5]. The development of HSV disease in HCT recipients has been associated with protracted cellular immunodeficiency (such as occurs with haploidentical/T-cell-depleted HCTs) [28], type of HCT (matched unrelated and mismatched) [29], and severity of GVHD [30]. Therefore, multiple factors contribute to the epidemiology of HSV infection in the setting of HCT.

Clinical manifestations

The manifestations of HSV infection are dependent on the anatomic site involved, the immune status of the host, and the HSV serotype. Oropharyngeal/orofacial infection predominates after HCT [2], followed by infection of the genital or perianal area [31]. Other sites that are infrequently involved include the esophagus, lung, liver, and central nervous system (CNS), usually in the setting of dissemination.

Oropharyngeal/orofacial infection

The majority (92%) of HSV infections present with oropharyngeal or orofacial lesions, irrespective of the use of antiviral prophylaxis [2,31]. In an observational study by van der Beek et al., the polymerase chain reaction (PCR) methodology was employed to assess oral viral shedding and its correlation with the presence of oral ulcers/mucositis in HCT recipients in the absence of antiviral prophylaxis [32]. Multivariate analysis revealed that the HSV-1 viral load was a positive predictor of ulcerations on the keratinized mucosa (odds ratio 1.35, $p = 0.0005$) but not for ulcerative mucositis. Mucositis following HCT is often confused with HSV infection. Woo et al., demonstrated that HSV does not play a major role in mucositis in HCT recipients receiving acyclovir prophylaxis [33]. Mucositis-related ulceration tends to involve non-keratinized mucosa, in contrast to HSV which affects keratinized mucosa.

Primary infection in the normal host is characterized by painful gingivostomatitis and pharyngitis that are often accompanied by fever and lymphadenitis. This presentation is uncommon in HCT recipients as disease is mostly due to reactivation. Patients may present with a typical vesicular rash located at the vermilion border of the lips, nasolabial fold (Figure 88.1a), or palate that progresses to ulcerative lesions. More serious oral disease may manifest as severe mucositis with painful deep ulcers (Figure 88.1b). In the setting of severe immunosuppression, HSV disease may spread to the esophagus or trachea. Bacterial or fungal (especially *Candida*) superinfection can occur. Pain is a common complaint. Response to treatment is typically slow and may take weeks to resolve.

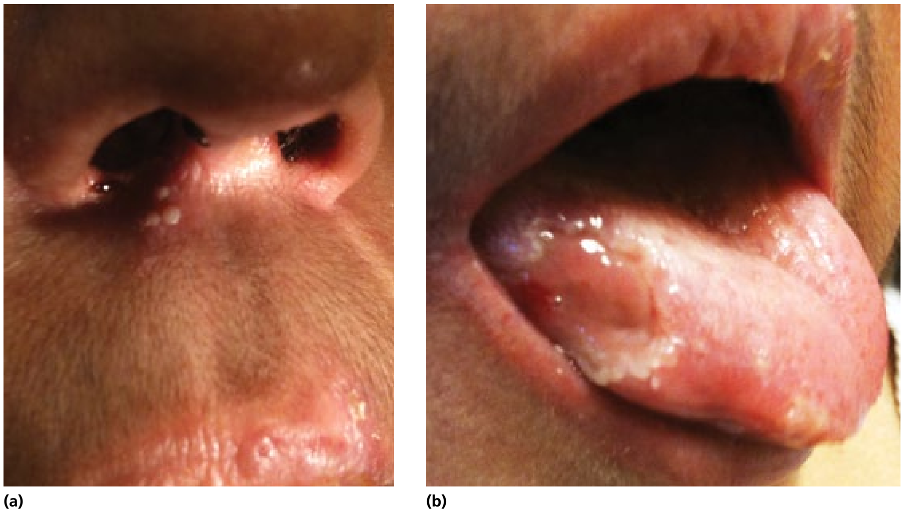
(a) (b)

Figure 88.1 (a) Vesicular lesions of HSV along the nasolabial fold in an HCT recipient. (b) HSV ulceration of the tongue caused by an acyclovir-resistant strain.

Genital/perianal infection

In the pre-antiviral prophylaxis era, about 6% of HCT recipients developed genital HSV [2]. However, with the use of antiviral prophylaxis, reactivation of genital HSV was noted in 15% of patients [31], possibly reflecting the relatively higher acyclovir resistance of HSV-2 compared with HSV-1.

Primary infection presents with fever, inguinal adenopathy, and vaginal or urethral discharge, and the symptoms do not differ based on the HSV type. However, HSV-2 is associated with more frequent recurrences of genital herpes compared with HSV-1, and may be associated with recurrent aseptic meningitis. As mentioned previously, primary HSV infection is uncommon after HCT, and genital HSV is primarily due to reactivation from the sacral ganglia. It usually presents as patches of vesicular lesions in and around the genital area and may be associated with itching, burning, or pain. Symptoms of urethritis or cystitis may be present. The vesicles typically break down to form shallow, painful ulcers that may coalesce to form large, deep ulcers. Infection can also involve the perianal skin, buttocks, and hips. Inguinal lymphadenopathy can occur. Rectal involvement may also occur and is typically associated with severe pain, tenesmus, and constipation, and may cause rectal bleeding. Acute episodes of genital HSV (both primary and recurrent) can be complicated with acute urinary retention, constipation, and neuropathic pain. This has been attributed to autonomic dysfunction related to infection [34]. The unilateral occurrence of lesions can be confused with varicella zoster virus (VZV) infection. Uncommonly, cytomegalovirus (CMV) infection may lead to ulcerative lesions in the genital/perineal area [35]. The differentiation between these conditions is important for appropriate management.

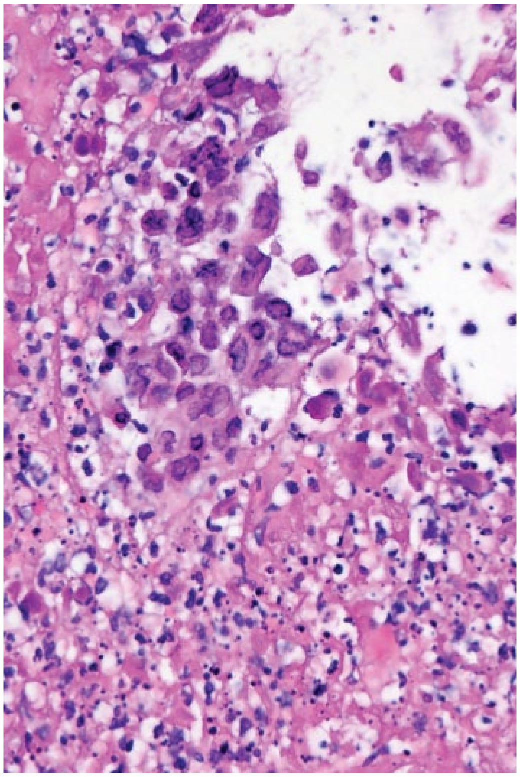

Figure 88.2 Histology of HSV esophagitis: ulceration with cellular changes depicting viral inclusions.

Gastrointestinal infection

This entity has become rather uncommon with the use of antiviral prophylaxis. The most commonly involved site is the esophagus. HSV esophagitis may develop as a consequence of direct extension from the oropharynx or by reactivation of latent HSV from the vagus nerve [36]. The symptoms of HSV esophagitis include persistent nausea and vomiting, dysphagia, and retrosternal pain [37], although acute GVHD, CMV, and *Candida* can cause similar symptoms [38]. Onset can be abrupt but distinguishable from heartburn. HSV causes ulcerative disease and endoscopic examination is necessary to establish diagnosis. McDonald et al. provide a detailed description of the endoscopic findings of HSV esophagitis [37]. The infection progresses through different stages: discrete raised vesicles initially, followed by ulceration, and eventually diffuse mucosal necrosis. However, biopsy of the abnormal area and culture is necessary to make a diagnosis, as clinical symptoms, radiographic studies and morphology seen on endoscopy cannot distinguish HSV infection from other etiologies, especially CMV or VZV (Figure 88.2).

Intestinal HSV infection is extremely uncommon after HCT, and the diagnosis is difficult to establish. In two of three HCT recipients with intestinal HSV infection, diagnosis was made by assessment of surgical specimens obtained at laparotomy [39,40], and one had an endoscopic examination with a colonic biopsy [41]. All developed disease in the first 50 days after HCT and had documented GVHD. Of the two patients who underwent laparotomy, one had ileus, diffuse abdominal pain, ascites, and bleeding [39], and the other had abdominal pain, diarrhea, and bleeding [40]. Both had involvement of the small bowel. Diagnosis was established by histological examination and confirmed as HSV-1 by culture. Both died from uncontrolled hemorrhage despite surgical intervention. The third case had documented genital HSV-2 breakthrough infection 36 days after HCT, and 11 days later developed diarrhea. Endoscopic examination revealed ulcerations in the ileocecal area and biopsy culture grew HSV-2 and CMV. However, the histology was negative for viral inclusions. Full recovery was achieved with antiviral treatment that was confirmed by repeat endoscopic examination with biopsies [41]. It is plausible that the patients with fatal infection probably had disseminated visceral infection, but no discernible site of reactivation was observed by the authors. However, the last case illustrates the potential involvement of the colon in conjunction with genital HSV-2 infection due to reactivation from the sacrococcygeal ganglia rather than disseminated infection.

Hepatitis is fairly rare but causes significant mortality. In one series, eight cases were identified over a 14-year period in an HCT population base of 3000 recipients [42]. HSV-1 and HSV-2 were equally distributed in this group. The most common presentation was fever, abdominal pain, and absence of herpetic skin lesions. The median peak aspartate aminotransferase level was 1565 units/L. In only two cases was the diagnosis considered ante mortem and treatment initiated. All of the cases were fatal. HSV hepatitis represents true dissemination as multiple organ involvement was found in the majority of the cases. In this study, cases from the preprophylaxis era occurred before day 20, whereas those who received prophylaxis did not develop disease until after day 40.

Respiratory infection

It is generally believed that HSV-1 respiratory infection results from an extension of oropharyngeal infection into the tracheobronchial tree. Necrotizing HSV pneumonia develops by extension of herpetic tracheobronchitis (which may be facilitated by the presence of an endotracheal tube), whereas diffuse interstitial

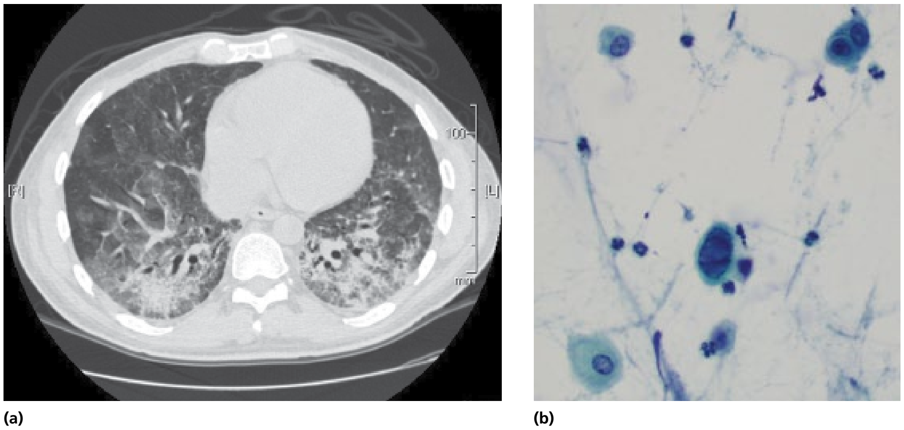

Figure 88.3 (a) HSV pneumonia in an HCT recipient: CT scan image shows mix of ground-glass interstitial infiltrates, nodular changes, and consolidation. (b) BAL fluid cytology from a patient with HSV pneumonia, depicting cellular changes of HSV with typical viral inclusions.

pneumonitis results from hematogenous dissemination [36]. Approximately 6–8% of interstitial pneumonia after HCT is attributed to HSV [43]. Both HSV-1 and HSV-2 can cause pneumonia [43,44]. Clinical presentation includes fever, dry cough, chest pain, dyspnea coupled with hypoxemia, and pulmonary infiltrates on imaging study (Figure 88.3a). Radiographic findings with high-resolution chest tomography (HRCT) scan can be diverse, such as diffuse ground-glass interstitial infiltrates, small centrilobular nodules, and air space consolidation [44]. HSV pneumonia has a high fatality rate (>80%) [43], which does not appear to have changed in the era of antiviral prophylaxis [45]. Diagnostic evaluation involves bronchoscopy with bronchoalveolar lavage (BAL) for viral culture and cytology (Figure 88.3b). Careful assessment needs to be made when attributing pneumonia to HSV based on respiratory cultures, especially in an individual at low risk for HSV pneumonia. This is important as oral shedding (asymptomatic or symptomatic) of HSV is commonly found in HCT patients and can lead to contamination of cultures by the virus.

Central nervous system

HSV encephalitis is a rare complication after HCT. It occurs in the general population with a frequency of 1 in 250,000–500,000 persons per year, and does not appear to be more common in HCT [46]. In the "normal host," the clinical presentation consists of a subacute onset of fever and focal, primarily temporal lobe, neurologic signs and symptoms. In an immunocompromised host, the presentation may be atypical, with a subacute but progressively deteriorating course [47]. However, an HCT recipient was recently described to have presented with classic HSV encephalitis manifesting with anomia, focal findings of temporal lobe encephalitis on magnetic resonance imaging (MRI) of the brain, abnormal electroencephalogram (EEG), and confirmed with positive cerebrospinal fluid (CSF) PCR for HSV [48]. CSF assessments with HSV PCR, MRI, and EEG are diagnostic mainstays. Other herpesviruses should be excluded, such as HHV-6, CMV, and Epstein–Barr virus (EBV).

Other syndromes

HSV-associated erythema multiforme (EM)

EM is a newly described entity in HCT, and considered to be an autoimmune phenomenon. Expression of the HSV DNA polymerase gene in skin lesions is considered to be the hallmark of this condition in the absence of local HSV replicative infection. An interesting observation of erythematous eruptions in the setting of HCT was reported by Burnett et al. [49]. Rash is a frequent event after HCT, and Burnett et al. described a diffuse erythematous rash (maculopapular, bilaterally symmetrical with predilection for acral regions, evolving into plaque-like lesions followed by desquamation) that occurred 3–15 days after HCT in 20 patients. Of the 19 patients with rash, 79% of skin biopsies had expression of HSV DNA polymerase (without molecular evidence of active HSV infection) compared with 75% in herpesvirus-associated EM. Therefore, it was concluded that this syndrome falls into the spectrum of EM. Chronic suppressive treatment with acyclovir is suggested in those with severe EM associated with HSV infection [50]. The therapeutic implication of this finding is unclear, since HCT recipients are usually on a prophylactic antiviral agent, and increasing the dose may not be justifiable in the absence of active infection.

Eye infection

HSV eye infections are uncommon in HCT. They may present with keratitis secondary to reactivation of HSV from the trigeminal ganglia or autoinoculation from a distant site of active HSV infection (such as oral or genital). GVHD of the eye may predispose to HSV infection due to impairment of local defenses or abnormal tear fluid [51]. HSV chorioretinitis is well described in the setting of HIV infection but rare in HCT. Progressive outer retinal necrosis can present with rapidly progressive visual impairment, leading to blindness, and requires an aggressive management approach in consultation with an ophthalmologist. VZV can cause a similar presentation and needs to be ruled out.

Herpetic whitlow

This is an HSV infection of the finger. Clinical manifestations include pain, swelling, erythema, fever, local tenderness, local lymphadenopathy, and the presence of lesions on the affected finger. This condition can result from autoinoculation or contact with bodily secretions (oral or genital) that contain virus. Management entails the use of an antiviral agent. Surgical debridement should be avoided.

Diagnosis

Identification of HSV infection relies upon both clinical suspicion and laboratory confirmation. Serology is of very little use in the post-HCT setting as HSV infection is mostly due to reactivation of latent infection. Its role in HCT candidates is to determine past HSV exposure. Moreover, it has been reported that the risk of HSV reactivation is proportional to the pre-HCT HSV immunoglobulin G (IgG) titer [52]. Glycoprotein G-directed IgG antibody differentiates between HSV-1 and HSV-2 [53].

The presence of characteristic vesicular lesions in clusters on an erythematous base near or around perioral or genital areas can lead to clinical diagnosis. However, this stage is often missed and ulcerative lesions are frequently encountered. Distinguishing oral HSV lesions from mucositis caused by HCT-related conditioning or alternative pathology may be difficult, while genital HSV may present with atypical lesions. In these situations, viral culture and/or PCR for HSV DNA should be obtained from the vesicle or ulcer base. Scraping of the lesion should be considered for rapid diagnosis. The specimen can be examined microscopically after Giemsa (Tzanck preparation), Wright, or Papanicolaou staining to assess for the presence of intranuclear inclusions and/or multinucleated giant cells suggesting herpesvirus infection. However, this does not differentiate HSV from VZV infection, and therefore the preferred test is detection of HSV (and VZV) antigen or DNA detection. The scrapings can be submitted for antigen detection by direct fluorescent antibody (DFA), peroxidase, or enzyme-linked methods. PCR is more sensitive than culture and should be considered when evaluating for suspected HSV infection [54]. In the instance of a respiratory tract specimen, BAL fluid should be examined for HSV-related cellular changes when HSV pneumonia is suspected.

The gold standard of diagnosis is virus isolation. The positive culture will also provide the opportunity to test for antiviral resistance. Culture for HSV is recommended when acyclovir resistance is suspected. Clinical specimens submitted for culture should be transported on ice and immediately inoculated into a cell culture system. A cytopathic effect may develop within 24–48 hours, but definitive identification may take from 48 to 96 hours. A more rapid culture technique is the spin-amplified culture, or shell vial culture, with subsequent staining for specific HSV antigen. The shell vial culture result can be reported in less than 24 hours. PCR is the method of choice for the diagnosis of CNS HSV infection [55].

Tissue biopsy is necessary to confirm the diagnosis of visceral HSV infection. The tissue should be submitted for culture and histologic examination. The demonstration of tissue pathology is important in differentiating between disease, shedding of virus, and contamination. Viral inclusions suggestive of HSV infection are often absent in necrotic cells seen on tissue sections [37] and, if detected, difficult to differentiate from CMV or VZV. The use of techniques such as immunostaining or *in situ* hybridization can help in confirming infection [37,39,40]. This approach may potentially expedite a diagnosis while waiting for viral culture.

Management of HSV infection in HCT

Acyclovir remains the drug of choice for treatment of HSV infection since its introduction into clinical practice in the early 1980s. Other antiviral agents in clinical use with activity against HSV are foscarnet and cidofovir (Table 88.1). The approaches to the management of HSV in HCT include (1) treatment of reactivated infection and (2) prevention with antiviral prophylaxis. These approaches have reduced the burden of disease. However, antiviral resistance to acyclovir and other agents is an emerging issue and poses therapeutic challenges [28,32,45,56–62].

Table 88.1 Antiviral prophylaxis and treatment for HSV infection

Prophylaxis
Acyclovir 250 mg/m^2 or 5 mg/kg intravenously every 12 hours, or
Acyclovir 400–800 mg orally every 12 hours, or
Valacyclovir 500–1000 mg orally every 12 hours
Treatment
Mucocutaneous HSV infection
Acyclovir 250 mg/m^2 or 5 mg/kg intravenously every 8 hours, or
Acyclovir 400 mg orally 5 times per day, or
Valacyclovir 500–1000 mg orally every 12 hours, or
Famciclovir 500 mg orally every 12 hours
Visceral/disseminated/CNS disease
Acyclovir 500 mg/m^2 or 10 mg/kg intravenously every 8 hours
Acyclovir-resistant HSV infection
Foscarnet 40–60 mg/kg intravenously every 8 hours
Acyclovir/foscarnet-resistant HSV
Cidofovir 5 mg/kg intravenously once weekly x 2, then every 2 weeks, or
Cidofovir 1 mg/kg intravenously once every other day or three times per week
Other considerations
In the setting of mucocutaneous infection that is severe, slowly resolving or intolerable side effects of systemic antiviral treatment, may consider topical 5% cidofovir gel

Acyclovir is a nucleoside analog and requires phosphorylation by viral thymidine kinase (TK) and cellular enzymes to result in the formation of acyclovir triphosphate. Viral DNA polymerase is competitively inhibited by acyclovir triphosphate, leading to its antiviral effect [63]. It is usually given intravenously but can be given orally if the patient is capable of taking and absorbing the oral formulation. Intravenous acyclovir was compared with placebo in one of the early double-blind studies [64], and shortened the time to resolution of pain (10 versus 16 days), crusting of lesions (7 versus 14 days), healing (14 versus 28 days), and duration of culture positivity (3 versus 17 days). This study is the basis for the current standard of therapy: acyclovir 250 mg/m^2 or 5 mg/kg intravenously every 8 hours for at least 7 days. Oral acyclovir therapy for mucocutaneous HSV given as 400mg five times per day for 10 days demonstrated similar benefit [65], although the oral route should be considered only if the gastrointestinal absorptive capacity is considered to be adequate. In immunocompromised HCT patients, antiviral treatment should be continued until mucocutaneous lesions have completely resolved. Valacyclovir (valyl ester of acyclovir), and famciclovir (prodrug of penciclovir) result in higher serum levels and can be used, although they have not been extensively studied in HCT patients. If the patient has severe mucocutaneous, visceral, disseminated, or CNS disease, acyclovir 10 mg/kg every 8 hours is recommended. Each dose should be infused slowly over 1 hour and adequate hydration maintained to avoid renal dysfunction, which can result from crystallization of the drug.

Acyclovir resistance should be suspected in patients who worsen despite adequate therapy with acyclovir, individuals with multiple episodes of recurrent HSV, and the highly immunocompromised (such as GVHD grade >2, haploidentical HCT) [28,57]. Foscarnet is the drug of choice for suspected or proven acyclovir-resistant HSV infection. It is a trisodium salt of phosphonoformic acid and inhibits viral DNA polymerase without requiring TK activity and therefore is active against TK-deficient HSV. It is dosed at 40 mg/kg every 8 hours, and for severe disease or visceral involvement, 60 mg/kg every 8 hours is recommended. The major and limiting toxicity of foscarnet is nephrotoxicity, making it essential to avoid other nephrotoxic agents and maintain hydration. It also causes electrolyte imbalance, mainly hypocalcemia, hypokalemia, and hypophosphatemia. All of these toxicities make it difficult to use foscarnet in the HCT setting. Foscarnet-resistant strains have been reported [56], and linked to a mutation in viral DNA polymerase, which affects binding of the drug to the active site [63]. In such cases, cidofovir has shown some benefit and is recommended at 5 mg/kg intravenously once per week for 2 weeks, and then once every 2 weeks [66]. It acts by inhibiting viral DNA polymerase. The major limiting factor is nephrotoxicity, attributed to accumulation of the drug in the renal cortex. Probenecid is given with cidofovir to reduce its nephrotoxicity. A dosing regimen of cidofovir 1 mg/kg three times weekly regimen has been described in treating adenovirus infection [67]. No dose-limiting nephrotoxicity was observed and all patients resolved infection. Hence this dosing regimen has been used for acyclovir- and foscarnet-resistant HSV infections in HCT by the authors. However, no data are available for HSV infections. Few reports documenting the success of topical cidofovir in clearing acyclovir/foscarnet-resistant mucocutaneous infection in HCT have been published [68,69]. Cidofovir-resistant HSV strains have been described in HCT recipients [61,62]. Fortunately, these isolates remained sensitive to both acyclovir and foscarnet.

Topical trifluridine, idoxuridine, and vidarabine are used mainly in the context of HSV keratitis. New drugs in development include CMX001 and helicase–primase inhibitors [70]. CMX001 is an orally administered lipid conjugate of cidofovir, with excellent activity against HSV-1 and numerous other DNA viruses (e.g. CMV, adenovirus, smallpox). Helicase–primase inhibitors (ASP2151 – recently compared with valacyclovir for the treatment of genital herpes) inhibit viral DNA synthesis by interfering with the helicase–primase of HSV [71]. However, none of these drugs are approved for clinical use at present.

Prophylaxis

Acyclovir prophylaxis should be offered to all seropositive allogeneic recipients to prevent HSV reactivation during the early post-HCT period, as recommended by the Centers for Disease Control and Prevention (CDC), the Infectious Diseases Society of America (IDSA), the American Society for Blood and Marrow Transplantation (ASBMT), and others [72,73]. Despite the official recommendations and the fact that most HCT centers use HSV prophylaxis, the decision regarding the use of universal prophylaxis is not without controversy. Significant variability in practice exists between transplant centers [4]. The recommended regimen is either acyclovir 200 mg orally three times per day or 250 mg/m^2 intravenously every 12 hours. Valacyclovir is a recommended alternative. Antiviral prophylaxis should commence at the beginning of conditioning therapy and be continued until engraftment or resolution of mucositis, that is, approximately 30 days after allogeneic HCT. The duration of prophylaxis can be extended beyond 30 days for those who develop GVHD or with "frequent HSV reactivation." Viral shedding and reactivation of HSV have decreased dramatically with prophylaxis, yet disease occurs when prophylaxis is stopped [3,5]. The frequency of reactivation and severity of disease are decreased when prophylaxis is extended beyond 30 days [74,75]. Delayed immunologic reactivity to HSV antigens and specific immune reconstitution have been observed with antiviral prophylaxis [5]. Whether this delay is detrimental or has benefit is unknown. Another argument for prophylaxis is the observation that resistant virus emerges on treatment but rarely with prophylaxis [76,77].

The efficacy of long-term antiviral prophylaxis has been evaluated [77,78]. Three cohorts of HSV and VZV seropositive patients received prophylaxis for either 1 month, 1 year, or longer than 1 year after HCT. The 2-year probability for developing HSV disease was 31.6, 3.9, and 0%, respectively. Hence long-term prophylaxis resulted in less disease/reactivation, and also prevented the emergence of drug-resistant HSV. Higher than recommended prophylactic doses were used (acyclovir 800 mg orally twice daily or valacyclovir 500 mg twice daily after engraftment) and no hematologic toxicity was noted. It is plausible that higher doses of antiviral agent used translated into increased serum levels, thereby preventing the emergence of resistance. Long-term (at least 1 year) prophylaxis is considered to be the standard of care for VZV prevention in HCT at most transplant centers. Hence the burden of recurrent HSV disease and antiviral resistance should decrease. There are reports of earlier engraftment in patients receiving acyclovir prophylaxis [5], but this has not been confirmed by others [52,79].

The ultimate preventive measure is the development of a vaccine that will prevent primary HSV infection in the general population, which has been a challenge [80]. Preferably, it would be a killed, subunit, replication-defective viral or naked DNA vaccine, as a live vaccine would have the unknown potential of reactivating during HCT. The most recent vaccine was not efficacious in preventing genital herpes [80], and there will be no effective vaccine in the near future. Also, it is unlikely that a vaccine will benefit those who are already seropositive, especially in the prevention of HSV reactivation in the profoundly immunocompromised seropositive population of HCT recipients.

Conclusion

Since the inception of acyclovir prophylaxis, the incidence of HSV reactivation and disease has decreased dramatically during the early period of HCT. However, prophylaxis as now administered (until engraftment or approximately 30 days) under current recommendations and has resulted in delay of HSV-specific immune reconstitution, and reactivation now occurs during the later period of HCT. However, severe, visceral, and disseminated HSV infection still occurs and demands rapid and specific diagnosis in order to initiate effective therapy. Although resistant virus is less likely to occur under prophylactic conditions, it remains a significant problem in treating patients with HSV infection after HCT. A possible solution to the late reactivation after early cessation of prophylaxis and the problem of emergence of acyclovir-resistant HSV may be to extend the duration of HSV prophylaxis (in addition to using higher doses than currently recommended). At the same time, this would protect the HCT recipient against VZV reactivation, which may be an even more devastating infection. However, further examination of this approach will have to be undertaken before a recommendation for longer duration of prophylaxis can be made.

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CHAPTER 89

Varicella Zoster Virus Infections

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Introduction

Varicella zoster virus (VZV), like other pathogens of the herpesvirus family, can cause severe infections in hematopoietic cell transplant (HCT) recipients [1,2]. As in other immunodeficient patients, serious VZV disease after transplantation is related to the compromise of T-lymphocyte function [3,4]. VZV infections are encountered in HCT patients who are experiencing their initial contact with the virus or who have recurrent disease due to the reactivation of latent virus. Primary VZV infection is manifest clinically as varicella or "chicken pox," in which the exposure of a susceptible individual, who has not had previous VZV infection, to the virus results in systemic symptoms of fever and malaise, and a characteristic vesicular rash. After primary infection, VZV establishes latency in cells of the dorsal root ganglia. Serum immunoglobulin G (IgG) antibodies to VZV provide evidence of a past primary infection and indicate that the individual is latently infected with the virus. The reactivation of endogenous latent VZV usually causes herpes zoster, in which the vesicular eruption appears in a localized, dermatomal distribution. Recurrent VZV infection also presents as atypical, non-localized herpes zoster in HCT recipients that cannot be distinguished from varicella by its clinical manifestations. Both primary and recurrent infections of VZV can cause significant morbidity and, in some cases, even mortality in HCT recipients.

In addition to describing the clinical patterns of VZV infection after HCT, significant progress has been made towards understanding viral pathogenesis and the host response to VZV among HCT recipients. Fortunately, most disease caused by either primary or recurrent VZV infection, when recognized promptly, can now be treated effectively with antiviral therapy. Although live attenuated VZV vaccines are available for the prevention of varicella in the pediatric population and zoster in the elderly population, these vaccines are not recommended for use in immunocompromised patients. Instead, an experimental inactivated varicella vaccine has been tested in HCT recipients. Prophylactic approaches using antiviral drugs have also been evaluated.

The virus

VZV is a member of the alphaherpesvirus subgroup of the herpesvirus genus. It is an enveloped virus that has a double-stranded DNA genome surrounded by an icosahedral capsid [5]. The VZV genome contains approximately 125,000 base pairs, arranged as long unique and short unique segments, each of which contains terminal repeat sequences. The VZV genome has coding regions for at least 70 distinct genes. Information about most of the VZV gene products is limited, but VZV proteins regulate viral gene transcription during replication and form the viral capsid, tegument, and virion envelope structures. Probable functions for some VZV proteins have been demonstrated by making targeted mutations in the viral genome and assessing the consequences; other gene functions have been deduced from homologies with herpes simplex virus, type 1 (HSV-1), which is the prototype of the alphaherpesvirus subgroup. Like HSV, replication of VZV usually involves synthesis and activation of a viral thymidine kinase which makes the virus susceptible to inhibition by the antiviral agent acyclovir and related drugs. However, since the thymidine kinase is not required for VZV replication, mutant strains that do not express this antiviral target can be selected by exposure to the drug and may cause infection similar to the wild-type virus. The viral glycoproteins gB, gC, gE, gH, and gI and also viral proteins with structural and regulatory functions, including the IE4, IE62, and IE63 proteins, are known to be targets of the host response following VZV infection. VZV is spread as cell-free virus to susceptible hosts, but is transmitted by cell-to-cell spread in the infected host. The VZV glycoproteins gB, gE, gI, and gH are important for cell-to-cell spread [6–11]. Other virulence factors have been identified by evaluating VZV mutants in human xenografts using the severe combined immunodeficient (SCID) mouse model of VZV pathogenesis [12].

Epidemiology

Primary VZV infection

Primary VZV infection, or varicella, is much less common than disease caused by VZV reactivation during the first year after HCT, accounting for only about 5% of VZV infections in this population. The lower incidence of varicella is due to the fact that more than 85% of individuals in the United States have had primary VZV infection by 8 years of age as a result of the annual varicella epidemics that occur in that and other temperate regions of the world. Nevertheless, if an HCT recipient has never had VZV infection before, the attack rate for varicella after close exposure to an index case will reflect the risk of transmission to any susceptible individual, defined as an individual who has not been infected previously. Attack rates range from about 30% with classroom exposure to 90% with household contact. Direct contact with

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Table 89.1 Incidence of VZV infections following HCT, from selected studies

Study	Patient population	Underlying disease	Transplant type	VZV infection[a]		Localized %)	Atypical zoster[b] (%)	Varicella (%)
				n	%			
Blume, 1980 [15]	Pediatric/adult	Leukemia	Allogeneic/ syngeneic	33	21			
Atkinson, 1982 [16]	Pediatric/adult	Leukemia/aplastic anemia	Allogeneic/ syngeneic	98	52			
Locksley, 1985 [1]	Pediatric/adult	Leukemia/aplastic anemia	Allogeneic	1394	17	85	15	0
Ljungman, 1986 [17]	Pediatric/adult	Hematologic malignancy	Allogeneic	73	36	91	7	2
Wacker, 1989 [18]	Pediatric	Leukemia/solid tumors	Autologous	236	23	75	13	18
Schuchter, 1989 [19]	Pediatric/adult	Leukemia/lymphoma	Autologous	153	28	77	20	2
Christiansen, 1991 [20]	Adult	Hodgkin disease	Autologous	28	32	100	0	0
Han, 1994 [21]	Pediatric/adult	Leukemia/lymphoma/ other	Autologous/ allogeneic	1186	18	62	32	
Koc, 2000 [22]	Adult	Leukemia/other	Allogeneic	100	41	100	0	
Steer, 2000 [23]	Adolescent/adult	Leukemia/other	Allogeneic	151	38	100	0	
Leung, 2000 [24]	Pediatric	Malignancies/other	Autologous/ allogeneic	109	30	42	40	18
Offidani, 2001 [25]	Adult	Leukemia/lymphoma/ other	Autologous	164	16	92	4	4
Tomonari, 2003 [26]	Adult	Hematologic malignancy	Cord blood	40	80	88	12	
Erard, 2007 [27][c]	Pediatric/adult	Leukemia/lymphoma/ other	Allogeneic	720	25	80	20	
			Autologous	212	21	87	13	
Kim, 2008 [28]	Adult	Hematologic malignancy	Allogeneic peripheral blood stem cell	192[d]	22	88	12	
Vandenbosch, 2008 [29]	Pediatric	Not available	Cord blood	37	46	65	35	
			Allogeneic (BMT)	77	31	96	4	
Su, 2011 [30]	Adult	Leukemia/lymphoma/ multiple myeloma	Mostly sequential autologous/ allogeneic; non-myeloablative	179	37	91	8	
Rogers, 2011 [31]	Adult	Hematologic malignancy	Autologous	56	16	68	32	

[a] Percentage of patients who developed any VZV infection.

[b] Including CNS, disseminated, or visceral disease.

[c] Including only patients who did not receive long-term acyclovir for VZV prophylaxis, but received acyclovir from day 7 before HCT to engraftment, if herpes simplex virus positive.

[d] 27/192 patients received long-term low-dose acyclovir prophylaxis.

lesions is not required since, in contrast to other herpesviruses, VZV is transmissible by the respiratory route. The incidence of primary VZV infection is higher in pediatric HCT recipients than adults and, as expected, the risk correlates inversely with the age of the child. In one series, 10 of 54 children (18%) with VZV infections after HCT had primary VZV infection demonstrated serologically [5]. Although 25 adult patients presented with a disseminated cutaneous VZV exanthem, only two patients had serologic evidence of primary VZV infection [1]. Although the possibility of reinfection cannot be eliminated in some cases, the epidemiologic evidence suggests that patients who have been infected previously are usually protected against infection following a new VZV exposure, presumably because VZV IgG titers are maintained despite immunosuppressive disease or therapies. The addition of the varicella vaccine to the routine childhood immunization schedule has reduced the extent of the annual epidemics, making it less likely that HCT recipients who have no pre-existing immunity to VZV will be exposed to varicella, and more likely that pediatric HCT recipients will have vaccine-induced immunity to VZV [13,14].

Recurrent VZV infection

The reported incidence of recurrent VZV infection after HCT ranges from 16 to 63% [1,15–31] (Table 89.1). Cord blood transplant recipients have the highest risk of VZV reactivation, with an incidence of up to 80% reported [26,29]. The incidence of recurrent VZV infection after HCT is higher than that observed among solid organ transplant recipients, which is about 7%, but it is comparable to rates of VZV reactivation in patients with Hodgkin disease receiving combined modality therapy. For purposes of comparison, the estimated annual incidence of herpes zoster in adults without underlying disease is 0.5% [22].

The reactivation of herpesviruses follows a predictable temporal pattern after HCT [2]. HSV-1 causes clinically apparent disease at about 2–3 weeks and cytomegalovirus (CMV) disease usually occurs during the second to third months after transplantation. Epstein–Barr virus (EBV) may also reactivate in the third month whereas VZV recurrences present at a median of 5 months after HCT. In general, the risk of recurrent VZV infection is highest between 2 and 10 months after transplantation, although cases have been reported within the first week and continue to occur after the first year (Figure 89.1). Locksley et al. found that 80% of patients who developed VZV infection following HCT had disease within 9 months [1]. When options for antiviral therapy were limited, 21% of these patients had visceral dissemination of the virus and 12% died from complications of recurrent VZV. In a review of 100 consecutive adult patients who received allogeneic HCT between 1992 and 1997, 41% developed VZV reactivation at a median of 227 days (range 45–346 days) [22]. Of these episodes, 12% occurred in the first 100 days; the attack rate was 59% among patients who survived for at least 2 years. In another retrospective analysis of VZV infection after HCT in children [24], 33 (30%) of the total 109 children who were transplanted during a 7-year period developed post-transplant VZV infection, including six cases of varicella and 27 cases of zoster. Twenty-four of these 33 (73%) children had VZV infection within

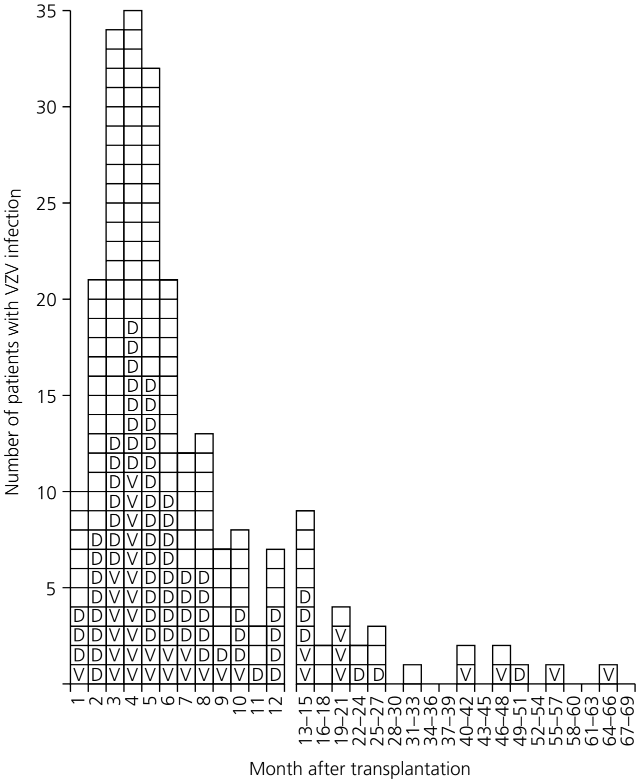

Figure 89.1 VZV infections by month after marrow transplantation: □, Uncomplicated herpes zoster; V, varicella; D, herpes zoster with subsequent dissemination. Source: Locksley et al. 1985 [1]. Reproduced with permission of Oxford University Press.

1 year following HCT. The cumulative incidences of post-transplant VZV infection at 1 and 5 years were 26 and 45%, respectively.

Factors that are associated with higher rates of VZV reactivation following HCT include genotypic non-identity for human leukocyte antigen (HLA) between donor and recipient, and acute or chronic graft-versus-host disease (GVHD), which are linked variables [23]. In one series, 64% of patients whose donor was HLA non-identical had herpes zoster compared with 44% of HCT recipients with matched donors and no GVHD. The risk of late VZV infection was increased fourfold (Table 89.2) [16]. In a second series, patients with limited chronic GVHD had a lower incidence of VZV reactivation than those with extensive chronic GVHD [22]. The presence of non-specific suppressor cells associated with chronic GVHD correlated with a higher risk of recurrent VZV (Table 89.2) [16]. A CD4 T-cell count <200 cells/μL and a CD8 T-cell count <800 cells/μL at 30 days after transplantation correlated with an increased risk of zoster within the first year [25]. The cumulative evidence indicates that patients undergoing allogeneic or autologous HCT have about the same overall risk of recurrent VZV disease although, as noted, cord blood HCT seems to pose the highest risk for VZV reactivation [26] (Table 89.1). In one study, 28% of autologous transplant patients developed recurrent VZV infection [19]. The initial manifestation of recurrence was localized disease in 77% of these patients while 23% had atypical non-localized zoster, which is comparable to the distribution of these clinical syndromes among allogeneic HCT recipients. Some 32% of patients who received an autologous HCT for Hodgkin lymphoma had VZV infection [20], which is consistent with experience in Hodgkin and non-Hodgkin lymphoma patients [32]. The somewhat higher incidence of zoster in lymphoma patients may account for the fact that patients who receive autologous HCT have rates of VZV reactivation that are similar to those in allogeneic HCT recipients even though GVHD is less common. Morbidity and mortality caused by VZV infection after autologous HCT cannot be compared with the original analyses of VZV-related disease after allogeneic HCT because antiviral therapy was available when the studies of autologous HCT patients were carried out. In the series of Schuchter et al., 15% of autologous HCT recipients had cutaneous dissemination, 5% had visceral dissemination, and 25% had morbidity, including postherpetic neuralgia (PHN) and neurologic dysfunction [19]. In a more recent series of pediatric HCT patients, 61 of 201 (35%) allogeneic HCT recipients compared with 10 of 109 (9%) autologous HCT recipients developed zoster. Risk factors for the development of zoster included age >10 years, allogeneic HCT, and total body irradiation in allogeneic recipients [33].

Table 89.2 Risk of recurrent VZV infection after HCT in relation to HLA matching of recipient and donor and the occurrence of GVHD in the recipient[a]

	HLA identical			HLA non-identical	Syngeneic
	No GVHD	With GVHD			
		No suppressor cells	With suppressor cells		
No. of patients	25	21	19	14	19
Late VZV infections (%)	24	38	47	36	11
VAV at any time (%)	44	57	79	64	21

[a] Non-specific suppressor cells were detected in co-cultures with donor lymphocytes, assessing their effect on the proliferation responses to alloantigens or concanavalin A.
Source: adapted from Atkinson et al. 1982 [16].

The underlying disease that provided the indication for HCT probably does not influence VZV reactivation. However, a history of symptomatic herpes zoster before marrow transplantation was associated with a higher risk of early post-transplant VZV disease among patients with Hodgkin disease undergoing autologous HCT [20]. VZV reactivation has not been found to predict relapse of the underlying disease in HCT recipients.

Reinfection with VZV

Natural immunity following varicella usually protects against systemic reinfection. For this reason, symptomatic reinfection with VZV is very rare among immunocompetent individuals. Surveillance of 9947 cases of varicella indicated that 4.5–13.5% of children presenting with varicella had a documented previous episode of varicella, suggesting that clinical reinfection may be more common than previously thought [34]. However, most of these cases of varicella were not laboratory confirmed and other childhood viral illness can also present with vesicular rashes. Furthermore, a study in adults showed that a history of previous varicella infection in adults was not reliable based on absence of VZV IgG antibodies in preillness serum specimens [35]. Reinfection probably occurs occasionally among severely immunocompromised patients who have had primary VZV infection previously [36]. However, this hypothesis is difficult to confirm because the distinction between atypical non-localized herpes zoster (which is much more common in immunocompromised patients) and reinfection requires a comparison of the VZV isolates causing the initial infection and the new episode by DNA sequence analysis. Based on clinical criteria only, children with leukemia have been reported to have second episodes of varicella following close

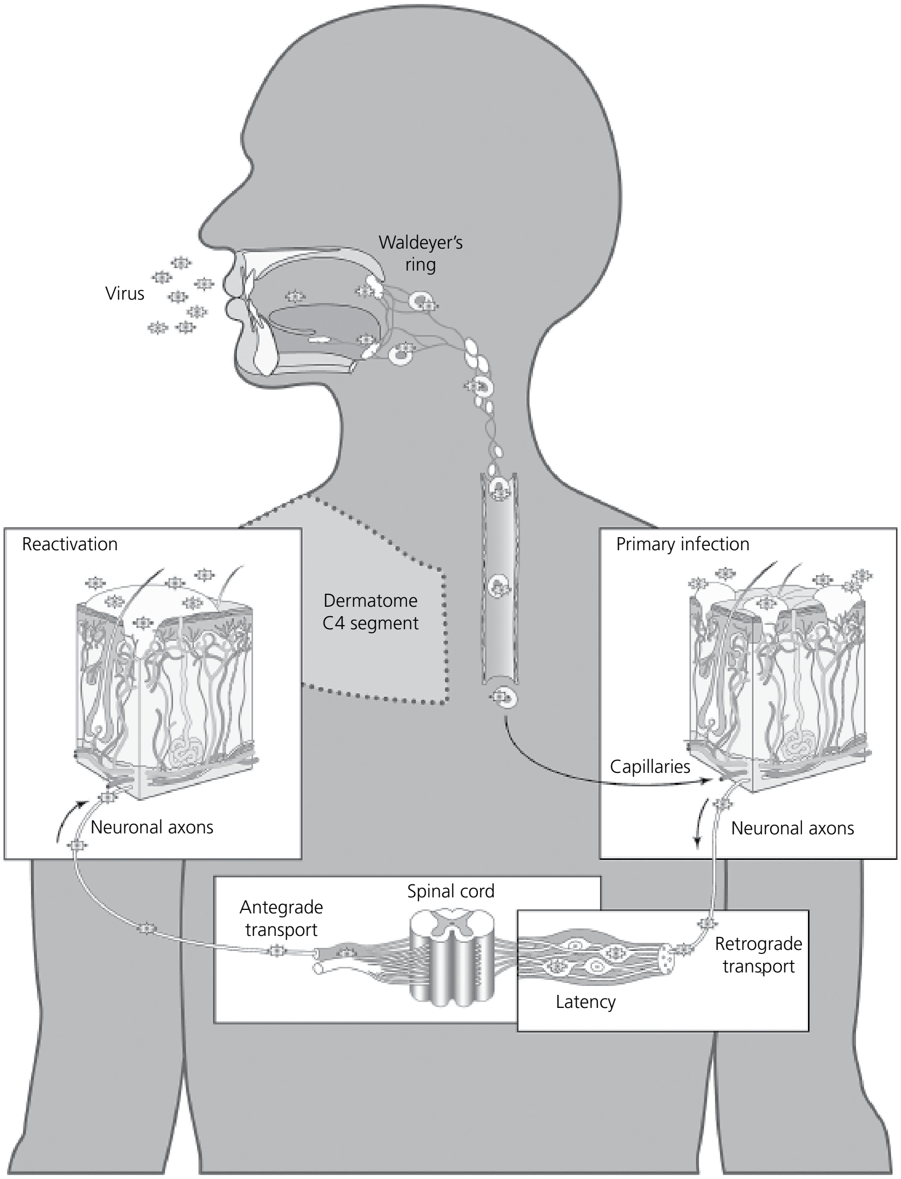

Figure 89.2 The pathogenesis of VZV infection in the human host. VZV infection is acquired by inoculation of mucosal epithelial cells. Since VZV is a T-cell tropic virus, infection of T cells in lymphoid tissues of Waldeyer's ring can amplify the infection and infected T cells can then transport VZV to the skin via a cell-associated viremia. Infection of skin produces the vesicular rash associated with chickenpox. During skin infection, VZ virions gain access to the sensory nerve cell body by retrograde axonal transport from fine nerve endings or via cell-associated viremia, to establish a lifelong latent infection within the sensory ganglia. Herpes zoster results from reactivation of latent VZV. During reactivation, VZ virions gain access to skin via anterograde transport along neuronal axons, causing a localized dermatomal rash. In immunocompromised patients, reactivation may result in T-cell infection and dissemination, with or without a dermatomal rash.

contact despite a past history of varicella and serologic evidence of pre-existing immunity. Nevertheless, anecdotal experience suggests that exposure of HCT patients who have had past primary VZV infection rarely causes any clinically apparent signs of reinfection [2].

Mechanisms of pathogenesis and viral immune evasion

Primary VZV infection

Events during the 10–21-day incubation period of primary VZV infection have been difficult to document with laboratory methods. The epidemiologic evidence suggests that infection is initiated by the inoculation of respiratory mucosal sites (Figure 89.2) [5]. Infectious virus is then presumed to be transported to regional lymphoid tissue, possibly undergoing a phase of local replication at these sites. VZV causes a cell-associated viremia, which appears to target T-lymphocytes in particular, although other cells of the immune system, such as dendritic cells, can become infected. Tonsillar T cells are highly permissive for VZV infection, suggesting that VZV could be transferred efficiently from respiratory epithelial cells into these local T-cell-rich tissues as a first stage of spread within the naïve host [37]. Cell-associated viremia has been demonstrated late in the incubation period, just before and after the appearance of

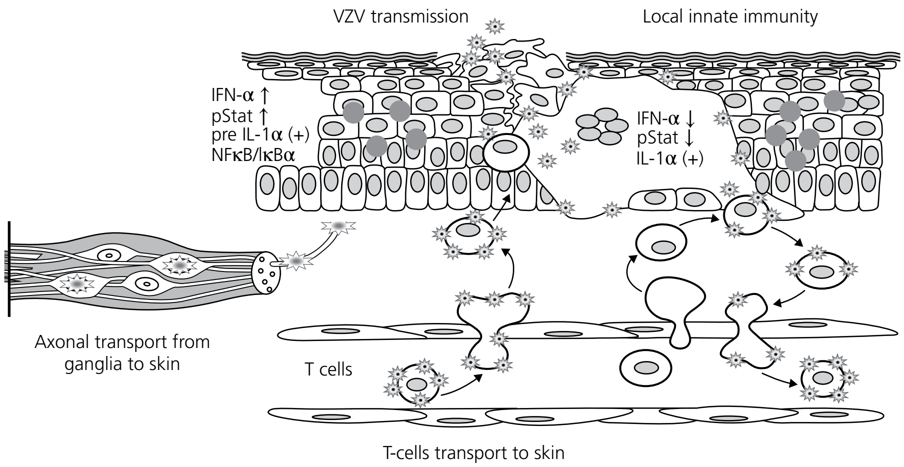

Figure 89.3 The pathogenesis of primary and recurrent VZV infection is modulated by innate immune responses of cutaneous epithelial cells. This diagram depicts new concepts about events in the pathogenesis of VZV infection of skin. During primary VZV infection, T cells within the tonsil lymphoid tissues become infected by VZV. Infected T cells transport the virus to the skin shortly thereafter, exiting through capillary endothelium by the usual mechanisms for trafficking of migratory T cells. The infected T cells then release infectious VZV at skin sites of replication. The remainder of the 10–21-day incubation period is the interval required for VZV to overcome the innate IFN-α and other antiviral responses in enough epidermal cells to create the typical vesicular lesions containing VZV at the skin surface. Signaling of innate responses, such as signal transducers and activators of transcription-1 (Stat-1), IL-1α and nuclear factor-kappa B (NFκB), in adjacent skin cells prevents a rapid, uncontrolled cell–cell spread of VZV. When uninfected T cells that are trafficking through skin in normal immune surveillance encounter early-stage cutaneous lesions, they may become infected and amplify viremia. When VZV reactivates from latency in sensory neurons, it is delivered to the skin by axonal transport. VZV replication in the involved dermatome encounters innate barriers as the initial host response, followed by the appearance of VZV-specific T cells that clear active infection. If these responses are delayed, the virus may enter migrating T cells to cause disseminated VZV infection.

varicella skin lesions. VZV was isolated by viral culture from 24% of samples of peripheral blood mononuclear cells (PBMCs) taken within 24 hours after the onset of rash [38]. Cell-associated VZV viremia was detected by in situ hybridization with a probe for VZV DNA, which showed viral DNA in lymphocytic cells, and by polymerase chain reaction (PCR) testing for viral DNA in PBMC specimens from otherwise healthy subjects tested immediately after the appearance of skin lesions [39]. VZV infection of tonsillar T-lymphocytes was enhanced in activated, memory CD4 T-lymphocytes that expressed skin homing markers, which suggests that cell-associated viremia may be particularly associated with infection of this subpopulation of T-lymphocytes [37]. When the cutaneous exanthem develops, VZV replication in epithelial cells may amplify viremia by transfer of infectious virus into migrating T-lymphocyte populations, leading to secondary 'crops' of skin lesions. These observations about the pathogenesis of primary VZV infection suggest that the efficiency with which cell-associated viremia and replication at skin sites is terminated, either by the host response or by antiviral therapy, is likely to influence the extent of the cutaneous exanthem and the risk of visceral dissemination (Figure 89.3).

The study of VZV pathogenesis in animal models has been hampered by the restricted replication of the virus in non-human species. Mice that have inherited SCID can be used to support grafts of human tissues that differentiate to contain the usual human cell populations. These animals, which are referred to as SCID-hu mice, provide a unique opportunity to examine VZV–cell interactions in intact human tissues independently of the effects of the host immune response on viral replication [40,41] (Figure 89.4; reviewed in [11,12,42,43]). Conditions in the model are similar to those in the period immediately after HCT, since VZV-specific immunity is absent. Experiments in the SCID-hu mouse model demonstrated that VZV infects CD4$^+$ and CD8$^+$ human T-lymphocytes, proving that VZV shares pathogenic mechanisms that are characteristic of both the lymphotropic and the neurotropic herpesviruses [42]. The tropism of VZV for human T cells and its infectivity for skin are both essential elements of its pathogenicity in human disease, but comparative analyses of VZV strains demonstrate that these tropisms are mediated by different virulence determinants. For example, the VZV strain used to make the live attenuated varicella vaccine, the vaccine Oka strain, was indistinguishable from low-passage VZV in its infectivity for CD4$^+$ and CD8$^+$ T cells whereas its pathogenic potential in human skin was reduced substantially compared with the parent Oka strain, as assessed by the extent of the cutaneous lesions, viral protein synthesis, infectious virus yields, and release of infectious virus. Transfer of VZV from infected T cells into skin, followed by the appearance of VZV skin lesions, was demonstrated in the SCID-hu model [44]. These experiments also revealed the substantial upregulation of interferon-α (IFN-α) production by epidermal cells adjacent to VZV-infected cells and blocking experiments with IFN-α/β receptor antibodies showed the biological importance of these responses. Investigations of VZV and HSV-1 replication in the SCID-hu model also revealed significant differences in cell tropisms that correlate with clinical observations about their pathologic effects in intact and immunocompromised individuals. In SCID-hu mice, VZV caused extensive necrosis in deeper dermal layers of skin implants, but HSV-1 was confined to the epidermis. In contrast to VZV, HSV-1 was not infectious for human CD4$^+$ or CD8$^+$ T -cells, which is consistent with the clinical differences, since primary HSV infection is not associated with viremia in the intact host. HCT recipients and other immunocompromised patients are also susceptible to cell-associated viremia when VZV reactivates, whereas HSV viremia is a rare occurrence during recurrent HSV infection even with intensive immunosuppression.

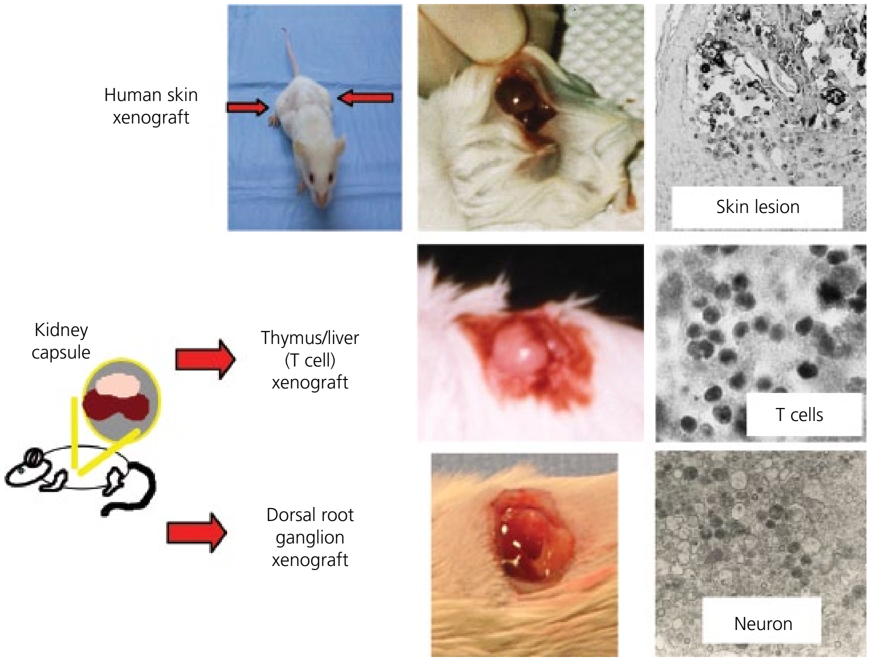

Figure 89.4 Modeling varicella zoster virus (VZV) pathogenesis in human tissue xenografts in vivo. Human tissue xenografts are maintained in mice with severe combined immunodeficiency. VZV infection of cells within their tissue microenvironments can be evaluated in mice with skin, T-cell (thymus/liver) or dorsal root ganglia xenografts. (For a color version of this figure, please see the color plate section.)

Recurrent VZV infection

The hypothesis that VZV becomes latent in sensory ganglia following primary infection and that herpes zoster is caused by its reactivation from latency was proved by using restriction enzyme analysis to demonstrate that a single VZV strain caused both varicella and a subsequent episode of herpes zoster in a child with Wiskott–Aldrich syndrome [45]. During the primary attack, VZV is postulated to pass centripetally from the skin to the corresponding ganglia; hematologic seeding of the ganglia secondary to cell-associated viremia is also likely. Once in the ganglia, VZV establishes latency, apparently without viral replication and without causing cell damage. In contrast to HSV, infectious VZV has not been recovered from ganglion tissue by explant culture or co-cultivation techniques. However, the presence of VZV nucleic acid sequences has been documented in human ganglia taken at autopsy from individuals with no evidence of recent VZV infection [5,46–53]. Multiple VZV RNA sequences have been detected by in situ hybridization and PCR, in contrast to HSV in which only limited gene transcription has been identified in latently infected neurons. Transcription of VZV genes corresponding to ORF4, 18, 21, 29, 40, 62, 63, and 66 have been reported in human dorsal root ganglia (DRGs) from autopsies, although observations about specific VZV genes have varied between studies [52,54,55]. ORF63 transcripts are the most abundant and the most commonly found [54]. Latent infection appears to be more common in the trigeminal ganglion than in any of the thoracic ganglia [50]. In addition to gene transcription, expression of VZV proteins in the cytoplasm of neurons has been described in autopsy specimens from adult subjects without zoster [48,55,56]. However, these observations of VZV protein expression during latency were not substantiated when antibody reagents were used that did not contain murine or rabbit endogenous antibodies to the human blood group A determinant [57]. Blood group A antigen expressed in human neurons resulted in non-specific reactivity. The extensive analysis by Mahalingam et al. identified very few IE63-positive neurons in only a few subjects [55], as was confirmed by Zerboni et al., indicating that VZV transcripts are translated occasionally, perhaps during abortive replication [58]. Basic questions about viral and host mechanisms involved in VZV neuropathogenesis and persistence in human sensory ganglion cells remain unanswered.

Investigations using molecular probes for VZV DNA sequences indicate that the virus persists in neurons [5,49,53]. The study of VZV neuropathogenesis has been facilitated by engrafting human DRGs under the kidney capsules of SCID mice [59]. DRG xenografts contained neurons and satellite cells within a typical DRG architecture. An initial phase of viral replication was characterized by infection of both neurons and satellite cells, inducing fusion of these cell subpopulations [11,60]. This acute process was followed within 4–8 weeks by a transition to VZV latency, characterized by the absence of infectious virus release, the cessation of virion assembly, and a reduction in VZV genome copies. Furthermore, VZV-infected T cells transferred virus from the circulation into DRG, suggesting that VZV lymphotropism facilitates its neurotropism.

The maintenance of VZV latency, as defined by the absence of symptomatic VZV reactivation, is also influenced directly by the host response to VZV, as is evident from the high incidence of VZV reactivation after HCT when VZV immunity is impaired. Although HSV reactivation causes little or no damage to ganglion cells, VZV reactivation is associated with extensive viral replication in the affected ganglia, producing pathologic changes including necrosis, inflammation, and destruction of neural cell bodies.

In contrast to HSV, CMV, and EBV, which can be recovered from asymptomatic patients by viral culture methods, technical problems have interfered with the detection of subclinical VZV reactivation. Episodes of subclinical VZV reactivation, consisting of cell-associated viremia in HCT recipients detected by VZV PCR, were documented in 19% of patients tested at a mean of 94 days after transplantation [61]. Disseminated VZV infection has been diagnosed at autopsy in HCT patients who had no cutaneous lesions [1,62,63]. However, testing PBMCs with the VZV PCR assay provided the first virologic evidence that these severely immunocompromised patients can experience VZV reactivation in the absence of clinically apparent cutaneous infection and resolve the infection without developing signs of visceral dissemination. Herpes zoster, like recurrent HSV, has been attributed to the spread of the reactivated virus along neural pathways from the site of latency in DRGs. Some immunodeficient patients with localized herpes zoster develop VZV viremia, presumably because T-lymphocytes become infected at the site of local cutaneous replication. Subclinical, cell-associated viremia in HCT patients without cutaneous disease suggests that the virus may also be taken up directly by circulating lymphocytes at the neuronal site of viral reactivation. This mechanism for causing viremia could account for the clinical observation of atypical, generalized herpes zoster in some HCT recipients. Since the activation of T-lymphocytes makes this cell population more permissive for VZV infection in vitro [37], cell-associated VZV viremia in HCT patients may be potentiated by the characteristic persistence of activated T-lymphocytes in circulation for a prolonged period after transplant [64].

Viral immune evasion

Like many viruses, VZV has mechanisms to inhibit recognition by the host immune system. We have found that VZV causes a significant reduction of the cell surface expression of MHC class I protein on infected fibroblasts and on infected T cells, as demonstrated in the SCID-hu model [65]. Although their synthesis was not affected, MHC class I molecules were retained in the Golgi in VZV-infected cells. This effect may enable the virus to evade $CD8^+$ T-cell immune recognition during VZV pathogenesis, including the critical phase of T-lymphocyte-associated viremia. In related experiments, we showed that VZV has the capacity to block the upregulation of MHC class II protein that is usually induced when cells are exposed to IFN-γ[66]. Induction of MHC class II expression is required for recognition of virus-infected cells by antiviral CD4 T cells. When skin biopsies of varicella lesions were analyzed by in situ hybridization, MHC class II RNA transcripts were detected in cells near the lesion but not in cells that were infected with VZV. VZV infection inhibited transcription of interferon regulatory factor (IRF-1) in the IFN-γ signaling pathway and expression of Stat1a and Jak2 proteins. The inhibition of MHC class II expression on VZV-infected cells in vivo may transiently protect cells from CD4 T-cell immune surveillance, facilitating local virus replication during the first few days of cutaneous lesion formation during primary or recurrent VZV infection, and could allow a period of viral replication in skin cells even when the host has VZV-specific CD4 T cells. VZV also interferes with the cell signaling processes that signal the initial induction of the IFN-α/β response through a mechanism dependent upon the IE62 protein. Conversely, VZV induced upregulation of Stat3 and production of the anti-apoptotic protein survivin [67,68]. Stat3 induction was required for the pathogenesis of skin infection, indicating that VZV, a non-oncogenic herpesvirus, has the same effect known to occur in EBV- and KSHV-infected cells.

The host response

Primary VZV infection is associated with the development of virus-specific IgG and IgM antibodies and the acquisition of cellular immunity, which can be demonstrated by proliferation of T cells that are stimulated with VZV antigens in vitro. Although VZV IgG antibodies can neutralize virus infectivity and function in antibody-mediated cellular cytotoxicity, humoral immunity seems to be less important in the host response to VZV than cell-mediated immunity. Progressive varicella occurs in immunocompromised children despite the production of VZV IgG and IgM antibodies, whereas these children fail to develop VZV-specific T-lymphocyte proliferation [69]. Immunocompromised patients, including HCT recipients, develop VZV reactivation in spite of high concentrations of circulating antibodies to VZV. No quantitative relationship between VZV antibody titers in the donor or the recipient and the subsequent development of herpes zoster has been established in HCT patients [70]. A retrospective study examined anti-VZV IgG titers after HCT and also measured VZV DNA copy numbers by real-time PCR during clinical VZV reactivation using cryopreserved serum samples [71]. No significant difference was found between anti-VZV titers in patients with VZV infection and in those without VZV infection at each time point after HCT. However, patients with disseminated zoster tended to have lower pre-existing anti-VZV titers and higher serum VZV DNA copy numbers than those with localized zoster cases. A strong inverse correlation was found between pre-existing anti-VZV titer and serum VZV DNA at onset. Therefore, it was concluded that pre-existing antibody does not prevent the development of VZV reactivation in HCT recipients but may contribute to decreased viral load at onset. Periods of diminished VZV-specific T-lymphocyte proliferation have been correlated with an increase in susceptibility to herpes zoster among immunocompromised patient populations, including HCT recipients. In the individual patient, the loss of T-cell proliferation to VZV is a necessary, but not a sufficient, condition for symptomatic reactivation. Conversely, VZV-specific T-lymphocyte proliferation has been correlated with a decreased risk of herpes zoster.

Analyses of VZV-specific cell-mediated immunity have demonstrated a gradual recovery of T-cell proliferation to VZV antigens, with a larger percentage of HCT patients having detectable responses as the interval following transplant increases [4,17,61,72,73]. The interval to recovery may be shorter in autologous HCT recipients. This reconstitution of cell-mediated immunity to VZV antigens is generally observed by 9–12 months after HCT, which correlates with the time when VZV reactivation and susceptibility to severe VZV disease become less common. In HCT patients, IFN-γ production was associated with the recovery of T-cell proliferation to VZV antigen and increased with time following transplantation. Interleukin (IL)-10 production by PBMC stimulated with VZV antigen was also observed consistently after HCT and was highest in patients who recovered T-cell proliferation to VZV antigen [72].

When VZV T-cell proliferation responses were followed prospectively in unvaccinated patients in a study of inactivated varicella vaccine, autologous transplant patients had a mean VZV stimulation index of 9.9 ± 2.32 within 30 days before transplantation, responses were undetectable at 30 days, and then increased to 8.0 ± 1.63 by 90 days; mean responses continued to increase over the first year (Figure 89.5) [32]. Evaluation of VZV immunity at 6 months after transplantation using a quantitative flow cytometry method showed that the frequencies of CD4 T cells that made tumor necrosis factor-alpha (TNF-α) in response to VZV stimulation was 0.19%, compared with 0.30% in VZV immune adults without underlying disease; the mean percentage of CD4 T cells that made IFN-γ was 0.11%, which was equivalent to frequencies in healthy adults [32] (Figure 89.6). These analyses

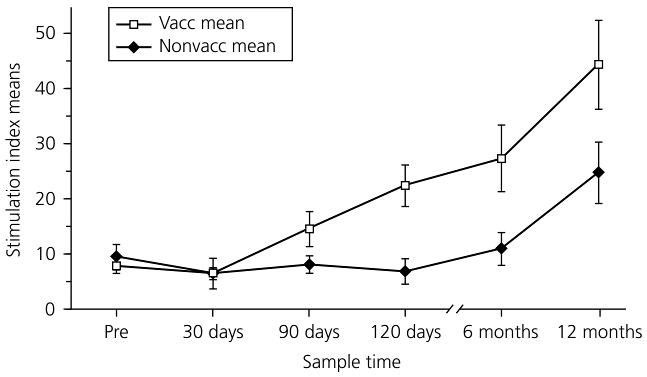

Figure 89.5 CD4 T-cell proliferation in response to VZV antigen. T-cell proliferation in response to VZV antigen, measured as the stimulation index, is shown as the mean stimulation index (*y*-axis) for vaccinated (□) and unvaccinated (◆) participants; the time of immunologic evaluation is shown on the x-axis in relation to time of HCT. Inactivated varicella vaccine was given pre-transplant and at 30, 60, and 90 days; the stimulation index for vaccinees at 30 days represents responses after the pre-transplant dose; at 90 days, three doses had been given, and at 120 days, 6 months, and 12 months, vaccinees had received four doses. The error bars represent standard errors. The numbers of patients tested at each time point were as follows: pre-transplantation, 58 unvaccinated/53 vaccinated; 30 days, 50 unvaccinated/49 vaccinated; 90 days, 43 unvaccinated/42 vaccinated; 120 days, 34 unvaccinated/39 vaccinated; 6 months, 30 unvaccinated/34 vaccinated; 12 months, 27 unvaccinated/33 vaccinated. Source: Hata et al. 2002 [32]. Reproduced with permission of the Massachusetts Medical Society.

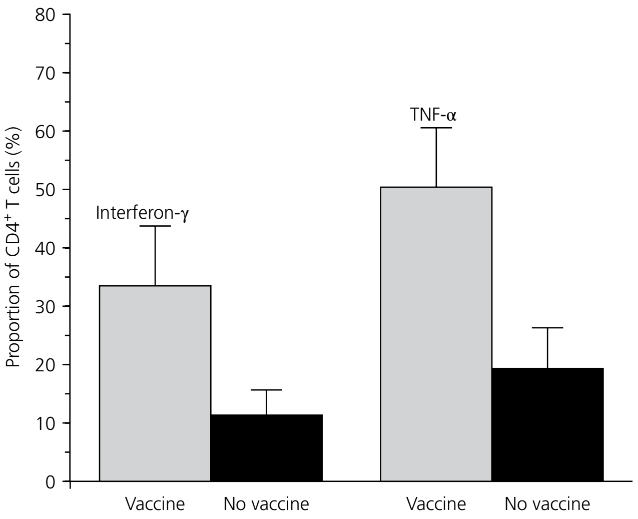

Figure 89.6 Intracellular production of interferon-gamma (IFN-γ) or tumor necrosis factor-alpha (TNF-α) in T cells incubated with VZV. The percentage of CD4$^+$ T cells that expressed CD69 and intracellular cytokine, IFN-γ (black bars) or TNF-α (gray bars), was determined at 6 months after HCT in 19 vaccinated and 19 unvaccinated patients. Results are shown as mean percent responder cells ± standard error. Differences in VZV-specific responder cell frequencies in vaccinated and unvaccinated subjects were analyzed by unpaired *t*-test: for IFN-γ, $p = 0.07$; for TNF-α, $p = 0.03$. Source: Hata et al. 2002 [32]. Reproduced with permission of the Massachusetts Medical Society.

excluded patients who developed herpes zoster, indicating that VZV-specific T-cell responses can recover either as a result of subclinical VZV reactivation, as documented by VZV PCR testing of HCT recipients who had no signs of infection, or through re-expansion of memory T-cell populations that persisted through the preparative regimen for transplantation [61]. In this prospective study, the risk of zoster was reduced by 19% per unit increase in stimulation index (SI) >1.6; an SI >5.0 correlated with >93% protection [32]. Pre-transplant immunity in both the donor and the recipient may facilitate the reconstitution of VZV-specific cell-mediated immunity [74].

Nevertheless, it is apparent that the recovery of virus-specific cellular immunity is delayed for months and recovery of VZV-specific T-cell immunity often does not occur until after the patient has had an episode of herpes zoster, which provides in vivo re-exposure to VZV antigens. Meyers et al. detected T-lymphocyte proliferation to VZV in 16 of 18 patients (89%) following symptomatic recurrences of VZV compared with 15 of 29 patients (51%) who did not develop herpes zoster [4]. When herpes zoster occurs, HCT recipients show an increase in VZV-specific CD4 T-cell proliferation. We found that the mean SI response before onset of herpes zoster was 2.2 ± 0.4 compared with 28.1 ± 6.68 when the episode of VZV reactivation had resolved [32], which likely explains why HCT recipients are usually not susceptible to repeated episodes of herpes zoster.

During the first year, HCT patients recover cytotoxic T cells that recognize and lyse autologous target cells expressing VZV proteins [61]. About 50% of HCT patients showed recovery of VZV-specific cytotoxic T-lymphocyte (CTL) function when tested at a mean of 155 days after transplant (Figure 89.7). However, the mean precursor frequency of T-lymphocytes that recognized the VZV IE62 protein or glycoprotein E (formerly designated gp I) was more than twofold lower among HCT recipients than the frequency of CTLs that recognized these viral proteins in PBMCs from healthy immune subjects. In these experiments, cultures from HCT recipients showed a significant reduction in the proliferation of CD4$^+$ T cells compared with the pattern of cell phenotypes in cultures from healthy subjects. CD8$^+$ T cells predominated in VZV-stimulated cultures, reflecting the relative increase in circulating CD8$^+$ T cells that is common after HCT. Although CD8$^+$ T-lymphocytes have been defined as the "classic" cytotoxic effecter cell, human CD4$^+$ cells also function effectively as antiviral CTLs against many viruses, including VZV [75]. The diminished CD4$^+$ T-cell response to VZV antigen may explain why the overall frequencies of CTL precursors specific for the IE62 or gE (gp I) proteins remained significantly lower after HCT than in healthy, VZV immune individuals. Helper and cytotoxic T-cell responses specific for the IE63 protein and also proteins encoded by open reading frames 4 and 10 are maintained for decades after primary VZV infection in the intact host [76,77], and their absence could be related to the high risk of VZV reactivation causing herpes zoster.

In contrast to virus-specific T-cell immunity, natural killer (NK) cell activity comparable to that of healthy subjects is recovered during the first few months after HCT [78]. Some HCT recipients had a predominance of NK cells, expressing CD16 surface antigen, in assays for cytotoxicity against VZV-infected targets [61]. The importance of NK cells in the host defense against VZV has been exemplified by cases of severe or fatal disseminated VZV infection in patients with an absence of circulating NK cells during the VZV infection or with innate NK cell deficiencies [79–82]. The capacity of lymphocytes from some HCT patients to lyse targets expressing VZV proteins by a mechanism that is not antigen specific may help to limit VZV replication prior to the recovery of virus-specific T cells.

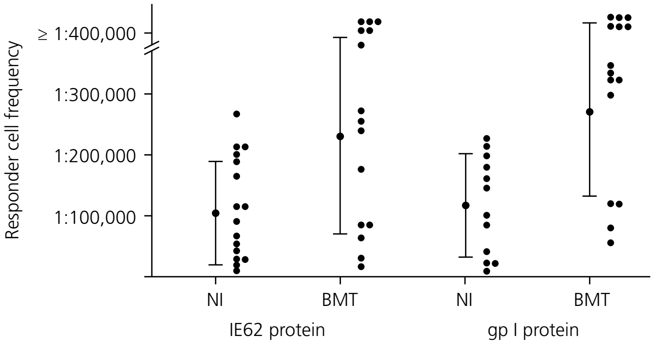

Figure 89.7 Precursor frequencies of cytotoxic T-lymphocytes (CTLs) specific for the immediate early protein (IE62) and glycoprotein I (gp I) of VZV in bone marrow transplant (BMT) recipients and healthy subjects (NI). The mean ± standard deviation for precursor frequency estimates are indicated next to the individual data points (dark circles) generated by testing individual BMT recipients and healthy immune subjects. Source: Wilson et al. 1992 [61]. Reproduced with permission of Oxford University Press.

Clinical manifestations

Primary VZV infection

The diagnosis of varicella is usually suspected clinically in both high-risk and healthy children when the characteristic vesicular exanthem appears [3,24,83,84]. The interval from exposure of the non-immune child to the appearance of the rash is about 14 days, with a range of 10–21 days; the incubation period may be shorter in immunocompromised children.

Because of its low incidence, specific descriptions of the clinical course of varicella after HCT are limited, but it is reasonable to generalize from the literature about varicella in other high-risk populations [83,84]. The initial manifestations of varicella in immunocompromised children are similar to those observed in healthy children. Prodromal symptoms may precede the rash by 24–48 hours, usually consisting of headache, irritability, malaise, and fever. Cutaneous lesions most often appear first on the scalp, face, or trunk, and are usually pruritic. Each lesion begins as a small erythematous macule that evolves into a vesicle of 1–4 mm diameter, on an irregular erythematous base, the classic "dewdrop on a rose petal." In high-risk children, including HCT recipients, the vesicles may be unusually large and can involve deeper skin layers. Vesicles on mucous membranes, including the conjunctiva, the oropharynx, the rectum, or the vagina are common even among otherwise healthy children. Varicella is typically accompanied by low-grade fever but temperature elevations may be as high as 40.5 ° C in high-risk patients, and fever often persists beyond the usual 3–4 days.

New vesicle formation typically continues for about 3 days, with a range of 1–7 days, among healthy children. This phase is often prolonged in immunocompromised children, with more than half of patients developing new lesions for more than 7 days [84]. Although the majority of immunocompetent children with varicella have fewer than 500 lesions, it is not unusual for patients on immunosuppressive therapy to develop more than 1500 lesions. Successive crops of varicella vesicles develop in a centrifugal pattern, appearing last on the extremities. These later crops of lesions tend to be more extensive in immunocompromised patients, and may involve the palms and soles in addition to arms and legs. In lesions that are resolving normally, the vesicle fluid rapidly becomes cloudy and the lesion may develop an umbilicated appearance as it becomes crusted. Among healthy children, the lesions that erupt later in the disease sometimes resolve after a maculopapular phase, but many of the late lesions also progress through the vesicular stage in immunocompromised patients. In general, the time to complete crusting of lesions is prolonged. Extensive hypo-pigmentation and scarring may be seen as the crusts resolve, presumably because of the involvement of deeper layers of the dermis in immunocompromised patients.

Patients who acquire the infection during the first 9–12 months after transplantation appear to be at highest risk of developing severe varicella [1]. The potential complications of varicella after HCT can be anticipated from the clinical experience with primary VZV infection in other immunocompromised patient populations. HCT recipients can be compared with patients whose immunosuppression is due to treatment of lymphoproliferative malignancies or solid tumors. In one large series of pediatric cancer patients, 32% developed visceral dissemination in the course of varicella, with VZV infection of the lungs, liver, and central nervous system (CNS) [83]. The mortality rate was 7% overall, with an increase to 25% in patients who developed varicella pneumonia. The risk for visceral involvement was significantly higher among patients whose absolute lymphocyte counts were less than 500/μL, and the rate of visceral dissemination increased to 71% when the lymphocyte count declined to less than 100 cells/μL. Visceral dissemination was associated with new lesion formation for a median of 10 days in more than half of patients who were evaluated before effective antiviral therapy for VZV was available. Based on the analysis of placebo recipients in the original studies of antiviral treatment of varicella in high-risk children, the mortality rate was 17%; 27% of the patients developed varicella pneumonia, 19% had hepatitis, and 6% had CNS disease [84].

Among the complications of primary VZV infection, varicella pneumonia is the most common cause of life-threatening illness. Respiratory symptoms, except for mild rhinitis and cough, are unusual in uncomplicated varicella and require urgent evaluation in the immunocompromised patient. Pneumonia most often develops within 3–7 days after the appearance of the rash and progresses rapidly to respiratory failure. Clinical signs of pneumonia may be limited to tachypnea, cough, and dyspnea with no accompanying abnormal findings by auscultation. The degree of hypoxemia may be marked even when abnormalities on the chest X-ray are minimal.

Varicella can be associated with abnormal liver function tests in the immunocompetent host but hepatic involvement is usually subclinical. In contrast, immunocompromised children are at risk of fulminant varicella hepatitis, with progression to hepatic failure.

Severe varicella is usually accompanied by signs of disseminated intravascular coagulopathy, including epistaxis, hematuria, and gastrointestinal bleeding. Hemorrhage into the cutaneous lesions is recognized as a poor prognostic sign in high-risk patients. Bleeding is most often due to disseminated intravascular coagulopathy and hepatic failure, but isolated thrombocytopenia can occur. Vomiting and abdominal pain are uncommon in children with uncomplicated varicella. Severe abdominal or back pain should be considered a sign of life-threatening infection, and may be associated with syndrome of inappropriate antidiuretic hormone (SIADH) secretion in the absence of any obvious CNS disease. This atypical presentation of VZV infection has been reported after HCT [85,86]. Although the pathogenic mechanism is not understood, disseminated VZV infection can cause pancreatitis and disease of the gastrointestinal tract, such as esophagitis, which may account for these symptoms, or pre-eruptive infection of sensory ganglia may occur. Varicella meningoencephalitis is unusual in immunocompromised children but when it occurs, it is usually associated with other signs of visceral involvement. Some patients develop a rapidly deteriorating course progressing to death. Symptoms of CNS disease most often occur between 2 and 6 days after the eruption of the rash and can progress rapidly to coma. Encephalitis may precede the appearance of the rash [87]. The clinical signs include vomiting, headache, altered sensorium, and seizures. The cerebrospinal fluid (CSF) usually shows a mild to moderate pleocytosis and elevated protein. SIADH may occur in patients who have meningoencephalitis [88].

Severe hypertension is a poor prognostic sign in immunocompromised patients with varicella. The mechanism is uncertain except in cases that are associated with nephritis. Adrenal cortical necrosis is noted at autopsy in patients with disseminated VZV infection.

In addition to complications directly related to viral replication, secondary bacterial infections are a risk in immunocompromised patients with primary VZV infection, as they are in otherwise healthy children. *Staphylococcus aureus* and *Streptococcus pyogenes* (group A streptococcus) are the most common pathogens, causing cellulitis, subcutaneous abscesses, and lymphadenitis. Varicella gangrenosa, a very rare syndrome of necrotizing fasciitis caused by *S. pyogenes*, is life threatening even in the intact host. Secondary bacterial infection of sites other than skin, including bacterial pneumonia, septic arthritis, and osteomyelitis, may follow varicella. Staphylococcal sepsis can occur, and children with indwelling catheters can develop line-related infections.

Although information is limited, varicella occurring late after HCT is usually not associated with complications. Four children who had varicella 2 years after transplantation had clinical disease that resembled primary VZV infection in children with no immunodeficiency, and four children who developed varicella 105–350 days after HCT had no complications [1,74]. However, progressive varicella with severe pneumonia was reported in one child who acquired varicella 6 years after successful HCT [89].

Recurrent VZV infection

Localized herpes zoster

Localized herpes zoster is the most common clinical presentation of VZV infection in HCT recipients who are seropositive at the time of transplantation, accounting for 85% of cases in 231 HCT recipients in the study by Locksley et al. [1]. The rash of localized herpes zoster is usually preceded by pain and paresthesias in the involved dermatome [5,84,90]. These symptoms may begin as long as 5 days before the eruption and vary from mild discomfort to very severe debilitating pain. The etiology of the initial pain is sometimes misdiagnosed as pleurisy, myocardial infarct, acute abdomen, or renal colic. In some instances the prodrome is not followed by any skin eruption [62], but a typical antibody response or viremia as detected by PCR is observed; this syndrome is referred to as "*zoster sine herpete*" [91,92]. In healthy subjects, herpes zoster involves the thoracic dermatomes in about half of the cases, and cranial nerve disease occurs in 13–20% of cases [84]. Recurrent VZV affects similar dermatomes in HCT patients, with thoracic dermatomal disease in 41–47% of cases and cranial nerve involvement in 16% (Table 89.3) [1,22,93].

Table 89.3 Distribution of dermatomal involvement with herpes zoster in HCT recipients and healthy individuals with recurrent VZV infections

	HCT recipients[a]	Healthy individuals[b]
No. of patients	195	116
Dermatomes involved (%):		
Cranial	16	15
Cervical	17	22
Thoracic	47	53
Lumbar	20	18
Sacral	12	8
Dissemination (%):		
Cutaneous	23	0
Visceral	13	0
Mortality (%)	7	0

[a] Source: adapted from Locksley et al. 1985 [1].
[b] Source: adapted from Brunell 1985 [93].

The cutaneous lesions of localized herpes zoster appear as clusters of varicella-like vesicles in one or several sites anteriorly and posteriorly along the dermatome; typically, the lesions do not cross the midline, but some cases of bilateral dermatomal involvement have been described. Discrete vesicles often enlarge to form confluent lesions. In immunocompromised patients, including HCT recipients, the local vesicular eruption may be very extensive, evolving to occupy the whole dermatome;, the lesions may become hemorrhagic, and the duration of cutaneous disease is prolonged [84]. In the normal host, new lesion formation usually continues for 3–7 days followed by a phase of pustulation and crust formation; complete healing is expected by 2–3 weeks. In high-risk patients, the average time for cessation of new lesion formation was 8 days, and crusting was not complete until an average of 18 days. Immunosuppressed patients may occasionally develop a chronic cutaneous reactivation of VZV that persists for months [90,94]. As clinical experience with severely immunocompromised patients due to human immunodeficiency virus infection increases, knowledge about the spectrum of disease caused by VZV continues to expand [95]. Many of these complications are now recognized in HCT recipients with VZV reactivation.

When VZV reactivation involves cranial nerves, complications are more likely in both immunocompromised and immunocompetent patients [3]. In one series, corneal damage, facial scarring, cranial nerve VII palsy, or hearing loss occurred in 35% of patients [1]. The distribution of the ophthalmic branch of the trigeminal nerve is a common site of herpes zoster. The development of vesicular lesions on the nose is a sign of involvement of the ophthalmic branch and indicates a risk for ocular complications. The clinical findings of ocular herpes zoster include conjunctivitis, keratitis, anterior uveitis, or, rarely, pan-ophthalmitis (reviewed in [96,97]). Although corneal lesions are common, blindness is an unusual complication of herpes zoster ophthalmicus. Loss of vision associated with herpes zoster is usually secondary to retrobulbar neuritis. Although it is less common than CMV retinitis, chorioretinopathy caused by VZV is observed after HCT. Progressive outer retinal necrosis (PORN), a distinct form of VZV necrotizing chorioretinitis, is found almost exclusively in patients with acquired immunodeficiency syndrome, but cases of VZV-related PORN after allogeneic HCT have also been reported [98,99]. VZV reactivation may produce facial palsy when it involves cranial nerve VII. Herpes zoster oticus is associated with VZV reactivation from the geniculate ganglion, producing the Ramsay Hunt syndrome with unilateral deafness and vestibular symptoms. Oral lesions of the palate may develop without any cutaneous lesions when the second branch of cranial nerve V is affected.

Cerebral angiitis is a syndrome of cerebral vascular inflammation with thrombosis and microinfarcts associated with VZV reactivation in cranial nerve ganglia (reviewed in [100]). In severe cases, massive thrombosis with contralateral hemiparesis may result [101]. Infarct in the distribution of the middle cerebral artery can be demonstrated by computed tomographic scan. Pathologic findings include a granulomatous inflammatory process in the arterial wall, mononuclear cell infiltrates, vascular necrosis, and viral particles within the smooth muscle cells of the affected arteries. The syndrome is rare and there is no evidence that HCT or other immunocompromised patients are at increased risk for cerebral angiitis. However, it is important to recognize this complication in patients who develop hemiparesis about 6–8 weeks after the symptoms of herpes zoster.

When the anterior horn cells are involved in VZV reactivation, inflammation and necrosis may follow with resulting motor deficits in about 10% of otherwise healthy individuals with cervical, thoracic, or lumbosacral zoster [92]. Transverse myelitis and ascending paralysis are rare neurologic syndromes caused by recurrent VZV infection with or without immunosuppression, but the immunocompromised patients tend to have poorer outcomes. In a review of 31 patients, all 14 immunocompetent patients had good outcomes, whereas only eight out of 17 immunocomopromised patients had good outcomes [102].

Post-herpetic neuralgia (PHN)

Immunocompromised patients are at increased risk of PHN, which is the most common complication of herpes zoster in all patient populations. The definition of post-herpetic pain varies from any pain lasting after the crusts of the cutaneous lesions have disappeared to pain that persists more than 2 months. Estimates of the incidence of PHN vary depending upon the definition used. In one study of healthy individuals, 9% of patients with herpes zoster developed PHN, whereas Locksley et al. observed PHN in 25% of patients with herpes zoster after HCT [1] and Offidani et al. reported an incidence of 32% [25]. Koc et al. found that PHN and peripheral neuropathy were the most common complications of VZV reactivation after HCT, occurring in 68% of 100 consecutively identified cases [22]. The risk factors associated with PHN after VZV infection among HCT recipients were evaluated by Onozawa et al. [103]. Advanced age was the only risk factor noted in autologous HCT, but advanced age, male gender, and GVHD prophylaxis with a tacrolimus-based regimen were associated with increased risks for PHN in allogeneic HCT [103]. PHN is uncommon in children, even in those who are immunocompromised [90,104,105]; only 2–3% of pediatric HCT recipients who developed herpes zoster have persistent pain [24].

Secondary bacterial infection of skin lesions and local scarring are common in HCT recipients with herpes zoster, occurring with an incidence of 17 and 19%, respectively.

Cutaneous and visceral dissemination

In contrast to otherwise healthy subjects, HCT patients often develop cutaneous dissemination, defined as more than five vesicular lesions beyond the primary dermatome. Cutaneous dissemination has been reported in 17–24% of HCT recipients and was equally common among those who had recurrent VZV during the early or late period after transplant [1,22]. HCT recipients are also at risk of visceral dissemination during episodes of VZV reactivation (Figure 89.1). Since it is a sign of VZV infection of circulating lymphocytes, cutaneous dissemination provides a marker for the risk for visceral dissemination. However, visceral dissemination also occurs in patients whose cutaneous lesions are localized to the primary dermatome. Without antiviral therapy, visceral dissemination was observed in 13% of HCT patients with herpes zoster [22]. The potential sites of visceral dissemination with recurrent VZV are the same as those with disseminated primary VZV infection. The clinical complications that result include pneumonia, hepatitis, disseminated intravascular coagulopathy, and encephalitis. The mortality that accompanies VZV reactivation is almost always due to viral pneumonia, but fatal fulminant hepatitis and disseminated intravascular coagulopathy without VZV pneumonia have been reported [106]. An early study showed that acute GVHD is the only risk factor associated significantly with VZV dissemination [1]. An episode of zoster might trigger dermatomal GVHD as an isotopic response [107]. VZV has also been identified as the causative agent of late interstitial pneumonia in HCT patients with chronic GVHD [108].

Occasionally HCT recipients present with signs of visceral dissemination 24–96 hours before the localized cutaneous eruption of recurrent VZV becomes evident, resulting in delayed diagnosis, or misdiagnosis as GVHD or other complications [109–111]. Among these patients, the clinical presentation is characterized by abdominal pain, often mid-epigastric or periumbilical, with or without associated nausea, vomiting, or fever. Pancreatitis, hepatitis, gastrointestinal hemorrhage, intestinal necrosis, disseminated intravascular coagulopathy, and SIADH have been reported in patients with abdominal symptoms preceding the rash [63,85,109–112]. In particular, a "triad" of severe abdominal pain, SIADH, and disseminated zoster has been described in HCT or other immunocompromised patients [111,113,114]. In a series of 10 cases, patients had abdominal pain and elevated aminotransferases and pancreatic enzymes, associated with nausea (60%), fever >38 °C (60%), and vomiting (50%) [115]. Four patients had skin rash, which appeared at an interval of 4–14 days after the onset of abdominal pain. Five patients had pneumonitis and VZV infection was fatal in five cases. Visceral dissemination can also occur without any signs of cutaneous disease [116]; in those cases, VZV PCR might aid in the diagnosis [62,113]. In the study of Locksley et al. [1], three HCT patients had fatal VZV infection and no skin lesions during the course of their illness despite evidence of widespread organ infection at autopsy. In a retrospective review [33] of 310 children undergoing HCT in a single institution over a 7-year period, 61 of 201 (32%) allogeneic and 10 of 109 (9%) autologous HCT recipients developed zoster. Of 37 with available data on alanine aminotransferase (ALT) values 4 weeks preceding the clinical diagnosis of zoster, 22 (59%) patients experienced a rising ALT level, unassociated with GVHD. Hence, although there are many causes of elevation of transaminases and abdominal pain post-HCT, the possibility of VZV reaction should be considered, especially among those with increased risk factors for zoster, to allow for earlier diagnosis and therapeutic intervention [33].

Immunosuppression also predisposes to CNS infection with recurrent VZV, although symptomatic neurologic complications of herpes zoster are rare [117,118]. Two of 100 consecutively identified patients with recurrent VZV after HCT developed VZV encephalitis [22]. From the series of 310 children with HCT [33], three of 73 patients for whom sufficient information was available had CNS involvement. Despite its rare occurrence, cases of fatal VZV encephalitis have been reported [119]. In some cases, neurologic symptoms preceded eruption of skin lesions [87]. In evaluating these patients, it is important to note that abnormal CSF findings are common even in immunocompetent patients with herpes zoster. Lymphocytic pleocytosis and elevated protein were observed in about 40% of healthy individuals who had no abnormalities by neurologic examination, whereas the incidence of herpes zoster-associated encephalitis is estimated to be only 0.2–0.5% in this population [20,120,121]. The clinical symptoms and signs of CNS disease are headache, photophobia, meningismus, and altered state of consciousness [120,121]. The neurologic symptoms usually appear within a few days after the exanthem. The temperature is usually normal or mildly elevated. Seizures are rare but the electroencephalogram may show diffuse slowing or epileptiform activity.

As would be expected, the occurrence of visceral dissemination increases the mortality of recurrent VZV infection substantially, from an overall death rate of 7% to 55%. The mortality rate is higher in patients who develop both pneumonia and encephalitis than in those patients who have encephalitis only.

Atypical non-localized herpes zoster

Atypical non-localized herpes zoster after HCT is sometimes categorized as varicella because the HCT recipient is presumed to lack VZV immunity, regardless of prior immune status. However, identifying the syndrome as a distinct clinical presentation is useful because the pathogenesis of infection and the timing of the host response may be affected by differences between the endogenous and exogenous routes of infection and residual immunity may modify the clinical course. The incidence of atypical non-localized herpes zoster is variable, with reports of its occurrence ranging from none to as many as 25% of episodes of VZV reactivation after HCT. Clinically, patients with atypical non-localized herpes zoster have cutaneous vesicles that are identical with those of varicella. The number of lesions is variable but the eruption is often extensive, involving face, trunk, extremities, palms, and soles. This syndrome occurs in both autologous and allogeneic HCT recipients, accounting for 21% of episodes of recurrent VZV in one series [20]. The morbidity of untreated infection is high, with 45% of patients developing visceral dissemination, resulting in a mortality rate of 28% during the period before antiviral therapy was available [1].

Second episodes of recurrent VZV

Second episodes of VZV reactivation after HCT are uncommon. About 2% of patients had two episodes of herpes zoster, occurring at an average of 25 months after transplantation, with a range of 4–41 months. Most of the reported second episodes after HCT have involved the same dermatome but reactivation from another ganglia may occur [1].

Subclinical reactivation of latent VZV

The occurrence of subclinical VZV reactivation in HCT recipients has been shown by using a PCR method to demonstrate cell-associated VZV viremia [61]. Subclinical VZV reactivation was detected in 19% of 37 HCT patients who were screened randomly and had no signs of infection. Two of the seven patients who had subclinical VZV viremia developed clinical signs of herpes zoster subsequently, at intervals of 60 and 130 days later. Three patients who had subclinical VZV viremia between 17 and 85 days after HCT had cleared the cell-associated viremia when they were retested by VZV PCR; two patients were not re-evaluated by VZV PCR but did not develop herpes zoster. Thus, five of seven patients (71%) who had subclinical viremia did not progress to clinical infection.

Laboratory diagnosis

The diagnosis of VZV infection can be made by a variety of techniques, including viral culture, viral antigen detection, and viral DNA detection. Serologic testing is useful to demonstrate that the patient has had prior primary VZV infection and is therefore harboring the virus in sensory ganglia, but serologic tests are not useful for the diagnosis of active VZV infection. PCR technology has revolutionized the diagnosis of many infectious diseases, including that of VZV, because it is sensitive and rapid. The caveat is that clinical specimens contain inhibitors that may cause false-negative results in PCR assays.

Viral isolation in tissue culture cells

VZV can be detected in clinical samples using standard tissue culture methods for viral isolation [122]. The highest yield for viral culture depends upon obtaining vesicular fluid along with infected cells from the base of the cutaneous lesion. Vesicular fluid can be collected in a tuberculin syringe or by using a cotton or a Dacron [poly(ethylene terephthalate)] swab. Swabs should be put in viral transport medium immediately, agitated, and pressed against the side of the vial to remove absorbed fluid; the swab should be taken out of the vial before it is sent to the laboratory. If storage for more than a few hours is required, the specimen should be kept on dry-ice or frozen at −70 °C; storing the specimen at −20 °C in a standard refrigerator freezer for 24 hours usually inactivates the virus.

The optimal recovery of infectious virus in tissue culture requires the use of diploid cell lines or human embryonic lung fibroblasts. The cytopathic effect appears within 2–4 days after inoculation. Since the average time to detection of the virus in tissue culture is 7 days, diagnosis by viral culture is not rapid enough to influence clinical decisions in most circumstances. The sensitivity of tissue culture for detection of VZV is also substantially less than for identification of HSV and CMV. Procedures such as centrifugation enhancement may shorten the time required to detect cytopathic effects. There are some differences in the morphology of plaques produced by VZV compared with these other herpesviruses but the identity of the virus isolate must be proved by immunofluorescence staining with virus-specific antisera. As in the case of CMV, the shell vial culture method improves the sensitivity of VZV isolation and permits earlier identification of positive specimens. This method combines centrifugation and staining with fluorescein-conjugated monoclonal antibodies to VZV; positive results may be available within 1–3 days after inoculation. VZV detection by tissue culture methods is less sensitive than the direct fluorescence antigen (DFA) test.

The likelihood of recovery of VZV from cutaneous lesions is directly related to the stage of the lesion, with clear vesicles being much more likely to be positive than specimens from lesions that have become pustular or crusted. Varicella lesions are usually positive for 3 days, whereas virus can be recovered for 1 week or longer in herpes zoster. VZV can be isolated from PBMCs by tissue culture inoculation of specimens from immunocompromised patients with recurrent and also primary VZV infections. In contrast to meningoencephalitis associated with primary VZV infection, the virus has been isolated from the CSF of patients with herpes zoster. Bronchial washings may yield VZV in patients with pneumonia. The lungs are the most common autopsy organ from which VZV has been isolated, but the virus has been recovered from many sites, including heart, liver, pancreas, gastrointestinal tract, brain, and eyes.

Direct detection of VZV antigens in infected cells by immunofluorescence

The optimal method for the rapid diagnosis of cutaneous VZV infection is to obtain epithelial cells from a fresh lesion and to stain the specimen using fluorescein-conjugated monoclonal antibodies to VZV antigens [122,123]. The VZV-specific monoclonal antibodies bind to viral proteins that are synthesized within infected cells; fixing the cells prior to staining makes the cell membrane permeable, allowing the detection of VZV proteins in the cytoplasm and the nucleus and also on the cell surface. This method is referred to as the DFA test. The most important step in obtaining the specimen is to disrupt the roof of the vesicle in order to collect intact cells from the base of the lesion. Cells can be recovered efficiently by rotating the blunt end of a wooden applicator stick in the unroofed lesion or by scraping with a scalpel and transferring the material to a glass slide. Unless at least five intact cells are visible on the slide, the specimen should not be considered adequate for processing by the DFA method. It is important to include parallel staining of a portion of the specimen with reagents to detect HSV as VZV and HSV lesions are often indistinguishable clinically, and to provide a control for the specificity of the assay. The proper interpretation of DFA slides requires experience; the most common error is to identify false-positive results because of lack of expertise at distinguishing background non-specific fluorescence. If the clinical course of the patient is not consistent with a diagnosis of VZV infection that was based on the DFA method, the laboratory result should be questioned. DFA and indirect immunofluorescence or immunoperoxidase methods can also be used to demonstrate the presence of VZV in properly prepared tissue sections of lung, liver, and other organs from patients with disseminated VZV infections.

Enzyme immunoassay methods are also useful for detecting VZV antigens in specimens from skin lesions [122]. When laboratory facilities for DFA testing are not available, the Tzanck stain and other cytologic methods can be used to detect multinucleated giant cells in a lesion scraping; however, it is essential to realize that false negatives are common and that these methods do not differentiate VZV from HSV infection. Herpesvirus particles can be detected by electron microscopy but few diagnostic laboratories are equipped to perform this procedure. The method is not rapid and the morphology of herpesviruses is too similar to distinguish these viruses by electron microscopy.

Viral DNA detection

VZV DNA sequences can be detected using radiolabeled or biotinylated nucleic acid probes for in situ hybridization or Southern blot procedures [39,124,125]. However, in recent years, PCR has become the diagnostic test of choice for detecting VZV in clinical samples from patients with varicella or zoster. PCR assays for VZV are commercially available for routine diagnosis and can be applied to blood, CSF, skin biopsies, and various other kinds of tissues [126,127]. PCR-based techniques have been used for VZV genotyping and to differentiate wild-type VZV strains from Oka vaccine strains in investigations of varicella outbreaks following immunization [128–130]. Furthermore, this technology is particularly useful for the early diagnosis of VZV infection in the CNS. Since CNS infection by different herpesviruses may lead to indistinguishable clinical manifestations, PCR assays to enable parallel detection of more than one of these herpesviruses have also been developed [131–133]. Other molecular methods under development to detect VZV DNA include loop-mediated isothermal amplification (LAMP) [134,135] and microarrays [136–138].

Serologic diagnosis

Serologic screening of prospective HCT recipients for VZV antibodies is a valuable tool to establish immune status before transplantation. Many serologic methods are available for measuring IgG antibodies to VZV [3,122]. The most sensitive serologic assay for the detection of VZV antibodies is fluorescent-antibody staining of membrane antigen (FAMA), but this is a research method only. Other methods that are relatively reliable for establishing immune status include enzyme-linked immunosorbent assay (ELISA) and anticomplement immunofluorescence (ACIF). Although the commercially available ELISA kits for detecting VZV antibodies have a high degree of specificity, they are not as sensitive as FAMA. These methods do not usually yield false-positive results, but 10–15% of immune individuals may be wrongly identified as not having had previous VZV infection. Complement fixation methods are not satisfactory for determining immune status. Latex agglutination is a relatively sensitive and specific method for establishing VZV immune status [139,140].

Although seroconversion can be documented with primary VZV infection and boosts in antibody titers accompany recurrent VZV, the serologic diagnosis of acute infection requires paired sera and is rarely helpful for clinical purposes. VZV IgM can be detected in patients with varicella, but the majority of patients with recurrent infection also produce VZV IgM, which can be detected intermittently in healthy asymptomatic individuals [141]. Therefore, testing for VZV IgM antibodies is not useful in clinical practice, and should not be used. The test is commercially available through some reference laboratories, but testing is rarely performed with the proper control of a known VZV IgM-positive specimen.

Quantitative antibody detection has been used to measure the immune response to VZV after vaccination in clinical trials of varicella vaccines. The immune response following natural infection is usually more robust, with VZV antibody titers up to >10-fold higher than those following vaccination. Glycoprotein (gp) ELISA assays that allow quantitative VZV antibody measurement have been developed to evaluate VZV-specific immune status [142,143]. A titer of 5 gp ELISA units/mL of gp ELISA VZV antibodies at 6 weeks post-vaccination in initially seronegative children has been proposed as an approximate correlation of protection from infection [142,144]. However, this method is not commercially available and is subject to false-positive results [145]. At present, there is no reliable serologic method to assess VZV immunity after varicella vaccination. Furthermore, the detection of VZV IgG antibodies after vaccination using commercially available methods does not provide evidence of protection against breakthrough varicella, which can be expected to occur in some vaccinated individuals with close exposure.

Antiviral therapy for VZV infection

Acyclovir

Acyclovir {9-[(2-hydroxyethoxy)methyl]-9*H*-guanine} has been the drug of choice for the treatment of primary and recurrent VZV infections. The antiviral activity of acyclovir against VZV follows the same pathway that mediates its interference with the replication of HSV. The metabolism of the drug to the triphosphate form by the viral thymidine kinase produces a compound that functions as a competitive inhibitor and chain terminator of viral DNA polymerase. However, although HSV-1 and HSV-2 isolates are usually inhibited in vitro by 0.125 and 0.215 μg/mL of acyclovir, respectively, the mean concentrations required to inhibit VZV isolates are often 0.82–4.64 μg/mL, with ranges as low as 0.3 and as high as 10.8 μg/mL [146].

The plasma concentrations achieved by the intravenous administration of acyclovir at doses of 10 mg/kg or 250–500 mg/m^2 range from 15 to 25 μg/mL [84]. These concentrations are several-fold above the in vitro inhibitory concentrations for most VZV isolates. In contrast, only about 20% of the oral dose of acyclovir is absorbed. Oral administration to pediatric patients at doses of 600 mg/m^2 given four times per day produced peak plasma concentrations of approximately 1.0–1.5 μg/mL [147]. An oral dose of 200 mg of acyclovir given five times per day to adults (approximately 115 mg/m^2 for an adult male) produces plasma concentrations of approximately 0.5 μg/mL; increasing the unit dose to 600 mg resulted in plasma concentrations of 1.3 μg/mL [148]. Thus, acyclovir concentrations required to inhibit some VZV isolates can be expected to be significantly above the mean peak plasma concentration achieved by oral dosing.

Antiviral treatment of varicella

The administration of intravenous acyclovir to immunocompromised children with varicella has the potential to terminate the cell-associated viremia that produces malignant progressive varicella in these patients and to prevent the onset of varicella pneumonitis, each of which is correlated with a high risk of fatal infection [3,5,83,149]. The dosage of intravenous acyclovir for varicella in high-risk patients is 500 mg/m^2 or 10 mg/kg given every 8 hours, with administration continuing for 7–14 days. When this dosage was tested in placebo-controlled trials, the effect of the drug on the number of days to defervescence and resolution of cutaneous lesions was not significant, but varicella pneumonitis was prevented in the acyclovir recipients [150,151]. Primary VZV infection in HCT recipients occurring within the first year after transplant should be considered to require intravenous acyclovir therapy. The goal of antiviral therapy for varicella in high-risk patients is to initiate drug treatment within 72 hours after the appearance of the cutaneous rash. Parents and patients need to be educated about the typical appearance of varicella lesions because many cases occur without any known exposure, and prompt diagnosis during the early phase of infection is important to the success of antiviral therapy.

Immunocompromised children who have been vaccinated are at risk for breakthrough vesicles and parents should also be educated about the signs and symptoms, even though vaccination is expected to provide some protection from severe disease. Since there are other causes of vesicular rashes in childhood and in immunocompromised patients, the clinical diagnosis should be confirmed by the laboratory using the DFA or PCR technique. Although most children do not have clinical signs of dissemination immediately, the interval during which preventive therapy can be initiated is very short. The decision to initiate antiviral therapy must be made before progression of the rash becomes obvious, because visceral dissemination takes place at the same time. The average period to onset of varicella pneumonitis is 6 days, with most cases occurring within 4–8 days among untreated high-risk patients. In addition to preventing life-threatening dissemination, acyclovir therapy can also minimize the extent of the cutaneous disease and shorten the time to complete healing significantly [83]. More rapid resolution of the cutaneous lesions may also reduce the risk of secondary bacterial infections.

Immunocompromised children or adults who present with signs of disseminated VZV infection should receive immediate treatment with intravenous acyclovir. The efficacy of acyclovir for the treatment of established varicella pneumonia or other visceral sites of infection has not been determined in a controlled trial. Five recipients of placebo in the original acyclovir trial who developed pneumonitis were placed on the drug approximately 6–8 days after the appearance of the varicella rash, and all of these patients improved after initiation of the drug [151]. However, in another series, three of four high-risk patients who were not treated until at least 5 days after the onset of the cutaneous lesions had evidence of visceral dissemination at the initiation of treatment; all three patients had progressive varicella and two patients died [152].

Beyond 9–12 months after HCT, it may be acceptable to monitor the clinical course of varicella without giving acyclovir, assuming that the patient has no evidence of GVHD and is not receiving immunosuppressive therapy. However, given the predictable benefits of a short course of acyclovir for varicella, even in healthy children, its administration is justified.

Antiviral treatment of herpes zoster

Acyclovir has been shown to be effective for the treatment of recurrent VZV infection in immunocompromised patients in placebo-controlled trials and through extensive clinical experience with the drug. The dose of acyclovir is 500 mg/m^2 or 10 mg/kg given every 8 hours intravenously. Therapy should be continued for 7–14 days or for 2 days after cessation of new lesion formation, whichever provides the longer treatment course.

In a placebo-controlled trial, local progression and progression of cutaneous dissemination was terminated with acyclovir therapy [153]. One of 52 treated patients had progressive VZV disease compared with 11 of 42 placebo recipients. Treatment resulted in a shorter time to cessation of new lesion formation, more rapid crusting and healing, and prevention of cutaneous and visceral dissemination in patients who presented with localized herpes zoster (Figure 89.8). Acyclovir decreased the duration of local viral replication, with viral cultures remaining positive for VZV for only 4 days after the initiation of treatment. Based on this experience, acyclovir therapy initiated within 72 hours after the onset of VZV reactivation can be expected to reduce the duration of new lesion formation in HCT patients to approximately 3 days. On average, early antiviral treatment should cause the cessation of acute pain within 4 days, crusting of lesions by 7 days, and complete healing by 2–3 weeks (Figure 89.8). Although early acyclovir treatment is likely to produce the best results, clinical benefit can still occur when therapy is delayed for more than 3 days [153]. None of 29 immunocompromised patients whose therapy was initiated more than 3 days after the onset of the rash had progressive herpes zoster compared with three of 17 patients in the placebo group.

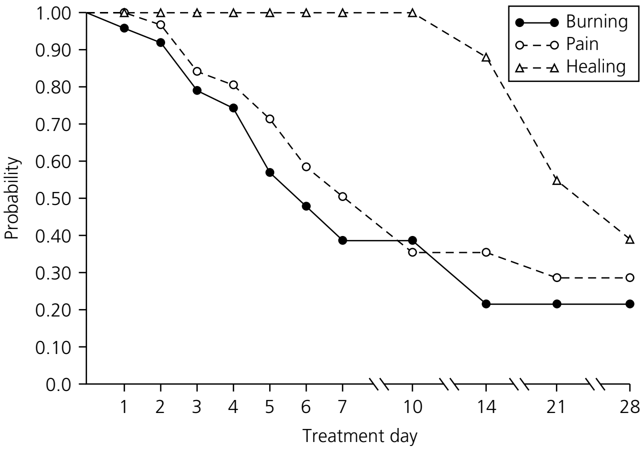

Figure 89.8 Probability of lesion burning (23 patients; •), of lesion pain (31 patients; ∘), and of not having total healing (31 patients; Δ) by treatment day. Source: Meyers et al. 1984 [277]. Reproduced with permission of Lippincott Williams & Wilkins.

Although the drug eliminates the life-threatening complications of VZV reactivation in most patients, relapse of herpes zoster is a clinical problem in some HCT patients who are treated with acyclovir. In one series, five of 40 patients (12%) developed new lesions with relapse occurring less than 4 days after treatment was stopped in three of the five patients [154]. Early acyclovir therapy may delay the recovery of VZV-specific immunity in some patients. Nevertheless, most patients respond to treatment with a second course of acyclovir.

The efficacy of oral acyclovir for herpes zoster in HCT patients has not been firmly established in prospective studies, but this route of administration is appropriate for selected patients who have localized herpes zoster occurring in the late period after HCT. The bioavailability of oral acyclovir is low, and this route of administration requires giving 800 mg per dose, five times per day. Most clinicians now prefer to use valaciclovir, the prodrug of acyclovir, or famciclovir, which have enhanced bioavailability (see below). Patients receiving oral acyclovir should be monitored for signs of progressive VZV infection and should be treated with the intravenous drug if complications arise. When patients are treated with the standard intravenous regimen, there is no need to provide further oral treatment after discharge. However, in some cases, the full course of therapy may be achieved by a combination of intravenous and oral agents. HCT recipients may have a higher rate of recurrence of pain after cessation of therapy.

Acyclovir toxicity

The safety of acyclovir has been established in numerous clinical trials. Although HCT patients also tolerate the drug well, the incidence of side effects is higher. In one series, gastrointestinal symptoms of nausea and vomiting occurred in 40% of treated patients [154]. Nephrotoxicity, defined as a 50% rise in serum creatinine, was also more common than in other patient populations treated with acyclovir; 10–25% of HCT patients receiving acyclovir are reported to have an abnormal serum creatinine, but these elevations may be caused by other medications given concurrently [154]. In any case, since acyclovir is excreted by glomerular filtration, other drugs that affect renal function, such as cyclosporine, can interact to cause elevated plasma concentrations of the drug.

The dosage and dose interval for acyclovir administration should be adjusted based on the relative impairment of creatinine clearance. The dosage interval should be lengthened to every 12 hours for clearances of 25–50 mL/min and to 24 hours for clearances of 10–25 mL/min; if the clearance is 0–10 mL/min, the dosage should be reduced to 250 mg/m^2, given every 24–48 hours. It is also important to maintain adequate hydration in patients receiving acyclovir to avoid precipitation of the drug in renal tubules. A few cases of acute neurotoxicity have been reported in patients with deficient renal clearance who were receiving acyclovir. Abnormal liver function tests should be considered possible evidence of VZV hepatitis rather than drug toxicity. Acyclovir does not have hematologic toxicity and does not interfere with engraftment in HCT recipients.

Resistance to acyclovir

Although VZV resistance to acyclovir is not common in HCT recipients, it has been reported in these patients and patients with the acquired immunodeficiency syndrome [155–157]. Among HCT recipients, the failure of VZV infections to resolve, or their recurrence shortly after antiviral therapy is discontinued, is usually a function of the limited host response and should not be attributed to acyclovir resistance in most cases. When it occurs, antiviral resistance is usually mediated by thymidine kinase mutations, but mutations of the viral DNA polymerase have also been reported [158,159]. Interestingly, thymidine kinase mutations can be both temporally and spatially compartmentalized. Brink et al. reported the finding of plasma samples with wild-type thymidine kinase sequences when CSF or skin vesicle fluid acquired on the same day contained mutant sequences [160]. Hence sampling for molecular diagnostics should be performed on plasma and also samples from other affected body sites whenever possible, if antiviral resistance is suspected.

Other antiviral compounds

Famciclovir and valaciclovir are nucleoside analogs and are prodrugs of penciclovir and acyclovir, respectively. Both have been licensed for the treatment of herpes zoster in the immunocompetent host [161–164]. Similarly to acyclovir, penciclovir [9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine] is phosphorylated by the viral thymidine kinase and acts as a competitive inhibitor of the viral DNA polymerase, but unlike acyclovir, it is not an obligate chain terminator. The advantage of these prodrugs is much better absorption after oral administration than acyclovir, coupled with a safety profile that resembles that of acyclovir; however, they are not useful for treating VZV infections caused by acyclovir-resistant strains [155–157]. Although controlled trials to establish the clinical efficacy of these antiviral agents in HCT patients have not been performed, valaciclovir or famciclovir may be useful in selected patients who are considered to be at low risk of visceral dissemination during recurrent VZV reactivation. Attention to the dosage of these very efficiently absorbed drugs is essential, since toxicity of valaciclovir, manifest as thrombocytopenic purpura and hemolytic uremic syndrome, has been reported in HCT recipients [165]. Plasma concentrations approach those achieved by intravenous administration of acyclovir unless the patient has altered gastrointestinal function, which may be an obstacle to their use in some HCT recipients. Famciclovir and valaciclovir require less frequent administration and are more convenient for patients, but these drugs are more expensive than oral acyclovir.

Foscarnet, a pyrophosphate analog, has antiviral activity against VZV through inhibition of the viral DNA polymerase and has activity against isolates that are not inhibited by acyclovir or famciclovir [95,155,166]. Its clinical use is complicated by potential nephrotoxicity and the emergence of resistance [167,168].

Cidofovir is a nucleotide agent that may be useful for resistant strains but clinical experience is limited. Serious side effects of this drug include severe nephrotoxicity, metabolic acidosis, neutropenia, and ophthalmic complications.

Ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG)] has in vitro activity against VZV that is equivalent to acyclovir, but clinical studies of its efficacy for the treatment of VZV infections have not been carried out because of its greater toxicity. However, it is possible that the administration of ganciclovir to HCT recipients who have CMV infection could alter the course of concurrent VZV infection.

Vidarabine, the first antiviral agent shown to have clinical benefit for the treatment of VZV infections in immunocompromised patients, inhibits VZV by a different pathway, but was less effective than acyclovir and is no longer available [154]. Brivudine, a nucleoside analog of thymidine and its arabinofuranosyl derivative sorivudine also have potent antiviral activities against VZV and HSV. A single daily dose of brivudine at 125 mg was found to be effective in the treatment of herpes zoster compared with acyclovir 800 mg five times per day or famciclovir 250 mg three times per day, all given orally [169,170]. However, the metabolite of brivudine and sorivudine blocks the degradation of the anticancer drug 5-fluorouracil (5-FU), resulting in the accumulation of 5-FU to toxic levels. The development of sorivudine was terminated in the United States because of such lethal interactions with 5-FU and the concern that it might be administered inadvertently to patients who were receiving this drug [171,172]. Brivudin is approved for sale in some European countries, including Germany and Italy, but its use is contraindicated in cancer patients taking 5-FU, its prodrugs (e.g. tegafur, capecitabine), or other antimetabolites. Brivudin is not licensed for use in the United States.

Human and recombinant leukocyte interferon were shown to have significant clinical efficacy in immunocompromised patients with herpes zoster in early studies and provide an alternative to the nucleoside analog drugs for the treatment of resistant VZV infection [173]. However, given the availability of other antivirals, this approach is now rarely employed, if at all.

It should be noted that despite the activities of these agents against VZV, there is no evidence that combinations of these antiviral drugs improve the treatment of VZV infection and adverse drug interactions may occur.

Anti-VZV drugs in development

A number of novel antivirals against VZV are currently under development, including the helicase–primase inhibitors (HPIs), bicyclic nucleoside analogs (BCNAs), an ester derivative of cidofovir, and a new nucleoside analog.

One of the novel HPIs, ASP2151 (also known as amenamevir), is a non-nucleoside oxadiazolylphenyl derivative with potent activities against VZV and also HSV-1 and -2. This drug is significantly more potent than acyclovir in vitro and in vivo (e.g. anti-VZV EC_{50} of ASP2151 at 0.038–0.10 μM compared with that of acyclovir at 1.3–2.7 μM) [174] and is active against acyclovir-resistant virus. It is orally available and has been evaluated in several clinical trials. A randomized, double-blind control trial showed that three-day or single-day courses of ASP2151 appear to be effective and safe options for the treatment of recurrent genital herpes compared with valaciclovir [175]. A dose-finding study that investigated the efficacy and safety of three different doses of ASP2151 in comparison with valaciclovir in subjects with herpes zoster was also completed, but no results have been reported (ClinicalTrials.gov Identifier: NCT00487682).

The BCNAs have emerged as the most potent anti-VZV agents reported, with in vitro anti-VZV activity at ≤1 nM concentration, at least 1000 times more active than acyclovir (reviewed in [176,177]). Whereas HPIs are equipotent against VZV and HSV, BCNAs are selective for VZV, as they require activation by VZV thymidine kinase-mediated phosphorylation, but are not recognized by HSV thymidine kinase. Furthermore, neither cellular cytosolic nor mitochondrial thymidine kinases can phosphorylate BCNAs, thus explaining their low toxicities [177,178]. FV100, the prodrug of a BCNA Cf1743, was well tolerated by healthy subjects in a randomized, double-blind, placebo-controlled trial that studied its pharmacokinetics and safety [179]. A phase II clinical trial to evaluate FV100 for the treatment of herpes zoster in comparison with valaciclovir was also completed in December 2010, but no results have been reported to date (ClinicalTrials.gov Identifier: NCT00900783). Despite the potency of BCNAs, thymidine kinase mutants of acyclovir or brivudine would likely confer cross-resistance to this class of antivirals [177].

CMX001 is a lipid ester of cidofovir with enhanced bioavailability and was also significantly more active against double-stranded DNA viruses (including herpesviruses) than cidofovir when tested in vitro [180]. The drug was found to be well tolerated by healthy volunteers [181]. When tested as salvage therapy in 13 immunocompromised patients with severe adenovirus infection, no serious side effects were attributable to CMX001 [182]. Early-phase clinical trials to evaluate its safety and tolerability in immunocompromised patients with CMV infection and with BK infection have been completed (ClinicalTrials.gov Identifiers: NCT00942305 and NCT00793598, respectively) and several other clinical trials to evaluate the safety and efficacy of this drug are ongoing. There are currently no clinical data that demonstrate the use of this drug for varicella or zoster treatment, but it might have a significant role in the treatment of acyclovir-resistant VZV, given its potency and safety profile.

Valomaciclovir is the diester prodrug of the acyclic guanosine derivative (*R*)-9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine (H2G). It has been tested in a randomized, double-blind study for the treatment of herpes zoster in immunocompetent adults in comparison with valaciclovir [183]. Valomaciclovir was tested in three different doses (1000, 2000, and 3000 mg daily). The two higher doses were found to be non-inferior to valaciclovir 1000 mg three times daily for the primary efficacy measure of "time to complete crusting of the zoster rash by day 28." However, for some of the secondary endpoints (time to cessation of new lesion formation and time to cessation of pain by day 120), no valomaciclovir groups were non-inferior. Further studies to evaluate the potential of this drug for VZV treatment are needed.

Treatment of PHN

Although acyclovir is clearly beneficial for the treatment of acute herpes zoster, its effect on the incidence of PHN has been more difficult to establish. Varying definitions of PHN have complicated assessments of antiviral and adjunctive therapies. A meta-analysis of several placebo-controlled trials of oral acyclovir therapy for herpes zoster in otherwise healthy individuals indicated that long-term pain is diminished [184,185]; however, a more recent Cochrane review concluded that oral acyclovir treatment during the acute phase of zoster does not significantly reduce the incidence of PHN [186]. The fact that acyclovir ameliorates the acute pain associated with herpes zoster more effectively than long-term pain suggests that a different mechanism is responsible for PHN. Continued VZV replication is not likely to account for post-herpetic pain. Instead, it has been suggested that the initial neuronal infection and associated inflammation, which can persist for months, result in deafferentation, ectopic activity of damaged afferents nerves, and enhanced excitability of neurons relaying central pain signals (reviewed in [187]).

Current approaches to the management of PHN (reviewed in [187–189]) include opioid analgesics [190], tricyclic antidepressants [191–194], and antiepileptics such as gabapentin [195,196], pregabalin [197,198], and divalproex sodium [199]. Topical application of lidocaine or capsaicin in the form of patches or cream can also provide some relief.

Although the administration of corticosteroids orally or intrathecally as adjunctive therapy may have some benefits in relieving the zoster-associated pain during the acute phase of disease [200–202], steroid treatment is ineffective in preventing PHN [202,203]. However, intrathecal corticosteroids might provide relief to some patients with intractable pain from PHN [204]. Medical interventions to treat PHN are often used in combination, or sequentially, because no single approach is beneficial in most patients. The benefits of some other approaches, such as neuroaxial and sympathetic blockade [205], spinal cord stimulation [206], intravenous lidocaine [207], or cryotherapy [208], have not been convincingly demonstrated. A number of other agents (e.g. the selective serotonin reuptake inhibitors duloxetine and venlafaxine, tramadol, or tapentadol) have also been used in the treatment of neuropathic pain including PHN, but clinical data from randomized controlled trials are currently lacking [187].

Prophylaxis for VZV infection

Varicella zoster immune globulin

Varicella zoster immune globulin (VZIG) is a passive antibody preparation containing VZV IgG antibodies that is prepared from high-titer immune human serum. VariZIG (Cangene Corporation, Winnipeg, Canada) is currently the only VZIG preparation available in the United States and was approved by the US Food and Drug Administration (FDA) in December 2012 for postexposure prophylaxis of varicella in persons at high risk for severe disease. The product is lyophilized, and when reconstituted properly is an approximately 5% solution of IgG. The dosage is one vial (125 units) per 10 kg body weight given intramuscularly with the minimum dose of 62.5 units for pediatric patients who weigh ≤2 kg and the maximum dose of 625 units for patients who weigh >40 kg. If VariZIG is not available, an alternative would be intravenous immunoglobulin (IVIg), which can be administered at 400 mg/kg [209].

Passive antibody prophylaxis with VariZIG or IVIg is indicated following exposure of VZV seronegative, immunocompromised children or adults who have not had previous VZV infection, including HCT recipients [165,210]. HCT recipients are in general considered non-immune, regardless of previous history of varicella disease or varicella vaccination in themselves or in their donors. However, if varicella or zoster develops after transplantation, they should then be considered immune [210] and passive antibody prophylaxis would not be indicated after exposure. Passive antibody prophylaxis should be administration within 96 hours and preferably within 48 hours after exposure, although VariZIG can be administered up to 10 days after exposure [211]. Varicella exposure is defined as household contact, shared hospital room, or indoor play for at least 1 hour with a child who is in the contagious phase of varicella, which is the interval from 2 days before to 5 days after the onset of the rash. If a patient has received any of the commercial preparations of high-dose IVIg (≥400 mg/kg) for other indications within 3 weeks before the exposure, it is not necessary to administer VZIG [185,210]. The duration of protection after administration of passive antibody prophylaxis is unclear, but it is thought to last at least one half-life of the Ig, which is about 3 weeks. Therefore, a second dose of passive antibody prophylaxis should be given if a new exposure occurs more than 3 weeks after a dose of VZIG has been given [210]. Although the risk of VZV transmission from an individual with herpes zoster is low, close contact between an HCT patient who has never been infected with VZV and a patient with recurrent VZV lesions also justifies the administration of VZIG.

Clinicians must be aware that severe varicella develops in some immunocompromised patients despite the timely administration of VZIG. The incidence of varicella despite VZIG prophylaxis is significantly higher for children with household exposures than for those who have less prolonged contact with the index case, and the attack rate is affected by the VZV IgG titer of the preparation [212]. In a placebo-controlled trial of intravenous acyclovir, one of six (17%) placebo recipients who had received ZIG (zoster immune globulin) or ZIP (zoster immune plasma) before entry required reassignment to open drug because of progressive varicella [151]. In the St. Jude experience, the risk of varicella pneumonitis was reduced significantly by passive antibody prophylaxis but 11% of children developed pneumonia despite receiving ZIG, ZIP, or VZIG [83]. Because of these risks, HCT recipients who develop varicella should be treated with intravenous acyclovir even if passive antibody prophylaxis was given at the time of exposure.

Since the live attenuated varicella vaccine is recommended for universal administration to children in the United States at age 12–15 months, some children will have had vaccine-induced immunity before HCT [213]. These children should be given passive antibody prophylaxis for exposure to wild-type varicella because the protective efficacy of varicella vaccine is not defined in these circumstances. High-risk children may also have close exposure to an immunized child or adult who has breakthrough varicella caused by the wild-type virus or, rarely, to a healthy contact with a varicella vaccine-related rash. Immunocompromised children who have such an exposure should receive passive antibody prophylaxis. However, depending on the timing of administration after vaccination, contacts treated with passive antibody prophylaxis can still develop subclinical or clinical varicella. In an early study that evaluated the efficacy of VZIG in immunosuppressed children after household exposure to varicella, up to 60% developed clinical disease despite detectable VZV antibodies [212]. Based on reports of clinical experience but without controlled trials, some experts have recommended using acyclovir (or valaciclovir) in combination with passive antibody prophylaxis or alone as postexposure prophylaxis for VZV exposure in susceptible high-risk children [214–216]. There is no indication that passive antibody prophylaxis will reduce the risk of VZV reactivation after HCT in patients who have serologic evidence of prior VZV infection. Passive antibody administration is not effective for the treatment of herpes zoster.

Acyclovir prophylaxis

The efficacy of acyclovir prophylaxis for preventing recurrent VZV infection in HCT recipients has been evaluated in a number of studies [23,27,217–228], but only four of them were prospective

Table 89.4 Selected studies on antiviral prophylaxis for prevention of VZV infection after HCT

Study	Randomized, double-blind, placebo-controlled?	HCT type	Antiviral drug/dose[a]	Total duration of prophylaxis (months)	No. of patients on prophylaxis/ no prophylaxis	No. of patients with VZV reactivation during prophylaxis	Cumulative incidence of VZV reactivation from day 0 of HCT (treatment group/ control group)
Ljungman, 1986 [217]	Yes	Allogeneic	Acyclovir 1200 mg qd	6 months	20/22	0	20%/27% at 1 year
Selby, 1989 [218]; Perren, 1988 [219]	Yes	Allogeneic	Acyclovir 800 mg q6h	6 months	42/40	0	32%/45% at 1 year
Sempere, 1992 [220]	No	Autologous	Acyclovir 400 mg tid	6 months	21/NA	0	32% at 1 year
Steer, 2000 [23]	No	Allogeneic	Acyclovir 200 mg tid ± IV ganciclovir 5 mg/kg tiw[b]	≥6 months	143/NA	0	32%/NA at 2 years
Kanda, 2001 [221]	No	Allogeneic	Acyclovir 400 mg qd	During immunosuppression (median of 152 days)	45/41	0	10%/33% at 1 year
Thomson, 2005 [222]	No	Allogeneic	Acyclovir 200 mg bid	During immunosuppression	247/NA	1	34%/NA at 5 years
Boeckh, 2006 [223]	Yes	Allogeneic	Acyclovir 800 mg bid	1 year	38 /39	(2)[c]	21%/31% at 2 years; 37%/36% at 5 years
Erard, 2007 [27]	No	Allogeneic	Acyclovir 800 mg bid or valaciclovir 500 mg bid	1 year	851/720	NA	8.8%/24.9% at 2 years
Erard, 2007 [27]	No	Allogeneic	Acyclovir 800 mg bid or valaciclovir 500 mg bid	During immunosuppression or ≥1 year	402/720	NA	4.5%/24.9% at 2 years
Erard, 2007 [27]	No	Autologous	Acyclovir 800 mg bid or valacyclovir 500 mg bid	≥1 year	450/212	NA	8.2%/21.7% at 2 years
Asano-Mori, 2008 [224]	No	Allogeneic	Acyclovir 200 mg qd	During immunosuppression or ≥1 year	137/105	1	20%/50% at ~3 years
Kim, 2008 [225]	No	Allogeneic	Acyclovir 400 mg bid or 200 mg bid	During immunosuppression (range 3–47 months)	100/166	2	6.6%/23% at 2 years; 9%/29% at 3 years
Oshima, 2010 [226]	No	Allogeneic	Valaciclovir 500 mg tiw	1 year	40/NA	2	18.5%/NA at 2 years
Uchiyama, 2010 [227]	No	Unrelated cord blood	Acyclovir 200 mg bid	During immunosuppression or ≥1 year	8/NA	(1)[c]	NA
Klein, 2011 [228]	Yes	Allogeneic or autologous	Valaciclovir 1000 mg bid	4–24 months	22/26	0	NA

Abbreviations: bid, twice per day; qd, daily; q6h, every 6 hours; tid, three times per day; tiw, three times per week; NA, not available.

[a] Patients in these studies might receive a higher dose of oral acyclovir or intravenous acyclovir during the early phase of HCT (e.g. until engraftment) before being started on prophylaxis. Please refer to references for details.

[b] 28/143 patients received acyclovir only, and 115/143 patients received both ganciclovir and acyclovir. Ganciclovir was given to patients with CMV^+ donors from engraftment to at least day 84, or to those receiving ≥20 mg of prednisone for GVHD treatment. For those patients receiving ganciclovir, acyclovir prophylaxis recommenced at the completion of ganciclovir prophylaxis. (See [23] for details.)

[c] Cases occurred when patients were off acyclovir.

randomized, double-blind trials (summarized in Table 89.4). The dose of acyclovir employed ranged from 200 to 3200 mg per day, and valaciclovir was also used in some studies. Most of these studies gave prophylaxis for a duration of 6–12 months, or until the end of immunosuppressive therapy. Overall, the administration of acyclovir (or valaciclovir), even at low doses, can effectively prevent VZV reactivation during the prophylactic period. Among all these studies on >2000 patients combined, there were only a few cases of breakthrough zoster infection, compared with a 30–60% incidence of VZV reactivation without prophylaxis [1,22,24] (Table 89.4). However, whether acyclovir prophylaxis confers any long-term benefits is more controversial. Regardless of the acyclovir dose or duration employed, prophylaxis with acyclovir in general delays but does not completely prevent VZV reactivation. Several earlier studies using "higher" doses of acyclovir found that VZV readily reactivated after cessation of acyclovir prophylaxis, with accumulative incidence in subsequent years not significantly different from the placebo group [217–219,223]. For instance, patients who underwent allogeneic HCT were randomized to receive acyclovir 800 mg twice daily or placebo for 1 year after transplantation in a double-blind controlled trial of the efficacy of long-term acyclovir prophylaxis [223]. Acyclovir significantly reduced VZV infection at 1 year after transplantation, with a hazard ratio (HR) of 0.16, but the postintervention observation at 2, 3, and 5 years showed no statistical difference in VZV infection between the two groups. In contrast, several more recent studies [27,28,221,224,226] have found a reduced cumulative incidence of VZV reactivation among the prophylaxis groups after cessation of antiviral therapy. Furthermore, the severity of clinical symptoms associated with VZV reactivation was also reduced, with fewer patients developing disseminated disease or VZV-related complications and mortality [224,225]. Most of these studies used low doses of acyclovir [28,221,224,226], and it has been theorized that administration of very low-dose acyclovir might permit subclinical VZV reaction to allow the recovery of VZV-specific T-cell immunity [61]. However, the favorable outcomes of low-dose acyclovir prophylaxis are not universal [222] and have not been validated by randomized prospective studies.

The most recent guidelines [216], developed jointly by the Centers for Disease Control and Prevention (CDC), the Infectious Diseases Society of America (IDSA), the American Society for Blood and Marrow Transplantation (ASBMT), and other agencies for preventing infectious complications after HCT now recommend long-term acyclovir to prevent recurrent VZV infection for the first year after HCT among VZV-seropositive allogeneic and autologous HCT recipients. As VZV reactivation after cessation of acyclovir prophylaxis appears to occur predominantly in patients who are continued on or have resumed immunosuppressive therapy [221,224,226], acyclovir prophylaxis may be continued beyond 1 year in allogeneic HCT patients, until all systemic immunosuppressive drugs are discontinued and the CD4 count exceeds 200 cells/μL [216]. A prophylactic regimen of acyclovir 800 mg PO twice daily or valacyclovir 500 mg PO twice daily is recommended [216], although lower doses of acyclovir also appear to be effective, as summarized in Table 89.4. Although prolonged administration of low-dose antivirals for prophylaxis might enhance the emergence of VZV strains that are resistant to the drug, acyclovir-resistant VZV strains have been reported only rarely in HCT patients [158,160], and genotypic analyses failed to identify a cause of resistance in up to 50% of the isolates with clinical resistance to acyclovir [158,159]. Patients with breakthrough infection reportedly responded well to therapeutic doses of oral acyclovir, valacyclovir, or famiciclovir, or IV acyclovir, without any evidence of developing acyclovir-resistant mutants [224–226].

VZV vaccines

In 1995, a live attenuated varicella vaccine (Oka strain) was approved for administration to healthy children in the United States. Experience from the last 10–20 years, with tens of millions of doses administered over the pre- and postlicensure periods, has demonstrated the effectiveness and safety of this vaccine in the immunocompetent population. From various investigations, the overall vaccine effectiveness ranges from 71 to 100% against varicella and from 95 to 100% against moderately severe and severe disease [229–231]. After the implementation of universal childhood vaccination, the incidence of varicella declined by 90% and related mortality declined by 66% [232,233]. Two live, attenuated VZV-containing vaccines are available in the United States for prevention of varicella: a single-antigen varicella vaccine (Varivax; Merck) and a combination vaccine for measles, mumps, rubella, and varicella (ProQuad; Merck). In 1996, the Advisory Committee on Immunization Practices (ACIP) recommended one dose of varicella vaccine for children aged 12 months to 12 years, but two doses, 4–8 weeks apart, for those aged ≥13 years [234]. However, despite the success of the one-dose regimen with a vaccine effectiveness of 85%, varicella outbreaks continue to occur in highly vaccinated school populations [235]. Furthermore, a two-dose regimen was found to be significantly more effective than a single injection with a 10-year observation period [236]. In 2006, ACIP started to recommend two doses of varicella vaccine as opposed to one dose for children, and also all susceptible adolescents and adults [210]. A second dose is also recommended for all persons who received one dose previously. Following the implementation of two doses, the incidence of varicella further decreased from 48.7 cases/100,000 persons in 2005 to 24.5 in 2008 [237].

In May 2006, a live attenuated vaccine (Zostavax; Merck) was also approved in the United States for prevention of zoster and PHN in adults aged 60 years or older. This zoster vaccine is also based on the Oka-Merck strain, but each dose contains 19,400 PFU, compared with 1350 PFU in the varicella vaccine. In a large prelicensure study that enrolled 38,546 adults, the vaccine reduced the incidence of zoster by 51.3%. The burden of illness due to zoster and due to PHN was also reduced by 61.1% and 66.5%, respectively [238]. Based on a multicenter study conducted in approximately 22,000 people who were 50–59 years of age [239], the FDA further approved the use of Zostavax in adults of this age group in March 2011. However, ACIP has declined to recommend its use among adults aged 50–59 years and reaffirmed its existing recommendation that herpes zoster vaccine be routinely recommended for adults aged 60 years and older as a single dose [240], regardless of prior history of zoster [241]. Unlike Varivax, a second dose of Zostavax does not boost VZV-specific immunity beyond levels achieved after one dose [242].

The live attenuated varicella vaccine was originally investigated in children with leukemia, in an effort to prevent the development of severe or fatal VZV infection in these patients [243]. Although the varicella and zoster vaccines have significantly changed the impact of VZV infection on healthy pediatric and adult populations, the use of live virus vaccines is in general not recommended for immunocompromised hosts [244], given the potential increased risks for adverse events from the live virus and decreased effectiveness of immunization. Disseminated infection and severe complications after vaccination against VZV have been reported in immunocompromised patients [245–248] and in children with underlying immunodeficiency not apparent at the time of immunization [81,249–253]. However, there are now accumulating data to suggest that live varicella vaccine may be immunogenic and safe in certain immunocompromised pediatric patient populations, including selected HCT recipients [254–256], HIV patients [257–259], and those status post-liver and/or intestine transplantation [260–262]. Guidelines on vaccination of HCT recipients [216,263–265] recommend that live-attenuated varicella vaccine can be considered in HCT recipients who are at least 24 months post-transplant, not receiving immunosuppressive drugs, and free of GVHD. Some also proposed additional immunologic criteria, such as a circulating CD4 cell count of >200 cells/μL, a normal T-cell proliferative response to selected antigens, and/or immunologic response to prior vaccination post HCT [255,256,265].

For Zostavax, a recent retrospective cohort analysis showed that the vaccine administered in the setting of concurrent treatment with biologics was not associated with a short-term increase in the risk for varicella or zoster; instead, a lower risk of zoster (HR 0.61) was observed over a median of 2 years follow-up, compared with unvaccinated controls [266]. Naidus et al. [267] also reported the use of Zostavax in 62 patients with hematologic malignancy, of whom 26 and 5 received an autologous and allogeneic HCT, respectively. A single patient developed zoster during the study period, and there were no documented adverse reactions associated with the vaccine. It should be emphasized that these were select patients without significant immunosuppression. Furthermore, 13 of the 31 HCT patients, who might have been perceived as at higher risk by their clinicians, received concurrent antiviral drugs at the time of vaccination. Larger, prospective, controlled studies to study the safety and efficacy of Zostavax in HCT recipients are needed and its use in HCT populations remains contraindicated [216].

Immunizing healthy household contacts who are not immune to varicella with the varicella vaccine can reduce the risk of household exposure of the HCT recipient to varicella in siblings [268]. The risk of transmission of the vaccine strain is low. After the first 10 years of postmarketing experience, with ~55 million Varivax doses distributed worldwide, there were only three cases of confirmed and two cases of suspected secondary transmission of the Oka VZV [252]. Secondary transmission of the vaccine strain from recipients of Zostavax has not been documented [269]. To our knowledge, no secondary transmission from either Varivax or Zostavax recipients to immunocompromised hosts has been reported to date. Furthermore, illness caused by the vaccine strain in immunocompromised individuals is treatable with acyclovir, whereas infection associated with wild-type VZV can be severe. Since the benefits of vaccination outweigh the potential risk of transmission of vaccine-type virus to immunocompromised persons, routine vaccination of susceptible household contacts of immunocompromised persons is recommended [216,244]. Healthcare workers in contact with HCT patients should also be evaluated for susceptibility to VZV and immunized. There are also no contraindications for eligible household members of immunocompromised persons or healthcare workers in contact with HCT patients to receive the zoster vaccine for the prevention of zoster and PHN. Any vaccine recipients, particularly healthcare workers and household contacts, should avoid contact with a susceptible immunocompromised person if a vaccine-related rash occurs [244]. Since some vaccine recipients become candidates for HCT, post-transplant reactivation of vaccine virus has been demonstrated in a few instances. However, the vaccine virus appears to reactivate less commonly than wild-type VZV [270]. Only two cases of zoster were reported during a 10-year follow-up of ~2000 healthy children vaccinated with Varivax [236]. If symptomatic reactivation occurs, the vaccine virus is susceptible to inhibition by acyclovir and episodes of vaccine-related herpes zoster can be treated with this drug [271].

Whether immunization with an inactivated preparation of the live attenuated varicella vaccine could substitute for the "natural" resensitization caused by VZV reactivation after HCT and whether the early restoration of immunity modified the clinical course of recurrent VZV disease have been evaluated in two prospective studies [32,272]. The administration of the live attenuated varicella vaccine to elderly individuals without underlying disease boosts VZV-specific immunity, and immunization has been evaluated as a strategy to reverse the decline of T-cell responses that is associated with the increased risk for herpes zoster in this population [238,273]. Although live attenuated varicella vaccine is contraindicated in most immunocompromised patient populations, the vaccine can be heat inactivated without loss of immunogenicity [274]. HCT recipients recover VZV immunity but reconstitution is delayed for months and often does not occur until after the patient has experienced herpes zoster [4,32,61,73]. A single-dose regimen of inactivated varicella vaccine induced short-term immunologic enhancement but did not result in clinical benefit. A three-dose regimen in which vaccine was given to allogeneic or autologous transplant recipients at 30, 60, and 90 days after HCT boosted cell-mediated immunity and modified the clinical severity of herpes zoster, but did not reduce the overall incidence of herpes zoster after HCT [272].

When inactivated varicella vaccine was given to autologous HCT recipients in a regimen that included a pretransplantation dose, and also doses given at 30, 60, and 90 days after HCT, the risk of herpes zoster was reduced and protection correlated with reconstitution of VZV CD4 T-cell immunity [32]. In this study, 19 of 58 unvaccinated patients (33%) developed herpes zoster compared with seven of 53 (13%) of vaccine recipients ($p = 0.01$) (Figure 89.9). The difference was 30% compared with 13% ($p = 0.02$) when two patients who developed zoster before transplant were excluded. VZV-specific CD4 T-cell proliferation was significantly higher in vaccinees by 90 days, following three doses of vaccine ($p = 0.04$), and the differences remained significant at 120 days, after four doses ($p = 0.0001$), and at 6 months ($p = 0.004$), and 12 months ($p = 0.02$) (Figure 89.5).

As noted in the section on host response, this prospective study demonstrated that VZV-specific CD4 T-cell proliferation was a correlate of protection against VZV reactivation [32]. The risk of zoster was reduced by 19% per unit increase in stimulation index >1.6, and a stimulation index >5.0 correlated with >93% protection. The relationship between a high stimulation index and reduced risk of herpes zoster could reflect the direct contribution of VZV-specific CD4 T cells to controlling VZV replication, since these cells make IFN-γ and other cytokines, and have cytotoxic function. Reconstitution of VZV-specific CD4 T cells by vaccination may also provide helper functions necessary to expand VZV-specific CD8 T-cell populations that are essential for preserving VZV latency.

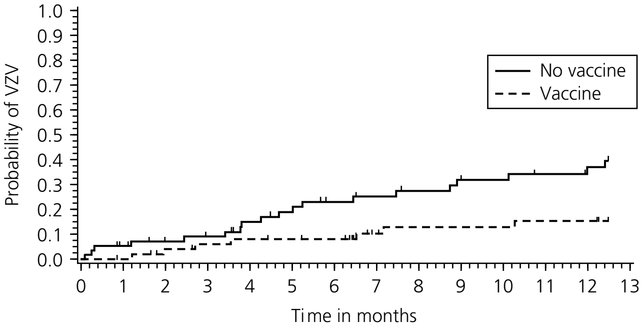

Figure 89.9 Occurrence of zoster in autologous HCT recipients randomized to receive inactivated varicella vaccine or no vaccine. The Kaplan–Meier plot shows the probability of zoster in study participants who were randomized to receive inactivated vaccine (dashed line) and those who were unvaccinated (solid line), from enrollment until 12.5 months after HCT. The incidence of zoster was significantly higher in the unvaccinated group ($p = 0.01$). When the two cases of zoster that occurred before transplant were excluded, the difference remained significant ($p = 0.02$). Source: Hata et al. 2002 [32]. Reproduced with permission of the Massachusetts Medical Society.

In terms of basic immunobiology, the reduced frequency of VZV reactivation observed after active immunization of HCT recipients establishes the critical role of virus-specific T-cell responses in preserving the balance between VZV and the host. Sustaining these host responses by repeated doses is likely to be necessary to preserve clinical efficacy against herpesvirus reactivation while immunosuppressive therapy continues. Immunization of HCT patients against herpesviruses represents a particular challenge because disease is usually due to the reactivation of endogenous viruses during the first several months after transplantation. However, this experience with inactivated varicella vaccine indicates that immunotherapy with vaccines against herpesviruses has the potential to reduce the morbidity caused by these common pathogens after HCT.

A multicenter phase III double-blind, randomized, placebo-controlled trial to study the safety and efficacy of the heat-inactivated varicella vaccine in autologous HCT recipients is in progress (ClinicalTrials.gov Identifier: NCT01229267). Other approaches to the development of VZV vaccines that would be safe in immunocompromised patients are also being pursued. For example, a recombinant VZV glycoprotein E subunit vaccine has been evaluated in a phase I/II clinical trial in healthy individuals. The vaccine was well tolerated and was more immunogenic than a live attenuated VZV vaccine (Varilrix) [275]. The potential benefit of boosting VZV immunity by vaccinating HCT donors before the transplant is also being explored [276]. Whether Zostavax vaccination of HCT donors (HLA-matched siblings) prior to donation can reduce the incidence of zoster in HCT recipients is being evaluated (ClinicalTrials.gov Identifier: NCT01573182).

Conclusion

Primary and recurrent VZV infections remain a serious threat to HCT recipients. Nevertheless, early recognition of the clinical signs of varicella and herpes zoster permits effective intervention with antiviral drugs. Immunization with inactivated varicella vaccine has the potential to reduce the morbidity of VZV reactivation after HCT.

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CHAPTER 90

Epstein–Barr Virus Infection

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Introduction

Epstein–Barr virus (EBV) is a gammaherpesvirus that is transmitted in saliva (and in cellular blood products with lymphocytes), infecting over 90% of the world's population by adulthood [1–3]. Primary infection is usually asymptomatic, but can present as infectious mononucleosis, especially when infection occurs in adolescence or adulthood. EBV persists for the lifetime of the host as a latent infection harbored by resting B lymphocytes. For most people there are no long-term consequences of infection. In healthy individuals, primary EBV infection and periodic EBV reactivation are met with robust cellular and humoral responses [4].

Patients undergoing hematopoietic cell transplantation (HCT) are at risk for developing EBV-associated lymphoproliferative diseases and lymphomas, collectively termed post-transplantation lymphoproliferative disorder (EBV-PTLD). Aspects of allogeneic (allo)HCT, particularly selective T-cell depletion of the graft or selective pharmacologic induction of T-cell dysfunction aimed at prevention or treatment of graft-versus-host disease (GVHD), have been associated with increased risk of EBV-PTLD. In addition, in some circumstances the use of EBV-naïve donors, human leukocyte antigen (HLA)-mismatched donors, or unrelated donors may also be associated with increased risk. This chapter reviews aspects of EBV biology, EBV-PTLD pathogenesis, diagnosis, treatment, and prevention, and also emerging treatment approaches to EBV-associated malignancies in the setting of HCT.

Aspects of EBV biology

EBV is an enveloped, double-stranded linear DNA virus with a genome length of approximately 180,000 base pairs, including a variable number of terminal repeats useful in determining EBV genome clonality in EBV-associated tumors [1]. EBV infects B lymphocytes, but can infect other cell types, including T lymphocytes and natural killer (NK) cells, and also epithelial and smooth muscle cells [1]. Infection of B cells involves CD21 binding (also known as complement component receptor 2). Linear double-stranded viral DNA is transported to the nucleus where the ends fuse to form a circular plasmid [1]. These plasmids serve as templates for the generation of linear double-stranded DNA concatemers, which are cleaved and packaged into capsids, then enveloped to form virions; or they may serve as templates for plasmid replication. Viral gene expression that gives rise to new virions is referred to as lytic or productive gene expression. Among the viral proteins expressed in lytic infection are the immediate early proteins ZTA and RTA that serve a regulatory role. Immediate early proteins trigger the expression of enzymes involved in replicating virion DNA, such the viral thymidine kinase and the viral DNA polymerase, and also the structural proteins that will ultimately form viral capsids. Among the approximately 80 open reading frames encoded by the virus, most are expressed during lytic infection. However, infection does not always or even usually result in lytic infection. Instead, the viral genome may enter latency. In latency, there is limited viral gene expression. When latency is established in dividing cells, viral plasmid replication is largely a function of the cellular machinery that replicates cellular DNA. The viral origin of plasmid replication is bound by a viral latency protein, Epstein–Barr nuclear antigen 1 (EBNA1), which is necessary for replication of the plasmid by the cellular machinery. Neither the viral thymidine kinase nor the viral DNA polymerase plays any role in this plasmid replication process. EBNA1 is the only viral protein required to maintain the latent genome. In latency, viral plasmids replicate approximately in tandem with the cellular genome during each cell cycle and are partitioned among daughter cells. Some of the genes expressed during latent and lytic infection are shown in Figure 90.1.

In vitro, EBV infects resting B lymphocytes and drives these cells to proliferate continuously in a process termed "immortalization," producing lymphoblastoid cell lines (LCLs). LCLs express six latency nuclear antigens (EBNAs), latency membrane proteins (LMP-1 and -2), and two small but abundant non-coding RNAs (EBER-1 and EBER-2), a pattern of expression termed Latency III or the growth program (Figure 90.2) [1]. In addition, a number of viral microRNAs are expressed [5]. When LCLs are injected into immunodeficient mice, EBV-B-cell lymphoproliferative disease develops [6].

During primary infection in people, there is an initial expansion of latently infected B-lymphocytes driven by the EBV growth program. Proliferation of the initially infected cells leads to expansion of the infected cell compartment, even in the absence of repeated cycles of virion production and new rounds of infection. Whereas in immunodeficient mice this EBV-driven B-cell proliferation is uncontrolled, leading to lymphoproliferative disease, in healthy people the infection elicits NK-cell, $CD4^+$, and $CD8^+$ T-lymphocyte cellular responses that limit the proliferation of infected cells [7,8]. Proliferating B-lymphocytes expressing viral antigens are eliminated. Viral genomes persist as nuclear plasmids

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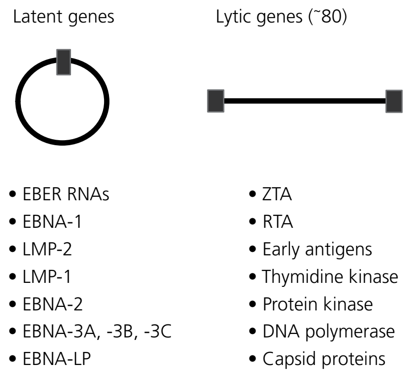


Figure 90.1 Genes expressed during latent and lytic EBV infection. In latent infection, no new virions are produced. Viral DNA is present as a closed circular plasmid. Latent gene expression is limited to non-coding Epstein–Barr virus-encoded RNA (EBER) transcripts, Epstein–Barr virus nuclear antigens (EBNAs), and latency membrane proteins (LMPs). In lytic infection, new virions are being produced. The viral DNA is present in a linear form. Approximately 80 viral proteins are expressed. These include transcriptional regulatory proteins (ZTA, RTA), viral kinases (thymidine kinase and protein kinase) responsible for phosphorylating antiviral nucleoside analogs such as acyclovir or ganciclovir, the viral DNA polymerase, which is necessary for the production of new virion DNA and is inhibited by these antiviral agents, and the structural proteins of the viral capsid.

in a subset of resting memory B-lymphocytes that constitute a lifelong viral reservoir. In these resting cells, there is little or no viral antigen expression. However, intermittent reactivation with virion production results in viral shedding into saliva, tears, and other secretions. In addition, new B-lymphocytes may become infected.

Antiviral drugs, such as acyclovir, ganciclovir, and foscarnet, inhibit the viral DNA polymerase and production of new virions. Phosphorylation of ganciclovir by viral kinases also inhibits cellular DNA polymerases and leads to cell death. These drugs that inhibit the viral DNA polymerase are effective in blocking production of new virions. However, as noted above, new virion production is not required for the spread of viral infection, as latently infected cells that harbor viral plasmids can proliferate. Hence these antiviral agents have no effects on such latently infected cells. The viral DNA polymerase is not required for genome maintenance in latency (and is not expressed in latency). The viral kinases that phosphorylate ganciclovir are not expressed in latently infected cells, hence ganciclovir and its congeners do not interfere with cellular DNA replication or lead to induction of cell death.

EBV-associated diseases

Infectious mononucleosis is the manifestation of primary infection. Present evidence suggests that signs and symptoms of mononucleosis reflect the immune response to viral infection. Oral hairy leukoplakia, a benign EBV-associated lesion that arises on the lateral margins of the tongue, is mainly seen in patients with HIV but can occur in transplant recipients.

A variety of other syndromes and diseases have been linked with EBV. Chronic active EBV (CAEBV) is a condition where the viral association is well established, whereas systemic lupus erythematosus and rheumatoid arthritis are examples where the viral association remains speculative. Among transplant recipients, EBV hepatitis, ulcerative enteritis, encephalitis, hemolytic anemia, and hemophagocytosis have all been reported but the associations are not well established [9–11].

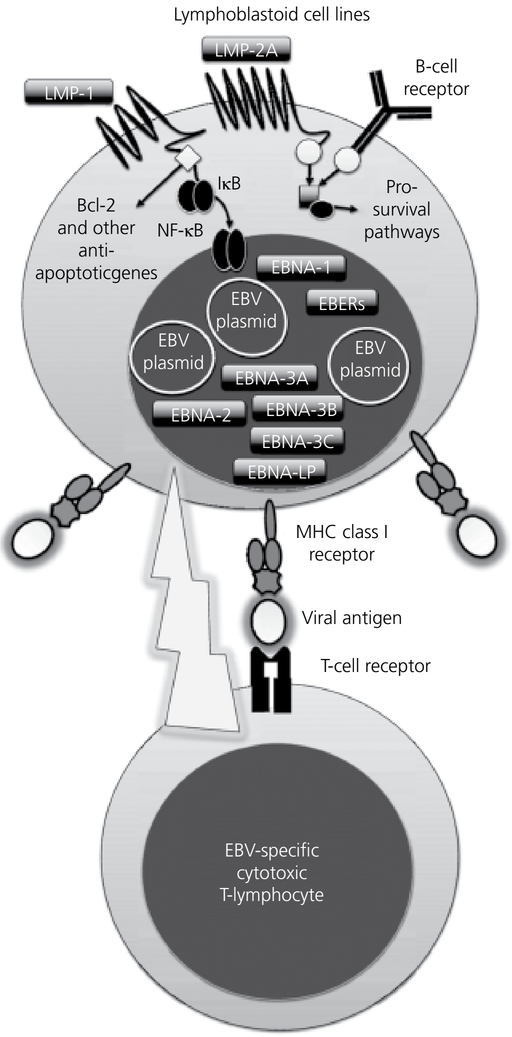


Figure 90.2 Schematic of Epstein–Barr virus latent gene expression by lymphoblastiod cell lines (LCLs). Latent membrane protein (LMP)-1 mimics the tumor necrosis factor receptor CD40 and activates the nuclear factor kappa B (NFκB) signaling pathway, modulating cell proliferation and apoptosis. LMP-2A mimics normal B-cell receptor (BCR) signaling, allowing dysfunctional B cells to escape regulatory checkpoints and survive. Immunodominant antigens are EBNA-3A, -3B, and -3C, which render the cell susceptible to killing by $CD8^+$ cytotoxic T-lymphocytes. Viral antigens are presented to T-lymphocytes on major histocompatibilty complex (MHC) I molecules.

EBV-associated tumors

In the context of tumors, the term “EBV-associated” is generally used to refer to tumors in which viral DNA, RNA, and protein are detected in tumor cells. Endemic Burkitt lymphoma, undifferentiated nasopharyngeal carcinoma, extranodal NK/T-cell lymphoma, and AIDS primary central nervous system lymphomas are consistently associated with EBV tumors [2]. Other malignancies such as Hodgkin lymphoma (HL) are variably associated with EBV. In North America and Western Europe, approximately 20–30% of HLs are EBV associated, whereas in some areas of Latin America and Africa, more than 75% are EBV associated [7,8]. Similarly, in patients with HIV, 90% of HLs are EBV associated. A variety of other T- and B-cell lymphoproliferative diseases, and also about 10% of gastric cancers, are EBV associated [12–14].

Table 90.1 Patterns of EBV latent gene expression

Latency	EBNA-1	LMP-1, -2	EBNA-2, -3A, -3B, -3C, -LP
I	+		
II	+	+	
III	+	+	+

EBNA, Epstein–Barr virus nuclear antigen; LMP, latent membrane protein; -LP, leader protein.

PTLD after solid organ or HCT transplantation is often but not always EBV associated. PTLD has been classified into four subcategories by the World Health Organization: early lesions, polymorphic PTLD, monomorphic PTLD, and classical HL-type PTLD [15]. Early lesions are universally EBV positive and typically arise in the first few months post-HCT, with histology that is akin to a reactive, infectious mononucleosis-like lymphoid expansion or plasmacytic hyperplasia [16]. Polymorphic PTLDs are also always EBV positive and are histologically comprised of a mixture of plasma cells, histiocytes, and activated B cells, or immunoblasts [16–18]. Early lesions and polymorphic PTLD are the most common PTLD histologic subtypes in post-HCT patients. Monomorphic lesions, many of which are EBV positive, resemble aggressive B-cell lymphomas, including diffuse large B-cell lymphoma or Burkitt lymphoma. Rarely, T-cell and NK-cell PTLDs can occur, 20–30% of which are EBV positive [9,19–22]. EBV-negative PTLDs, when they occur, are of the monomorphic subtype. Primary central nervous system lymphomas account for less than 1% of all PTLDs, are nearly always EBV positive, and tend to present with involvement of the brain parenchyma rather than leptomeningeal involvement [23]. HL-type PTLD is virtually all EBV positive, is of the mixed cellularity histology, and typically arises several years after transplantation.

EBV-PTLDs may arise very early in the post-transplant period when cell-mediated immunity is lacking or pharmacologically suppressed. Especially early after transplantation, these are B-cell proliferations with viral latent gene expression similar to that of LCLs, with a latency III pattern [24]. The viral antigens that drive B-cell proliferation are highly immunogenic and their expression speaks to the absence of cytotoxic T-cell control in these patients [25]. Whereas in the most profoundly immunocompromised patients, EBV-driven polyclonal B cell expansion may be sufficient for EBV-PTLD to develop, in other cases, particularly those that arise more than a few weeks after HCT, oligoclonal or monoclonal proliferations are more characteristic findings. Oligoclonal or monoclonal EBV-PTLDs often show more restricted patterns of viral latency gene expression (latency II, or latency I in cases of Burkitt lymphoma EBV-PTLD), and cellular mutations, deletions, or translocations are present in many cases. Patterns of EBV latent gene expression are shown in Table 90.1. Thus EBV-PTLD represents a spectrum of lesions with regard to viral gene expression, clonality, and histologic type.

In the alloHCT setting, EBV-PTLD typically arises in *donor* B-lymphocytes, although lesions of host-cell origin have been described and may be more common in non-myeloablative alloHCT [26–28]. High-dose HCT conditioning destroys the viral B-cell reservoir and, at least in some cases, may eliminate the host virus [29]. EBV-PTLD has also been described in the setting of autologous HCT when the preparative regimen or stem-cell processing procedure has included selective T-cell depletion (as used for the treatment of T-cell malignancies).

Allogeneic HCT and EBV-PTLD risk factors

The incidence of EBV-PTLD following HLA-matched, related donor alloHCT without T-cell depletion of the graft is less than 1% in adults and slightly higher (around 3%) in children [27,30,31]. However, in some series the incidence can be as high as 15–30% [27,30,32]. Commonly recognized risk factors are related to the donor, the preparative regimen, graft manipulation, GVHD prophylaxis, the occurrence of GVHD and its treatment, and the patient.

Rates of EBV-PTLD using unrelated donors has been reported to be higher than with related donors, although on multivariate analysis the risk may be limited to unrelated-donor HCTs that also utilize T-cell-depleted grafts or antithymocyte globulin (ATG) [33–35]. The incidence of EBV-PTLD in patients receiving HLA-mismatched grafts has been reported to be 10–15%, compared with 1% in those receiving HLA-matched grafts [32,35,36]. However, this increased risk with HLA mismatch may only apply to those approaches that also use T-cell depletion and/or ATG; multivariate analyses have shown that patients who receive HLA-mismatched grafts but no selective T-cell depletion or ATG have the same risk for EBV-PTLD as those receiving fully HLA-matched grafts [35].

Umbilical cord blood (UCB) grafts contain mostly naïve T cells that lack virus-specific cytotoxic T-cell activity. In this respect, UCB grafts resemble T-cell-depleted grafts. However, EBV generally does not cross the blood–placenta barrier and therefore the graft does not harbor infected cells. The incidence of EBV-PTLD after UCB HCT varies but may be as high as 5% [28,37–39]. ATG conditioning may increase the risk [28,38,39].

ATG, which is commonly used both in HCT conditioning regimens to promote engraftment and decrease GVHD and in the treatment of GVHD, is associated with an increased incidence of EBV-PTLD [11,28,33,35,39–41]. ATG is commonly used in the treatment of aplastic anemia and prior therapy with ATG has been associated with increased risk for EBV-PTLD after alloHCT, with a cumulative incidence of EBV-PTLD of 4% in those receiving one prior course and 25% in those receiving two prior courses [42]. In the treatment of severe aplastic anemia without alloHCT, ATG has also associated with the development of EBV-associated lymphoproliferative disorders [43,44].

Some approaches to HCT graft manipulation are associated with an increased incidence of EBV-PTLD (10–15%). Especially high rates of EBV-PTLD were seen historically with the use of an immunorosette technique using tetramers of anti-CD2 and anti-CD3 monoclonal antibodies that resulted in selective T-cell depletion [45]. Other approaches are associated with a risk similar to that with unmanipulated grafts (typically less than 1%) [26,27,35,36,40,46]. Depletion of both B and T cells, either by counterflow elutriation or by use of antibodies, results in much lower rates of EBV-PTLD similar to those associated with unmanipulated grafts [45]. Perhaps B-cell depletion, by reducing the number of lymphocytes that harbor EBV, reduces the need for T-cell control. Lending support to this hypothesis is the observation that GVHD prophylaxis with high-dose post-transplantation cyclophosphamide (given post-HCT days 3 and 4) has not yet been associated with EBV-PTLD, even among those receiving conditioning with ATG [47]. In a single-institution series of almost 800 consecutive patients who received high-dose post-transplantation cyclophosphamide as part of their GVHD prophylaxis following HCT, none developed EBV-PTLD [47].

Patients with congenital primary immunodeficiencies, such as X-linked lymphoproliferative disease, Wiskott–Aldrich syndrome, ataxia–telangiectasia, severe combined immunodeficiency, and other T-cell immunodeficiencies, are at high risk for EBV-associated lymphoproliferative disorders before alloHCT. AlloHCT is often the only curative option, although risk for developing recurrent EBV-associated lymphoproliferative disorders post-HCT, including EBV-PTLD, remains high. In a multivariate model adjusting for other risk factors, including selective T-cell depletion of grafts and the use of ATG, patients undergoing HCT for primary immunodeficiencies had a relative risk for EBV-PTLD of 2.5 compared with those receiving HCT for hematologic malignancies [36]. However, the incidence varies greatly as a function or the preparative regimen and details of immunosuppression [48].

CAEBV is a lymphoproliferative disorder first described in Japan, but now recognized to afflict persons worldwide. In patients with CAEBV, primary EBV infection is followed by chronically elevated EBV viral copy number in the blood, with persistent and progressive manifestations including hepatitis, cytopenias, hemophagocytic syndrome, splenomegaly, lymphadenopathy, and pneumonitis [49]. EBV-associated B- or T-cell lymphoproliferation may progress to aggressive lymphoma. AlloHCT is believed to be the only potentially curative treatment, although as with primary immunodeficiencies, these patients are at high risk for EBV lymphoproliferation after HCT [49]. It should be noted that CAEBV is not the same entity as chronic fatigue syndrome. Chronic fatigue syndrome has no generally accepted viral etiology and has a much better prognosis than CAEBV.

EBV-PTLD presentation and diagnosis

The clinical presentation of EBV-PTLD can vary from indolent to aggressive and from localized to disseminated. EBV-PTLD can present as a mononucleosis-like illness with fever, lymphadenopathy, pharyngitis, and malaise. Fulminant disease may be associated with hepatic transaminitis, respiratory compromise, and multi-organ dysfunction. Extranodal involvement may include Waldeyer's ring, the gastrointestinal tract, liver, bone marrow, central nervous system, skin, and lungs [28,41,50]. Central nervous system involvement may be parenchymal or leptomeningeal. Pulmonary involvement may manifest as a diffuse infiltrate or nodules. Definitive diagnosis requires biopsy with EBER in situ hybridization or LMP-1 immunohistochemistry. Whereas EBER RNAs are abundantly expressed across the spectrum of EBV-PTLD, LMP-1 is not expressed in a subset of these tumors.

The first year following HCT is the period of highest risk for EBV-PTLD, presumably reflecting the duration of time to establishment or re-establishment of an EBV-specific cellular response [24,26,51–53]. The median time from HCT to EBV-PTLD varies but many reports indicate peaks between 60 and 150 days post-alloHCT [28,30,32,40,54,55]. GVHD is a risk factor for EBV-PTLD [27,30,33,35,41]. Furthermore, treatment of GVHD with drugs that selectively deplete T cells, such as anti-CD3 monoclonal antibodies, increase the risk [27]. Whereas the risk of EBV-PTLD declines with time and is rare after 1 year, chronic GVHD is associated with prolonged risk over years [35].

EBV monitoring

EBV DNA can be readily detected and quantified in peripheral blood by real-time polymerase chain reaction (RT-PCR). In healthy individuals, EBV DNA is predominantly detected in the lymphocytes, or peripheral blood mononuclear cells (PBMCs), on the order of 1–50 infected cells per million PBMCs [56]. With immunosuppression, the number of infected cells increases [57]. There is also evidence that copy number per infected cell increases, perhaps reflecting increased lytic viral infection [58]. Monitoring EBV DNA in PBMCs may provide a guide to those at highest risk for EBV-PTLD, but some patients with high copy number do not develop any associated disease [59,60]. Therapies that deplete B cells, such as the anti-CD20 monoclonal antibody rituximab, deplete the cells that harbor virus. Hence serial monitoring of EBV DNA in PBMCs in the setting of rituximab treatment almost inevitably shows a decline in PBMC EBV DNA viral copy number, even in the face of progressive EBV-PTLD [57]. Hence monitoring PBMC viral copy number may identify patients at high risk but is of limited value following interventions that involve rituximab and other B-cell-targeted therapies.

EBV DNA is also detected in plasma or serum. Healthy EBV-seropositive adults rarely have detectable cell-free EBV DNA in blood. However, in patients with EBV-associated malignancies, such as undifferentiated nasopharyngeal carcinoma, EBV-positive HL, and extranodal NK/T-cell lymphoma, EBV DNA is usually detected in plasma or serum [61–63]. Prior to treatment, the level of cell-free EBV DNA in plasma has emerged as a useful prognostic marker that complements anatomic staging in nasopharyngeal carcinoma [62]. The cell-free EBV DNA detected in such patients is not virion DNA but viral DNA released from tumor cells. In other settings (acute infectious mononucleosis, HIV infection), virion DNA may be present in serum or plasma [64]. The sources of viral DNA are illustrated in Figure 90.3. As with monitoring in PBMCs, high levels or rising levels of EBV DNA in plasma or serum raise suspicion for an established or evolving EBV-PTLD [40,65].

There are no established, generally accepted guidelines for monitoring EBV DNA in the HCT setting. Some institutions monitor whole blood, others PBMCs, others serum or plasma. Some institutions report values in viral copies per microgram of DNA, while others report viral copies per volume of blood or cell-free blood. Others report "low-level" or "high-level" EBV DNA (with inter-institutional variation in the thresholds for these categories). Some institutions regularly monitor the EBV DNA in post-HCT patients whereas others monitor only in the setting of particular signs or symptoms.

EBV DNA in blood is often used to monitor the response of EBV-PTLD to treatment. Plasma EBV DNA has been shown to be a more reliable measure of treatment response than EBV DNA in PBMCs [60,65]. Rapid change in EBV DNA levels with EBV-PTLD treatment is common, but does not consistently correlate with survival outcomes [55].

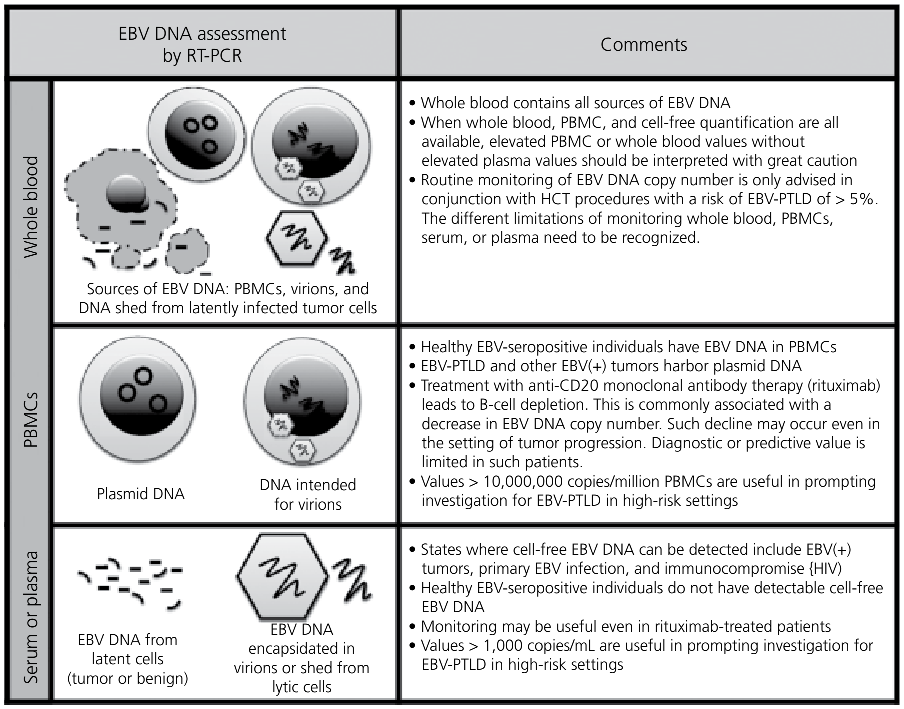
Treatment and prevention

EBV-associated non-neoplastic diseases

Acyclovir, ganciclovir, or their congeners interfere with the production of EBV virions and salivary shedding, but randomized controlled studies provided no evidence that the duration of symptoms associated with infectious mononucleosis is affected by such antiviral therapies [66]. These agents are very effective in treating or preventing oral hairy leukoplakia.

EBV-PTLD

Withdrawal or reduction of immunosuppression in the early post-HCT period may not lead to quick rebound of a previously immunosuppressed immune system as sometimes occurs in solid



	EBV DNA assessment by RT-PCR	Comments
Whole blood	Sources of EBV DNA: PBMCs, virions, and DNA shed from latently infected tumor cells	• Whole blood contains all sources of EBV DNA • When whole blood, PBMC, and cell-free quantification are all available, elevated PBMC or whole blood values without elevated plasma values should be interpreted with great caution • Routine monitoring of EBV DNA copy number is only advised in conjunction with HCT procedures with a risk of EBV-PTLD of > 5%. The different limitations of monitoring whole blood, PBMCs, serum, or plasma need to be recognized.
PBMCs	Plasma DNA DNA intended for virions	• Healthy EBV-seropositive individuals have EBV DNA in PBMCs • EBV-PTLD and other EBV(+) tumors harbor plasmid DNA • Treatment with anti-CD20 monoclonal antibody therapy (rituximab) leads to B-cell depletion. This is commonly associated with a decrease in EBV DNA copy number. Such decline may occur even in the setting of tumor progression. Diagnostic or predictive value is limited in such patients. • Values > 10,000,000 copies/million PBMCs are useful in prompting investigation for EBV-PTLD in high-risk settings
Serum or plasma	EBV DNA from latent cells (tumor or benign) EBV DNA encapsidated in virions or shed from lytic cells	• States where cell-free EBV DNA can be detected include EBV(+) tumors, primary EBV infection, and immunocompromise {HIV) • Healthy EBV-seropositive individuals do not have detectable cell-free EBV DNA • Monitoring may be useful even in rituximab-treated patients • Values > 1,000 copies/mL are useful in prompting investigation for EBV-PTLD in high-risk settings

Figure 90.3 Epstein–Barr virus (EBV) copy number quantification by real-time polymerase chain reaction (RT-PCR) and guidelines for interpretation. Whole blood contains EBV DNA in peripheral blood mononuclear cells (PBMCs), cell-free DNA, and potentially virions. PBMCs may contain either latent or lytic EBV DNA. In PBMCs that are latently infected, EBV DNA exists as circular plasmids that rely on cellular polymerases for replication only in tandem with cell cycle. In PBMCs that are lytically infected, EBV DNA is linear and undergoes replication using viral polymerases. Cell-free components of blood are serum or plasma. Cell-free EBV DNA can be shed or extruded from latently infected cells, which can include tumor cells or benign lymphocytes. Cell-free EBV DNA can also be present as encapsidated, infectious virions, or exist as free fragments of EBV DNA shed from dying lytically infected cells.

organ transplant recipients. GVHD may develop or worsen [54]. However, the approach is sometimes effective and is used in conjunction with other treatment strategies in some institutions [38,67]. Reduced immunosuppression may be more effective later in the post-HCT course, when partial immune reconstitution has already occurred [68]. In a large series from the European Group for Blood and Marrow Transplantation center, a lack of reduction of immunosuppression upon PTLD diagnosis was associated with a poor response to rituximab-based therapy and higher mortality [69]. There is growing interest in the antitumor and potential antiviral activities of mammalian target of rapamycin (MTOR) inhibitors, such as sirolimus and everolimus. Although restricted to case reports, PTLD remissions have been reported in solid organ transplant patients who have had their immunosuppressant regimen changed from a calcineurin inhibitor to an MTOR inhibitor, in some cases coupled with rituximab therapy [70,71]. Similarly, Syk tyrosine kinase inhibition may be a future therapeutic strategy in EBV-PTLD, as the EBV latency membrane protein 2 (LMP2) is a B-cell receptor mimic that sends survival signals to EBV-positive PTLD cells through the Syk tyrosine kinase pathway [72].

Rituximab for the treatment of post-HCT EBV-PTLD is often effective and has dramatically changed survival outcomes, where reported mortality rates once approached 100% [27,36]. Rituximab is well tolerated and does not threaten the allogeneic graft as does combination cytotoxic chemotherapy. One systematic review estimated the efficacy for treatment in the HCT setting at 63% [73]. “Incipient” EBV-PTLD, characterized by the development of lymphadenopathy or fever in the setting of a rising EBV DNA viral load, has been treated with rituximab, CTL infusions, or both [74]. In small series, reduction of immunosuppression in combination with rituximab therapy has also been reported [10,55].

Donor lymphocyte infusion (DLI) is often effective in EBV-PTLD [31,42,75]. The major complication of DLI is GVHD [40]. Sometimes very modest DLI doses have proved effective [76]. Note that the effect is generally not a GVH effect, since most cases of EBV-PTLD are donor-cell proliferations. DLI leads to the control of EBV-positive B-lymphocyte proliferation, presumably by targeting viral antigens. In EBV-seropositive healthy persons, a small fraction of T cells have EBV specificity [77]. In some instances, donors who were not the alloHCT stem-cell donors have been used. In these cases, there may be both GVH-related antitumor effects and antiviral effects. DLI for EBV-PTLD is typically combined with rituximab therapy.

Amplification of cellular responses to EBV ex vivo allows a more specific adoptive immunotherapy approach, with less risk of GVHD than with DLI [78]. EBV lymphoblastoid cell lines from normal donor B cells were used as stimulators of cytotoxic T-lymphocyte (CTL) expansion [79]. Responses to a multitude of viral antigens, particularly the immunodominant latency antigens EBNA-3A, -3B and -3C that are commonly expressed in EBV-PTLD, occur following CTL infusion. In the post-HCT setting, gene-marked CTLs have been shown to persist in recipients for years after infusion [80].

Advances in the field have included genetically modified CTLs that proliferate and persist despite concurrent immunosuppression

Table 90.2 Treatment options for EBV-PTLD

Recommended	Rituximab EBV-specific CTL infusion or DLI Standard lymphoma chemotherapy
Limited utility	Reduction of immunosuppression
Not recommended	Anti-herpesvirus agents (e.g. ganciclovir) Treatment based on EBV DNA monitoring without confirmatory biopsy

PTLD, post-transplantation lymphoproliferative disorder; CTL, cytotoxic T-lymphocyte; DLI, donor lymphocyte infusion

or that have a “safety switch” where a drug can be administered to induce CTL suicide should GVHD occur [81–83]. UCB HCT patients have some of the highest rates of viral complications due to the relatively naïve immune system derived from cord blood and a lack of experienced, virus-specific CTLs in the graft. Recent work in adoptive immunotherapy has now extended to UCB transplants, overcoming some barriers that the predominantly naïve UCB-derived T cells posed when trying to expand such cells successfully into virus-specific CTLs [84]. CTLs have now been generated to have EBV-specific cytotoxicity, and also cytotoxicity against other viruses that are problematic in the post-HCT period, such as adenovirus or cytomegalovirus [85,86].

The time required to expand EBV-specific CTLs ex vivo has been a limitation, previously taking several weeks. Current efforts in adoptive immunotherapy have therefore also focused in decreasing the production time of CTLs. Virus-specific CTLs have been produced in a time span of 10 days using a method of donor PBMC stimulation with viral peptides and pro-proliferative cytokines [87]. Patients have been successfully treated with virus-specific CTLs that were isolated in a matter of hours from frozen donor material or third-party donors using HLA multimers and magnetic beads [88]. CTLs have now been isolated from the whole blood of donors and made ready for adoptive immunotherapy in as little as 30 hours using selection methods based on EBNA-1-specific interferon-gamma secretion that do not rely on ex vivo expansion [89].

Cytotoxic chemotherapy is used in the treatment of post-alloHCT EBV-PTLD in patients who fail to respond to antibody and cellular therapies or where cellular therapies are not available. In patients who are able to tolerate combination chemotherapy with rituximab, long-term disease-free survival can be achieved in some [90]. However, chemotherapy is often not well tolerated or patients are too unwell to begin such therapies. Treatment options for EBV-PTLD are listed in Table 90.2.

Preemptive intervention with reduction in immunosuppression, adoptive cellular therapy, antibody therapy, or combinations of these approaches been explored in association with HCT approaches with particularly high incidences of EBV-PTLD. Reduction of immunosuppression may lead to GVHD or graft rejection. However, in a prospective study of pediatric HCT patients who were monitored for EBV in PBMCs and had preemptive reduction of their immunosuppression if EBV copy number crossed the protocol’s threshold, no increase in GVHD was observed among those who had their immunosuppression reduced [59]. Even a single dose of rituximab may be sufficient to prevent EBV-PTLD in patients with rising, very high EBV viral copy numbers [73]. Responses can be quick, with EBV DNA viral copy number falling to zero within 2 weeks of the first rituximab dose [91]. In patients with primary immunodeficiencies, weekly EBV monitoring and preemptive rituximab have been reported to result in reductions in EBV-PTLD-associated morbidity and mortality [54]. In a retrospective study comparing PTLD events pre- and post-implementation of a protocol monitoring EBV and administering preemptive rituximab, the EBV protocol was associated with lower EBV-related mortality and fewer cases of monomorphic PTLD [92]. Adoptive cellular therapies with EBV-activated CTLs have also been used effectively in this setting.

EBV-specific CTLs have been used prophylactically in high-risk patients post-HCT with great success [54,78,93]. Prophylactic EBV-specific CTLs are particularly used in patients receiving HCT for primary immunodeficiencies or CAEBV or in other high-risk groups, such as those receiving T-cell-depleted grafts [49,54,78].

The role of prophylaxis with antiviral drugs such as acyclovir or valganciclovir in preventing EBV-PTLD is uncertain. However, insofar as these agents are routinely used for other reasons and yet EBV-PTLD remains a problem, we know that their efficacy remains limited at best. Prophylactic post-HCT rituximab has been used in high-risk patients, which may decrease the incidence of EBV-PTLD by reducing the burden of EBV-infected B cells, but definitive evaluation of the risks and benefits is still awaited [38].

Treatment of other EBV-associated tumors

The successes in the use of EBV-specific CTLs for the treatment of EBV-PTLD in HCT have stimulated interest in adoptive T-cell therapy for other EBV-associated tumors. With regard to such immunotherapeutic approaches. the tumor cells must present antigens that can be effectively killed by cognate T cells. In EBV-associated HL, major histocompatibility complex (MHC) class I molecules are consistently expressed and HL cells are susceptible to lysis by antigen-specific CTLs. However, much of the HL tumor consists of regulatory T-lymphocytes, which release cytokines and chemokines that disrupt cellular immune responses. Thus, although adequate antigen presentation occurs, dysregulated immune responses can damp the efficacy of EBV-specific CTLs.

The pattern of viral antigen expression varies by tumor type, most of which are more restricted than in EBV-PTLD. The viral latency antigens typically targeted by EBV-specific CTLs expanded in vitro using LCLs, namely EBNA-3A, EBNA-3B, and EBNA-3C, are not expressed in most EBV-associated malignancies. In Burkitt lymphoma, only EBNA-1 is expressed by tumor cells. In HL, EBNA-1 and the latent membrane proteins LMP-1 and LMP-2 are expressed. In EBV-associated nasopharyngeal carcinoma (NPC), the LMPs are less consistently expressed. In EBV-PTLD, there is generally a match between the viral antigens expressed by tumor cells and the antigens targeted by EBV-specific CTLs. In patients with EBV-associated HL or EBV-associated NPC, a more restricted, and often less immunogenic, array of antigens are available as CTL targets. These patients have also been shown to have decreased EBV-specific T cells compared with healthy controls, suggesting that bolstering immune function with CTLs may have antitumor activity. EBV-specific autologous CTL therapy has had some successes in these patients, with demonstration that expansion of CTLs is feasible, even in heavily treated patients [94]. Infusions also appear to be well tolerated and safe, and there is evidence that CTLs are able to home to the tumor site [95]. Some NPC patients treated with autologous CTLs have been able to mount an antitumor, LMP-2-specific immune response with stabilization or regression of refractory tumors [94,96].

Similar results have also been reported with EBV-positive HL, with LMP-2-specific CTLs able to induce remissions in some patients with relapsed HL [95].

Conclusion

EBV is ubiquitous and most adults harbor latent EBV infection in a small percentage of B-lymphocytes. EBV drives the proliferation of infected lymphocytes and, in the setting of impaired cellular immunity post-HCT, EBV-PTLDs can develop. The major risk factors for EBV-PTLD are the use of selectively T-cell-depleted grafts and selective anti-T-cell agents. Monitoring strategies, if used, should be employed with an understanding of the limitations in peripheral blood EBV DNA quantification as a surrogate detection method. The prognosis of patients with EBV-PTLD has improved substantially and currently available treatment options, namely antibody and adoptive cellular therapies, are often effective.

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CHAPTER 91

Respiratory Viruses After Hematopoietic Cell Transplantation

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Introduction

Community-acquired respiratory virus infections are an important cause of morbidity and mortality after hematopoietic cell transplantation (HCT) [1–3]. The infection epidemiology in HCT recipients usually parallels that observed in the community, as these viruses circulate in immunocompetent individuals (including healthcare personnel and family members). Respiratory syncytial virus (RSV), influenza viruses, human coronaviruses (HCoVs), human bocavirus (HBoV), and human metapneumovirus (HMPV) have a seasonal distribution, whereas human rhinoviruses (HRhVs) and parainfluenza virus infections often occur year around (Figure 91.1, Table 91.1) [4,5]. The most significant impact on morbidity and mortality after HCT has been from RSV, parainfluenza viruses, HMPV, and influenza viruses as these viruses frequently cause lower respiratory tract disease (LRD), which is associated with high mortality [1,6–11]. LRD may also occur with HRhVs, HCoVs, and HBoV, but the frequency appears to be low [5].

Molecular detection techniques have largely replaced traditional virologic methods [12–16]. One feature of respiratory virus infections is that the clinical signs and symptoms at presentation are not specific. This syndromic nature of illness therefore requires multiplex testing platforms [17–19]. For non-molecular methods in particular, specimen handling is important for maximal diagnostic yield. Nasal wash or swab specimens should be placed on ice or in a refrigerator immediately and transported to the laboratory without delay to allow for specimen set-up within 2–4 hours [20]. Non-molecular methods available for testing include standard viral cultures (results available in several days), shell vial centrifugation cultures using specific monoclonal antibodies (results after 1–3 days), direct fluorescent antibody tests (results after 2 hours), and enzyme immunoassays (results after 2 hours). On tissue sections from lung biopsy or autopsy specimens, virus-specific monoclonal antibody staining, viral cultures, or polymerase chain reaction (PCR) can be used. The role of non-molecular assays is diminishing as PCR- or Luminex-based platforms allow for multiplex testing with short turnaround times [21,22]. Infection control guidelines are not discussed here as they have been reviewed by an international expert panel [23–25].

Respiratory syncytial virus

Clinical significance

RSV is an RNA virus (paramyxovirus) that causes a wide range of respiratory diseases, ranging from life-threatening bronchiolitis in infants and potentially fatal pneumonia in transplant recipients to a mild upper respiratory tract infection (URI) in immunocompetent adults and older children. The virus has also been implicated in a number of respiratory illnesses in immunocompetent or mildly immunosuppressed individuals, such as otitis media, exacerbation of chronic obstructive lung disease, and community-acquired pneumonia in elderly persons. In patients receiving cytotoxic chemotherapy for hematologic malignancies with or without HCT, RSV causes URI [26], which may progress to fatal pneumonia [27]. RSV has also been linked to late airflow obstruction, a debilitating condition of accelerated loss of lung function after HCT [28]. During the respiratory virus season, the incidence may be as high as 10% and both allogeneic and autologous transplant recipients may be infected [1,29,30]. The risk of acquisition continues throughout the post-transplant period (Figure 91.2a) [5,31].

Risk factors

Winter season and male gender are risk factors for acquisition of RSV in HCT recipients [1,32]. URI precedes pneumonia in most patients and approximately 20–50% of patients with RSV URI progress to pneumonia after a median of 7 days [30,33]; however, in 20% of patients with RSV pneumonia, URI is not present or very mild or occurs only concurrently with the onset of pneumonia. Risk factors for progression to LRD are neutropenia, lymphopenia, unrelated donor status, and fungal co-infections [1,33]. Corticosteroids at moderate doses (less than 1mg/kg) appear to have minimal impact on progression in cohort analyses [34]. One study in pediatric HCT recipients indicated that asymptomatic shedding of RSV can occur [35]. Two studies in adult HCT recipients, one of which used weekly PCR-based surveillance, did not detect asymptomatic shedding [12,36]. Among patients with LRD, oxygen requirement at LRD diagnosis and bone marrow as cell source were negative predictors for outcome [6]. RSV RNA is detectable in serum or plasma in patients with LRD and may serve as a marker for disease severity [37,38].

Treatment and prevention

Without treatment, RSV LRD is associated with a high fatality rate in highly immunosuppressed HCT recipients [27]. In a large multivariable analysis, RSV LRD (but not URI) was independently

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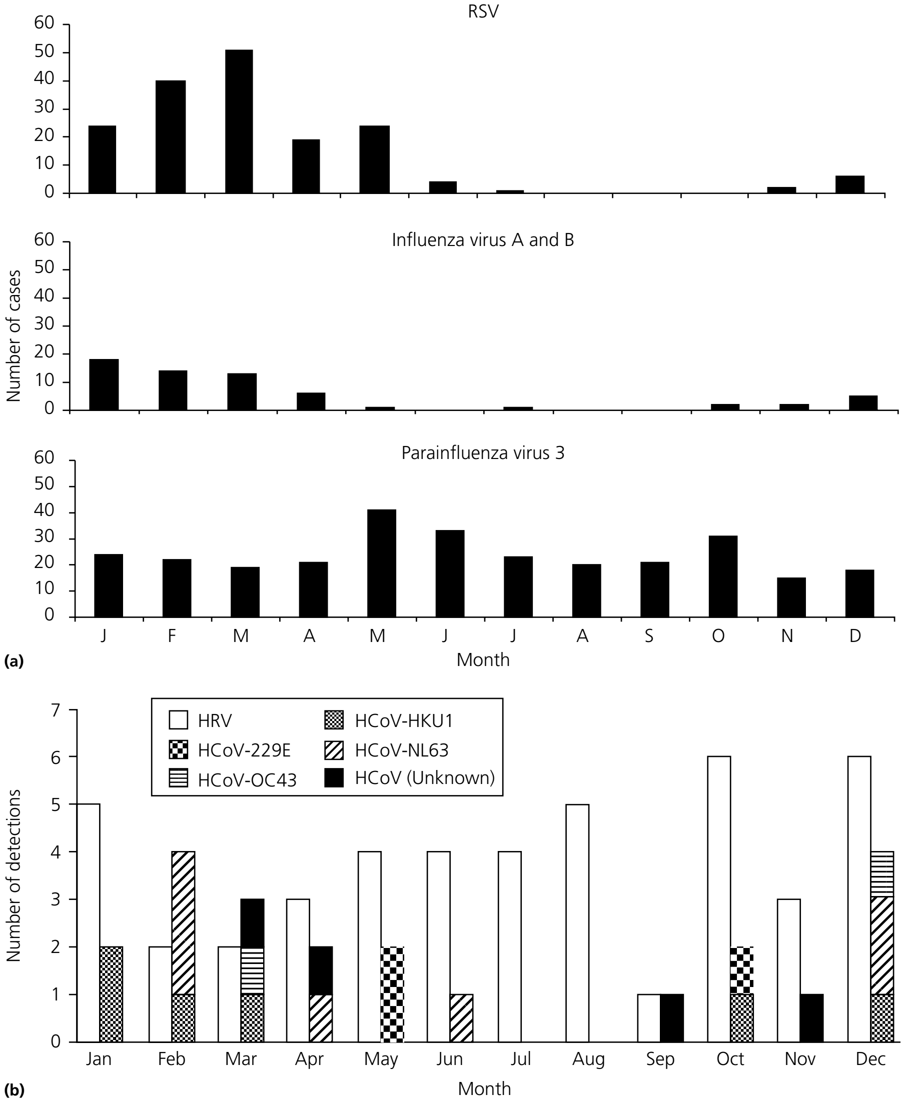

Figure 91.1 (a) Monthly distribution of RSV, influenza A and B, and parainfluenza virus 3. Source: Boeckh 2008 [4]. Reproduced with permission of John Wiley & Sons. (b) Monthly distribution of and rhinovirus and coronavirus. Source: Milano et al. 2010 [5]. Reproduced with permission of American Society of Hematology.

Table 91.1 Characterization of respiratory virus infections among hematopoietic cell transplantation

Virus	Incidence (%)	Seasonality	Proportion of LRD (%)	Proportion of long-term shedders (%) (duration[a])	Proportion of copathogen in LRD (%)	Mortality from LRD (%)	Ref.
RSV	2.6–12	Yes	24–56	32 (14 days)[b]	34–64	15–47	[1,5,6,32,33,37,47,183–185]
Metapneumovirus	2.5–7	Yes	17–41[b]	14 (54 days)[c]	16–38	12–43	[9,65,66,155,185–188]
Parainfluenza virus	1.2–9	No	30–44	34 (21 days)	39–53	17–57	[7,8,32,71–73,76,78,185]
Influenza virus	1.3–7	Yes	5–52[d]	24–33 (14 days)	13–60	25–43	[10–12,32,39,91,92,185,189–191]
Rhinovirus	0.5–22	No	13–50	56 (4 weeks)	48–100	40–100	[5,32,128–130,155,185,192]
Coronavirus	11	Yes	4.5	63 (4 weeks)	30[b]	NA	[5,136]
Bocavirus	2.6[e]	Yes	17	NA	NA	NA	[155]
WU/KI polyomavirus	WUPyV, 8; KIPyV, 17–26	No	NA	WUPyV, 13; KIPyV, 37 (4 weeks)	WUPyV, 14; KIPyV, 32	NA	[167,193]

NA, not available.

[a] Duration indicates time period that was used to define long-term shedding in these studies.

[b] Includes other immunocompromised patients.

[c] Among cancer patients.

[d] Includes the patients receiving oseltamivir at URI stage.

[e] Unpublished data.

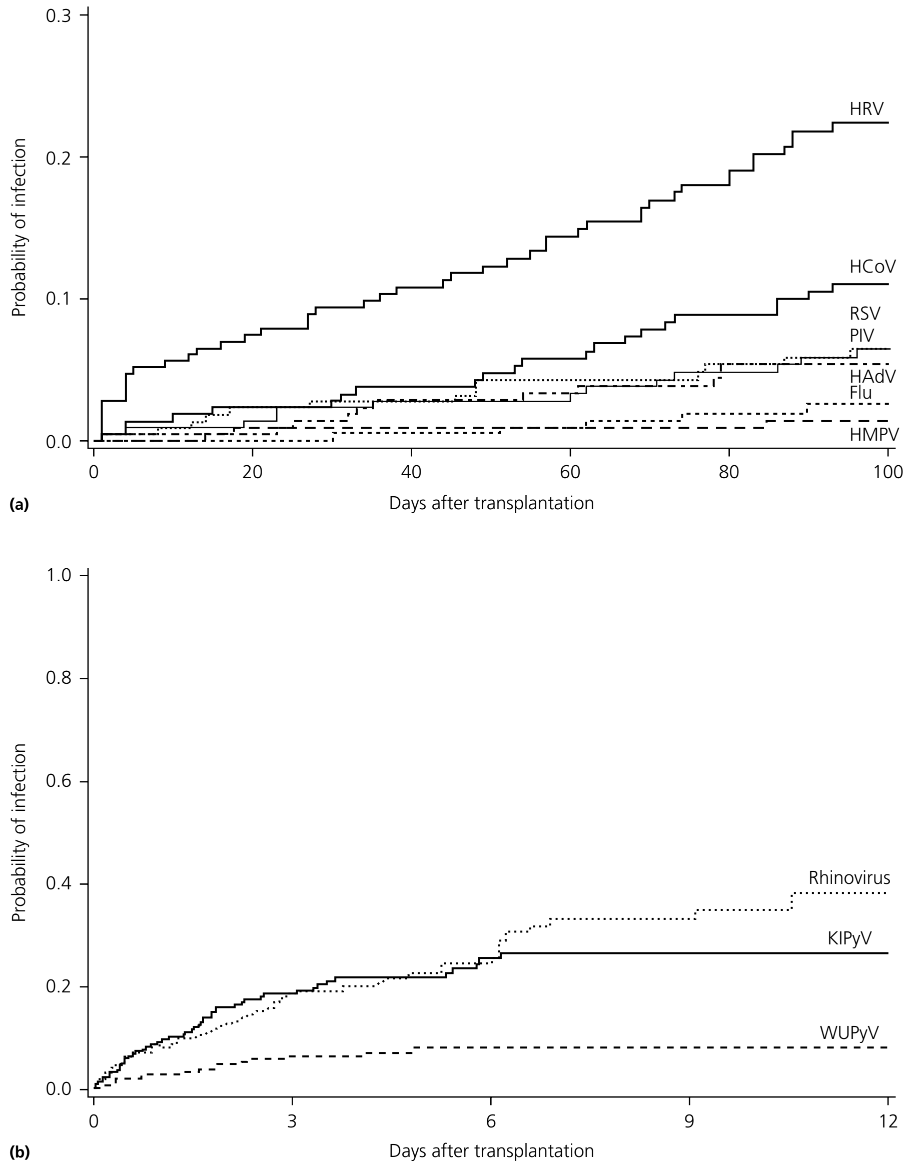

Figure 91.2 (a) Incidence of respiratory virus infections after HCT. Source: Milano et al. 2010 [5]. Reproduced with permission of American Society of Hematology. (b) Incidence of WUPyV and KIPyV after HCT. Source: Kuypers et al. 2012 [166]. Reproduced with permission of CDC.

associated with mortality [39]. However, recovery from RSV LRD without specific treatment has been reported in autograft recipients with multiple myeloma [40]. These differences are likely due to less intense immunosuppressive regimens or other poorly defined innate immune mechanisms. Pulmonary copathogens are commonly detected in patients with RSV LRD [6,33] and require aggressive treatment. No adequately powered controlled trials exist for the treatment of RSV infection and LRD in the HCT setting. Available evidence comes from cohort studies and small randomized trials [6,33,41]. The data suggest that treatment of early pneumonia (i.e. prior to oxygen requirement or mechanical ventilation) is associated with improved outcomes (Figure 91.3). This is supported by a recent multivariate analysis that showed that patients who require oxygen at the time of LRD diagnosis have a significantly higher mortality [6]. Intermittent short-duration (2g over 2 hours three times per day) or continuous aerosolized ribavirin is considered the treatment of choice for RSV LRD [3,33]. A small randomized trial recently showed that intermittent aerosolized ribavirin is more effective than continuous administration to prevent progression to LRD [42]. With the use of aerosolized ribavirin, the 30-day all-cause mortality is approximately 30–40% [6]; however, better outcomes have been reported in patients with good prognostic factors at the time of diagnosis. Systemic ribavirin alone does not seem to be effective for the treatment of pneumonia [3,43,44], although the small sample size of available studies prevents the analysis of smaller effects of systemic treatment. Better response rates are reported for systemic treatment of RSV URI; however, small sample sizes limit the strength of the data [3,45].

The role of concomitant intravenous immunoglobulin, RSV-specific immunoglobulin, or palivizumab, an RSV-specific monoclonal antibody directed at the F protein of RSV, is not well supported. A recent multivariate analysis of 82 patients with RSV LRD who were treated with aerosolized ribavirin did not show a benefit of palivizumab or pooled immunoglobulin (Figure 91.4) [6]. Although this is the largest

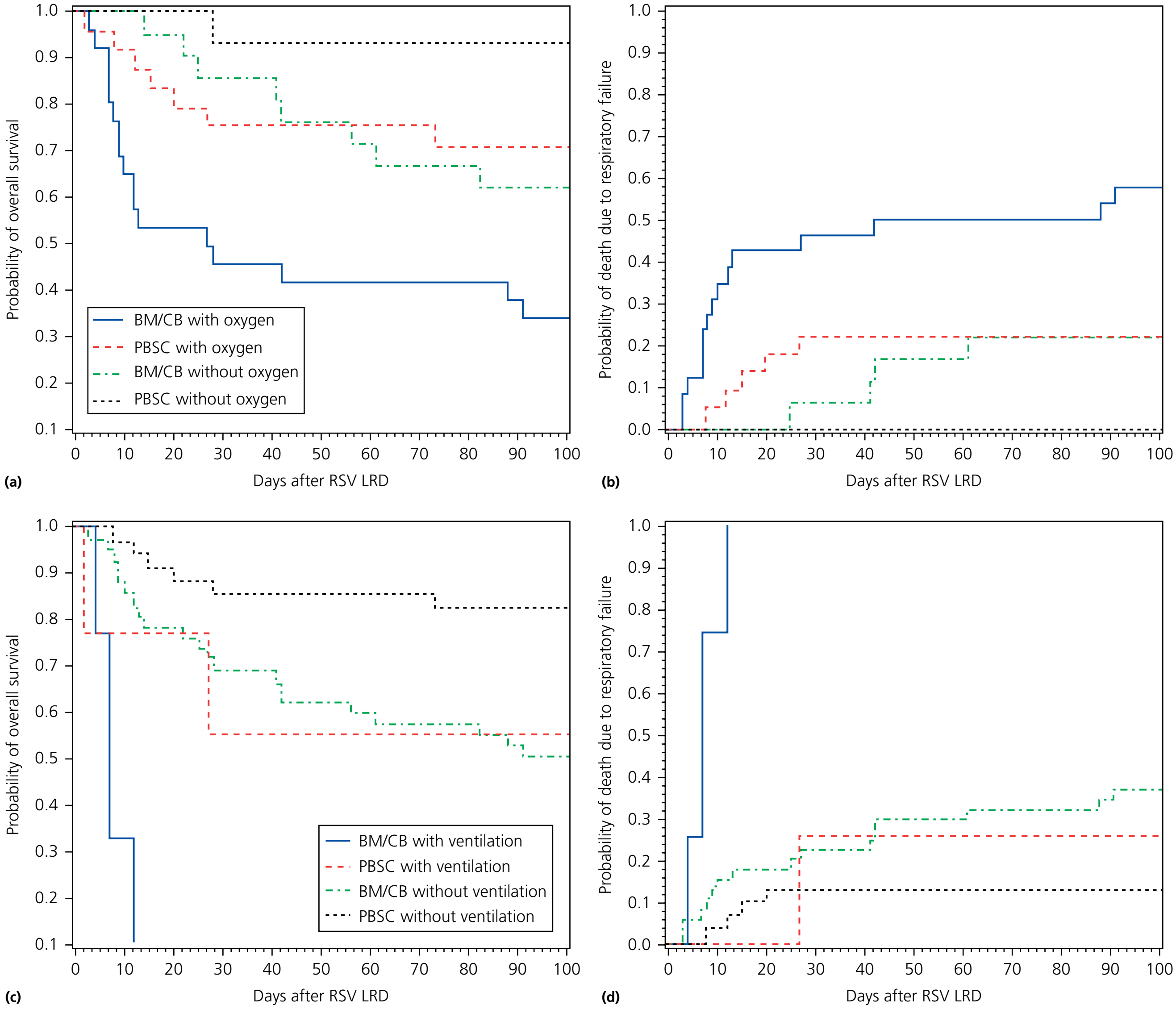

Figure 91.3 Outcomes after RSV LRD by cell source and oxygen requirement or mechanical ventilation. Source: Seo et al. 2013 [6]. Reproduced with permission of Elsevier.

outcome analysis to date, the sample size was not large enough to detect smaller effects. This is consistent with other small reports [3,46,47].

There are several factors that may account for differences in outcome in the available cohort studies. The most important factor seems to be the timing of initiation of therapy. Several studies suggest that treatment that is started when acute lung injury was already present was less successful [6]. Another factor that appears to be important is the stem-cell source. RSV LRD in recipients of peripheral blood stem cells (PBSCs) has a significantly better response to treatment than marrow transplant recipients [6]. Overall, survival of patients with RSV LRD is >90% in PBSC transplant recipients who do not require oxygen at the time of diagnosis (Figure 91.3). Moderate doses of corticosteroids (up to 1mg/kg) do not appear to be associated with poorer outcome in patients with LRD [6], hence reduction of steroid doses may not be required.

Owing to the high mortality of RSV LRD, much interest has focused on prevention and early treatment. Possible strategies are similar to those employed for the prevention of cytomegalovirus (CMV) disease. Although it is well established that RSV URI precedes pneumonia in most cases [33], limited information is available on the extent of asymptomatic shedding of respiratory viruses in HCT recipients [12,35,36]. A small controlled study of preemptive antiviral therapy based on RSV URI showed a decline in viral load but no significant difference in RSV pneumonia; the study was prematurely stopped owing to slow accrual [41]. A recent review of the literature with a pooled analysis suggests a beneficial effect of preemptive ribavirin [3]. Lymphopenia and other factors have been identified as risk factors for progression to LRD and an immunodeficiency scoring index has been suggested [33,34,48]. Hence a more targeted strategy would be to treat only patients who are at higher risk for progression. Some centers have adopted such a strategy, even though randomized trials have not been conducted. Whether palivizumab given preemptively to patients with RSV URI prevents progression to pneumonia has not been studied in a randomized fashion. Evidence ratings in recent American Society for Blood and Marrow Transplantation (ASBMT) and European

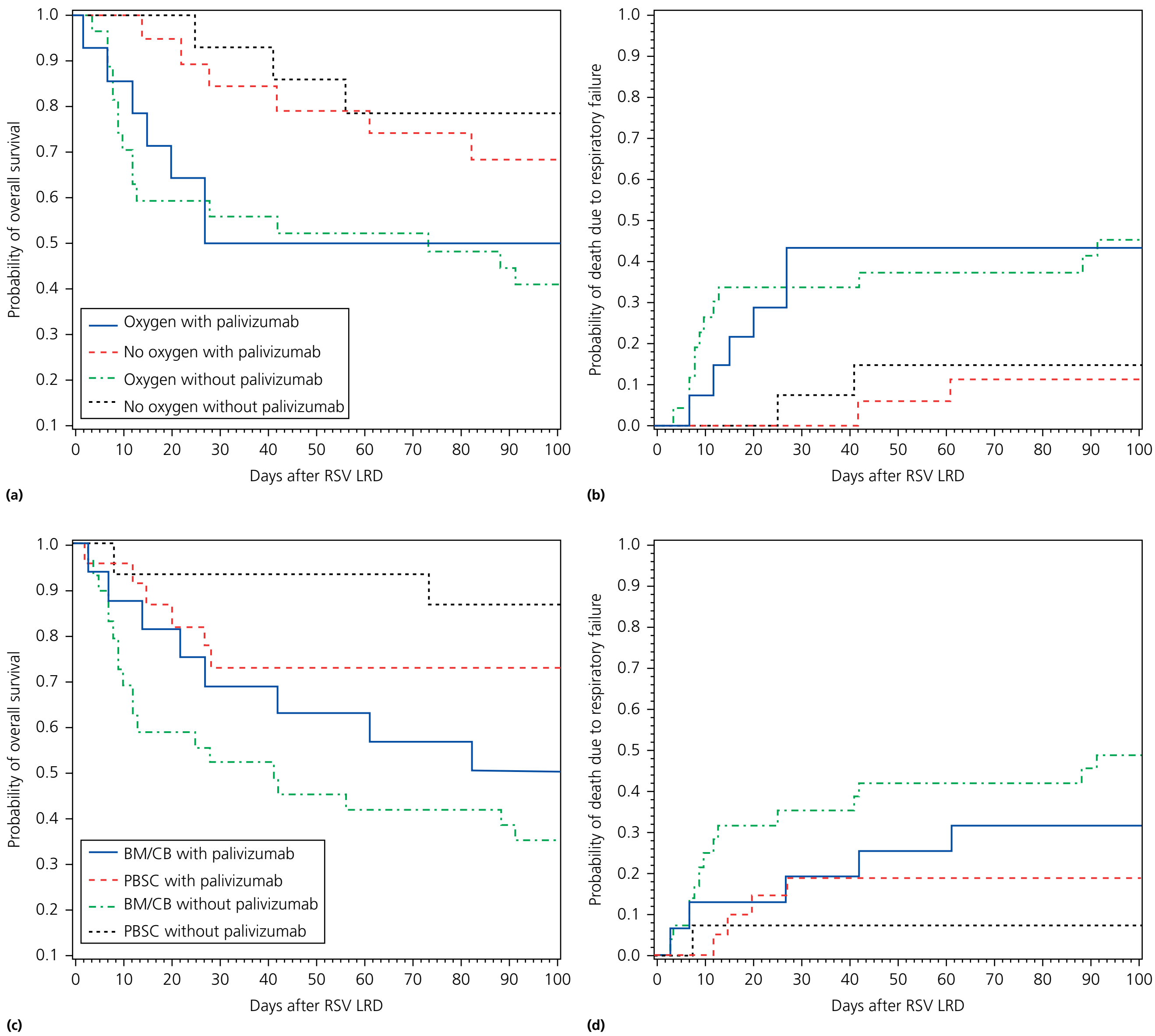

Figure 91.4 Palivizumab effect on RSV LRD by oxygen requirement or stem-cell source. Source: data from Seo et al. 2013 [6].

Conference on Infections in Leukaemia (ECIL) guidelines are shown in Table 91.2 [24,49].

Prophylactic measures recommended throughout the respiratory virus season include isolation of infected patients, hand washing prior to every patient contact, educational efforts targeted at healthcare personnel and family members, and avoiding patient contact of healthcare personnel and family members with uncontrolled secretions [50]. Whether pharmacologic prophylaxis (e.g., palivizumab, RSV-Ig) throughout the respiratory virus season is effective in preventing infection and disease in HCT recipients has not been studied. Some centers give monthly palivizumab to small children (aged <4 years) undergoing HCT while RSV is circulating in the community, similarly to prevention strategies employed in other children at high risk for RSV bronchiolitis [51].

In patients who acquire RSV before transplant, HCT is generally postponed until resolution of symptoms and cessation of viral shedding, especially when a myeloablative allogeneic HCT is planned. Both ASBMT and ECIL guidelines suggest postponing transplantation in all patients with URI symptoms [24,49]. This approach is supported by a study of pre-transplant RSV infection, which showed rapid progression to RSV pneumonia and high mortality after HCT in patients whose transplant procedure was not postponed [52]. However, there is some evidence that low-risk autologous transplant patients may be transplanted without adverse outcome [53]. Also, outcome of respiratory virus infections appears to be less severe with non-myeloablative or reduced-toxicity conditioning regimens [32].

The management of patients with pre-transplant respiratory virus infections who have persistent low-level viral shedding and subsequently undergo high-risk transplant procedures that cannot be further delayed is poorly defined. Options include close monitoring by PCR, the use of secondary prophylaxis (i.e., RSV-Ig, palivizumab), or continued treatment with systemic ribavirin. Some pediatric HCT programs use antibody preparations in these situations while oral ribavirin has been used for adults. However, no systematic evaluations of these approaches have been performed and the strength of the evidence is at the expert opinion level (CIII) [49].

Table 91.2 Overview of antiviral agents and antibody preparations against respiratory viral infections after HCT

Virus	Drugs	Clinical use in HCT recipients[a]	Strength of evidence[b]	Comments
RSV	Ribavirin	RSV LRD Preemptive therapy for URI	*A-II* B-II	Monoclonal antibody was not effective in a recent cohort study [6]
	RSV-specific immunoglobulin	Combination therapy for RSV LRD	B-III	
	RSV-specific monoclonal antibodies (palivizumab)	Combination therapy for RSV LRD	B-III (*C-II*)	
	Pooled immunoglobulin	Combination therapy for RSV LRD	B-II	
Metapneumovirus	Ribavirin	HMPV LRD	C-III	No apparent effect for pneumonia
Parainfluenza virus	Ribavirin	Parainfluenza virus LRD	B-III	Preemptive therapy has not been tested
	Pooled immunoglobulin	Combination therapy for parainfluenza virus LRD	B-III	
	DAS181	Parainfluenza virus infection	Experimental use	No licensed drugs
Influenza virus	Neuraminidase inhibitors (oseltamivir, zanamivir)	Influenza URI Influenza LRD Prophylaxis Combination therapy	B-II (*A-II)* B-II (*A-II)* A-II *C-III*	M2 inhibitors are only effective against influenza A Prophylaxis is recommended only during outbreaks in patients <6 months after HCT
	M2 Inhibitors (amantidine, rimantidine)	Used in combination with neuraminidase inhibitors and ribavirin	*C-III*	
	Ribavirin	Used in combination with neuraminidase inhibitors and M2 inhibitors	*C-III*	
Rhinovirus	Capsid-binding agents (vapendavir)	No data in HCT recipients	Experimental use	Drug is not FDA approved
Coronavirus	Ribavirin Interferon Corticosteroid Protease inhibitors	No data in HCT recipients	Experimental use	Combination therapy has been used in serious SARS cases
Bocavirus	No drug available			
WU/KI Polyomavirus	No drug available			
Measles Virus	Pooled immunoglobulin	Prophylaxis after exposure to a measles case	*C-III*	Vaccination is common.

[a] All clinical results in HCT recipients are from non-randomized studies.

[b] Evidence grading based on CDC/IDSA/ASBMT/EBMT/SHEA or ECIL guidelines; other gradings are assigned by the authors and listed in italics (if no society grading is available or recent studies change evidence in the opinion of the authors).

Human metapneumovirus

Clinical significance

Human metapneumovirus (HMPV) is a negative-sense non-segmented RNA paramyxovirus [54]. HMPV has two genetic groups, A and B, and each group has two more subgroups, A1, A2, B1, and B2 [54–57]. By age 5 years, virtually all children are seropositive. The virus can cause URI and LRD during the winter season [58,59]. Generally, healthy adults with HMPV infection are asymptomatic or have slight upper URI symptoms [60]; however, serious LRD associated with HMPV has been reported in immunocompromised patients [61–63]. HMPV infection occurs in approximately 5% of HCT recipients (Figure 91.2a) [5]. HMPV disease in HCT recipients immediately post-transplant typically presents with several respiratory symptoms, including fever, nasal congestion, and cough. Once pneumonia develops, rapidly progressive pulmonary infiltrates are frequently accompanied by hypotension, septic shock, or both [61]. Radiographic findings consist of a mixed pattern: small multiple nodules and ground-glass opacities are frequently observed [9,64]. Patients with centrilobular nodules were associated with mild symptoms in one study [9]. Histological assessment showed that diffuse alveolar damage with hyaline membrane formation, foci of bronchiolitis obliterans organizing pneumonia, and diffuse alveolar hemorrhage are common [61].

Risk factors

The progression rate to LRD is poorly defined. Small studies suggest that it may occur in 20–40% of patients with URI (Table 91.1) [65,66]. Among HCT recipients undergoing bronchoalveolar lavage (BAL) for work-up of clinical and radiographic pneumonia, 3–4% had HMPV detected [61]. Most of these patients were previously classified as having idiopathic pneumonia syndrome [61]. Whether asymptomatic shedding occurs is controversial [12,67].

Prevention and treatment

There is no proven treatment for HMPV disease, which may be fatal in HCT recipients [61]. Although intravenous immunoglobulin (IVIg) has been shown to neutralize HMPV effectively in vitro, there are no human studies of its efficacy in vivo [68]. The role of corticosteroids is also not defined. Ribavirin has been shown to have in vitro antiviral activity against HMPV [68] and some case reports supported its use [69,70]. However, clinical data from a non-randomized retrospective analysis suggest no major therapeutic effect of aerosolized ribavirin [9].

Parainfluenza viruses

Clinical significance

Parainfluenza virus, an enveloped paramyxovirus containing single-stranded RNA, is classified into four serotypes. Of the four types of parainfluenza viruses, parainfluenza virus 3 is most common (~80%), followed by serotypes 1, 2, and 4. The incubation time is 1–4 days. Parainfluenza virus infections remain detectable throughout the summer season (Figure 91.1a) [4]. The incidence may be 1–9 % during HCT (Figure 91.2a) [7,71–73]. The main radiographic findings are multiple small nodules in a peribronchial distribution [74].

Risk factors

Virus acquisition is a dependent on exposure to infected persons. However, higher infection rates have also been described in recipients of unrelated or mismatched related donors, with reduced-intensity conditioning regimens, or with acute or chronic graft-versus-host disease (GVHD) [7,71]. T-cell-depleted patients have been reported to have an increased risk of all respiratory virus infections, including parainfluenza virus infections [75].

Similarly to RSV, URI is the predominant presentation. Progression to LRD seems to be less common than with RSV

(Table 91.1) [30]. Late airflow obstruction has been linked to parainfluenza virus URI and LRD [28]. The most important risk factor for progression from URI to LRD is lymphopenia [32,76]. Recipients of non-myeloablative conditioning appear to have a small risk of progression during the first 3 months after HCT [32]. Parainfluenza virus 3 pneumonia is often associated with serious pulmonary copathogens such as *Aspergillus fumigatus* [71]. Pulmonary copathogen was another important factor for the progression in a cohort including non-transplant patients [8]. Male gender, mismatched related or unrelated donor, cord blood transplantation, neutropenia, and chronic GVHD are also associated with a high incidence of LRD [7,8]. Parainfluenza virus 3 pneumonia may also occur after autologous transplantation, mainly in the setting of CD34 selection or use of high-dose steroids [71]. Factors associated with poor outcome after pneumonia include low monocyte counts and high-dose steroid use for pneumonia [76]. Aggressive diagnostic work-up (e.g. BAL) and therapy are indicated in patients with suspected parainfluenza virus pneumonia. In a large retrospective analysis conducted in the 1990s, both URI and LRD due to parainfluenza virus 3 were associated with overall mortality in multivariate models [71].

Treatment and prevention

Mortality of pneumonia is approximately 20–50% [7,71,73,76,77]. Randomized treatment studies have not been performed. In a recent retrospective analysis that employed multivariate models, neither aerosolized ribavirin nor intravenous immunoglobulin led to improved outcome of pneumonia [8,76]. Systemic ribavirin has only been reported in case reports or small series [78,79]. Whether earlier antiviral treatment (e.g. preemptive treatment for URI) is effective in the prevention of pneumonia or late airflow obstruction is unknown [8,78]. The association of high-dose steroid treatment with progression to LRD or mortality might suggest that reduction of immunosuppression may be useful [8,71,76]. The effect of steroids has not been seen in all studies and such an approach has not been tested.

DAS181, a sialidase fusion protein, was developed for treatment of influenza and is also active against parainfluenza viruses [74,80]. Inhaled DAS181 was used in only a few cases after HCT; improvement of symptoms and decrease of viral load were detected [81,82]. BCX2798 is a novel hemagglutinin–neuraminidase inhibitor and showed a protective effect for parainfluenza virus 1 in animal models [83].

Infection control is the mainstay of prevention strategies. Unfortunately, current infection control practices seem to be far from perfect in keeping parainfluenza viruses out of HCT units, as indicated by the high incidence. Parainfluenza virus has been notorious as a cause of persistent outbreaks in HCT units [79,84–87]. Possible explanations for the difficulty in preventing parainfluenza virus from entering HCT units include lack of a vaccine for healthcare workers and close contacts, very mild or lack of symptoms in immunocompetent individuals with prolonged shedding, prolonged asymptomatic shedding in infected patients, and persistence of the virus on environmental surfaces [12].

Influenza viruses

Clinical significance

Influenza viruses belong to the family of orthomyxovirus and are enveloped, single-stranded, pleomorphic RNA viruses. Influenza is classified into three major types, of which type A is most common, followed by type B [88–90]. Influenza type C is uncommon even in the immunocompetent population; there are no reports of influenza C in HCT recipients. Influenza virus infections seem to be less common than RSV and parainfluenza virus infections (Figure 91.2a). Seasonal variations of antigenic and susceptibility patterns are a typical feature of influenza viruses. Studies conducted during the H1N1 pandemic outbreak in 2009 suggested that the 2009 H1N1 strain led to higher rates of LRD, hypoxemia, and prolonged viral shedding [11].

Risk factors

Acquisition of influenza is increased in patients with high-risk underlying disease [39]. Progression to severe LRD is similar to RSV and parainfluenza virus (Table 91.2) [90], and risk factors for progression are age, lymphopenia, and lack of antiviral therapy [10,11,91,92]. In contrast to parainfluenza virus infection where corticosteroids may increase the risk of progression [71], LRD appears to be less common and severe in patients with influenza infection who received corticosteroids (≥1mg/kg) for GVHD [11]. Also, non-myeloablative transplant recipients appear to be less likely to develop LRD [32]. Interestingly, the clinical presentation in HCT often lacks myalgia and high fever which are commonly seen in immunocompetent individuals (Figure 91.5) [12]. Influenza RNA detection in plasma or serum is associated with higher mortality in patients with LRD [93].

Treatment and prevention

Effective prevention is available for influenza, which may explain the lower incidence. Healthcare personnel, family members, and visitors are advised to be vaccinated against influenza early in the season [94]. Antiviral therapy is available for influenza virus infection. owing to the recent emergence of resistance against the M2 inhibitors amantidine and rimantidine (which have a more favorable side-effect profile), these agents should not be used to treat influenza A [95–97]. Neuraminidase inhibitors (e.g. zanamavir, oseltamivir) are active against influenza A and B [98,99]. The treatment duration is recommended as 10 days since prolonged viral detection frequently occurs in HCT recipients [94,96]. Uncontrolled studies suggest that preemptive therapy with neuraminidase inhibitors may be effective in preventing progression to LRD [11,92,100]. Whether higher doses of neuraminidase should be used in severe cases of influenza disease is not well defined [96]. Some centers prefer higher doses owing to uncertain absorption and overall poor outcome; however, the strength of the evidence is low (expert opinion, CIII).

Oseltamivir-resistant strains (mainly H275Y mutation) have emerged in pandemic 2009 H1N1 and also seasonal H1N1 [101–103]. This mutation was detected in 24% of HCT recipients with prolonged virus shedding [103]. Combination therapy with oseltamivir, amantadine, and ribavirin is safe and offers potential for improved treatment efficacy in immunocompromised patients [104]. A novel neuraminidase inhibitor, peramivir, showed early improvement of symptoms and non-inferiority to oseltamivir [105,106]. Two HCT recipients with oseltamivir treatment failure were successfully treated with peramivir [107,108]. Intravenous zanamivir is also another option for oseltamivir-resistant cases. The efficacy was reported in some reports and an early switch from oseltamivir to zanamivir may result in better outcomes [100,109–111]. DAS181 and laninamivir are investigational drugs and a phase II study has reported the efficacy of these drugs for influenza [112,113]. Nitazoxanide is a new class of anti-influenza drug and is

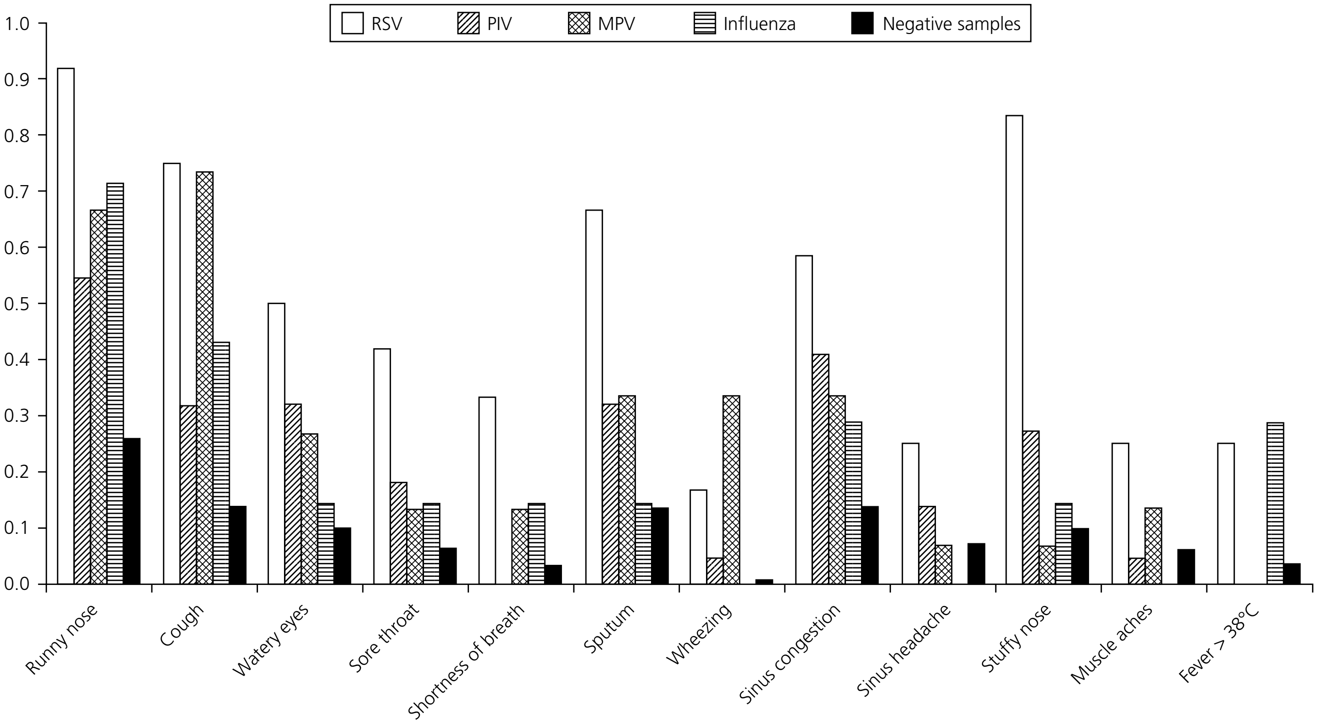

Figure 91.5 Symptoms associated with respiratory virus infection after HCT. Source: Peck et al. 2007 [12].

already approved by the US Food and Drug Administration (FDA) for the treatment of parasites. The anti-influenza mechanism is the inhibition of maturation and intracellular transport of the viral hemagglutinin protein [114]. Nitazoxanide has shown efficacy for the strains that are resistant to currently approved anti-influenza drugs and synergy with neuraminidase inhibitors [115]. A phase III trial to evaluate the efficacy and safety of nitazoxanide with or without oseltamivir is ongoing. The use of hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) as lipid-lowering agents has been associated with reduced mortality from influenza among the general population [116]; no data exist for immunocompromised patients.

Widespread chemoprophylaxis of susceptible immunosuppressed patients in outbreak situations has been recommended [23] and two studies using oseltamivir for prophylaxis suggest that this approach may be safe and effective [117,118].

Vaccination is generally recommended 6 months after HCT [94,119] and single-dose vaccination seems sufficient even after HCT [120,121].

A recent randomized study showed that pretransplant recipient vaccination improved the influenza-specific IgG levels [122].

Human rhinoviruses

Clinical significance

HRhV is classified as a picornavirus characterized by small, naked, single-stranded RNA. There are three groups (HRhV-A, -B, and -C) and approximately 100 serotypes that usually cause mild upper respiratory tract symptoms in immunocompetent hosts. HRhV-C has been reported to cause more severe disease in immunocompetent individuals than HRhV-A and -B [123,124]; however, no data exist for HCT recipients on viral strains and disease severity [125,126]. Using culture techniques, HRhV is infrequently reported as a cause of respiratory infection in HCT recipients. With the widespread use of reverse transcription PCR-based techniques, it is now the most common respiratory virus after HCT (Figure 91.2a) [5]. In a large prospective study, 13% of the HCT recipients had HRhV infection without symptoms and prolonged viral shedding (more than 3 weeks) was very common (Figure 91.6) [5]. Although HRhV is frequently detected in upper respiratory tract illness, some evidence suggests that it may also be a significant cause of LRD [127–129] and that pretransplant infection may lead to adverse outcomes [52]. Exact data on the rate of progression are lacking. Earlier reports showed conflicting results regarding HRhV ability to cause LRD after HCT [128,130]. However, several case reports and a recent initial analysis of a larger cohort showed similar outcomes to those reported with RSV, influenza, and parainfluenza viruses, independent of copathogens [17,127,129].

Risk factors

Factors associated with progression from URI to LRD or mortality have not been defined.

Treatment and prevention

There is currently no licensed drug or vaccine available for the treatment or prevention of HRhV infections. A capsid-binding agent, pleconaril, showed mild reduction of the duration and severity of symptoms and of viral load among healthy individuals, although the drug is not approved as a result of adverse effects and drug interactions [131–133]. BTA-798 (vapendavir), another capsid-binding agent, was evaluated in phase II clinical trials and significant reduction of symptoms was achieved in high-risk patients. Rupintrivir, an inhibitor of HRhV replication, failed to show significant effects in naturally infected patients [134].

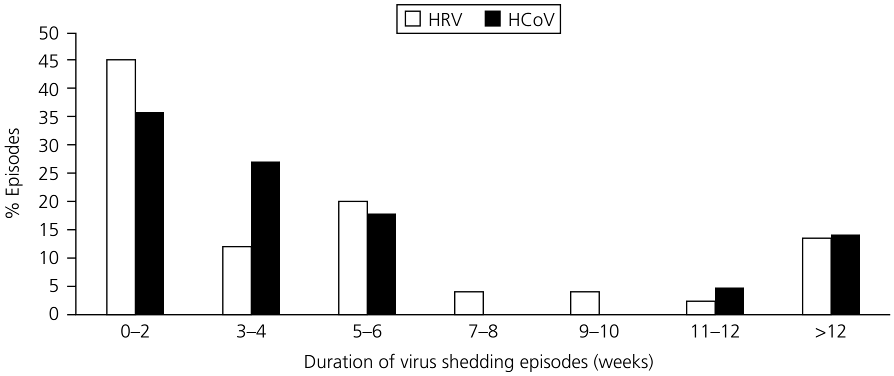

Figure 91.6 Duration of rhinovirus (white) and coronavirus (black) shedding. Source: Milano et al. 2010 [5].

Human coronaviruses

Clinical significance

HCoV is an RNA virus that can cause URI, croup, wheezing, and pneumonia in immunocompetent individuals. Fairly recently, four new strains were described, SARS-CoV (identified in 2003), NL63 (2004), HKU1 (2005), and EMC (2012), in addition to the previously described OC43 and 229E strains [135,136]. Among 823 patients admitted to hospital, 5.7% had HCoV detected; HCoV infection occurred in 8.8% of immunocompromised patients compared with 4.5% of immunocompetent patients [136]. Fourteen of the 47 HCoV-infected patients (30%), including three HCT recipients, were also infected with other pathogens (RSV and *Aspergillus*; influenza A; RSV and HRhV), and these patients had more severe diseases [137]. In the HCT setting, HCoV is the second most common respiratory virus with a day 100 cumulative incidence of 11% (Figure 91.2a) [5]. In a prospective surveillance study, asymptomatic shedding was as high as 41% and the median duration of viral shedding was 4 weeks (Figure 91.6) [5]. Only a few cases of HCoV LRD after HCT have been reported [138–141] and the significance of HCoV as a lower respiratory tract pathogen remains poorly defined in HCT recipients. Prospective studies in immunocompetent hospitalized patients showed that 5.4% of BAL samples were HCoV positive [142]. HCoV infections have also been associated with long-term decline in FEV1 in lung transplant recipients [143]; however, no data exist for HCT recipients.

Risk factors

Factors associated with progression from URI to LRD or mortality have not been well defined.

Treatment and prevention

There are no specific treatments for HCoV. The clinical experiences with severe acute respiratory syndrome (SARS) suggest that ribavirin, interferon, or hydrocortisone may improve outcome, especially when used in combination [144–148]. Protease inhibitors (lopinavir/ritonavir) were also used with ribavirin and showed improved outcome in SARS patients compared with historical controls [149].

Human bocavirus

Clinical significance

HBoV is a human parvovirus originally identified by a random PCR amplification/cloning technique from hospitalized children in Sweden [150]. HBoV is divided into four species [151]; HBoV1 is mainly detected in the respiratory tract and HBoV2–HBoV4 in stool. HBoV has been detected worldwide [152–154] and appears to be common in young children [151]. Symptoms associated with HBoV infections include rhinorrhea, cough, fever, wheezing, hypoxia, and diarrhea. HBoV infection was detected in 2.6% of HCT recipients (Figure 91.2a). A recent prospective study showed that HBoV was commonly detected in pediatric cancer patients with LRD [155]. However, since HBoV is frequently detected with other viruses [156], there is uncertainty as to whether HBoV is a pathogen.

Risk factors

High viral load in nasopharyngeal aspirates and blood samples was suggested to be related to LRD in immunocompetent children [156]. To date, there is no information on the risk factors of HBoV infection in immunocompromised patients.

Treatment and prevention

No antiviral agents exist for HBoV. Corticosteroids were not effective in improving symptoms and the duration of hospitalization among immunocompetent children with HBoV infections [157].

WU and KI polyomaviruses

Clinical significance

Human polyomaviruses, WU (WUPyV) and KI (KIPyV), were described in 2007 and frequently detected in respiratory specimens [158,159]. Infection by WUPyV and KIPyV generally occurs by age 5 years [160]. The prevalence in healthy children was 2–3% for WUPyV and 0.5–3% for KIPyV [161,162]. Incidence rates are similar between patients with and without respiratory symptoms [163–166]. Among HCT recipients, the 1-year cumulative incidence of WUPyV and KIPyV was 8 and 26%, respectively (Figure 91.2b) [167]. Seasonality was not detected [167]. Sputum production in KIPyV and wheezing in WUPyV are distinctive symptoms in HCT recipients [167]. Viral load for KIPyV was higher in immunocompromised patients than healthy children, whereas viral load for WUPyV was similar regardless of immune level [161]. The significance of WUPyV and KIPyV as a cause of LRD in HCT recipients is uncertain.

Risk factors

An important risk factor for acquisition in HCT recipients is age <20 years [167]. HCT patients who acquired KIPyV were more likely to have had another respiratory virus infection in the preceding 2 weeks [167].

Treatment and prevention

No treatments are available for WUPyV and KIPyV.

Measles virus

Clinical significance

Measles virus (rubeola), a paramyxovirus, is a recognized cause of giant-cell pneumonia, developing with and without rash, and severe central nervous system (CNS) disease in immunocompromised hosts, including HCT recipients [168–171]. The incidence of measles is extremely low at most centers owing to the widespread use of measles immunization. In 1997, an outbreak among HCT recipients in Brazil was reported. Among the 122 susceptible patients, severe clinical diseases occurred in one patient and mild symptoms were reported in seven patients [172]. Exanthema was present in all these patients, although it was often atypical. Koplik spots could be observed in five patients. All but one patient had fever and non-productive cough [172]. Recently, outbreaks of measles have been increasing owing to low vaccine coverage [173–175] and HCT recipients may be at increased risk of acquisition.

Risk factors

The key risk factor for acquisition is exposure to an infected individual in the setting of poor vaccine coverage or waning vaccine immunity. In a European study, 80–90% of measles cases were unvaccinated [175]. Risk factors for clinical disease outcome have not been described.

Treatment and prevention

The use of ribavirin has been reported for measles in two critically ill immunocompromised patients; however, no systematic assessment of this intervention can be made [176]. Assuring that all patient contacts have protective antibody titers (either by natural infection or vaccination) is the most important preventive measure. Immunity to measles continuously diminishes after HCT, thereby increasing the risk of acquisition [177–179]. Therefore, the use of vaccine after HCT is recommended after systemic immunosuppression has been discontinued [23]. At a median of 2.4 years after HCT, 49% of the HCT recipients responded to the vaccine [180]. Immunoglobulins may be used after an exposure [181].

Conclusions and future perspective

The increasing use of multiplex molecular diagnostic platforms has significantly increased our knowledge of the incidence, risk factors, and significance of respiratory viral infections after HCT [17]. However, important questions remain. Although many studies analyzed the outcomes and risk factors for LRD by respiratory viruses, the definition of LRD differs between studies. The strictest definition consists of LRD symptoms, positive radiography. and viral detection in the lung, while LRD symptoms plus a positive nasopharyngeal sample are frequently defined as LRD. Thus the current LRD includes a different and broad range of the diseases, which makes comparison of outcome results between centers difficult to interpret. Definitions of diagnostic criteria based on outcome studies should be established [76].

Molecular diagnosis is now used at many centers. At the same time, many open questions remain regarding the significance of some viruses that are now easily detectable and highly prevalent. For instance, studies are needed to define risk factors for progression to LRD for HMPV, HRhV and HCoV. Also, the significance of HBoV remains poorly defined. Molecular diagnostic methods have also provided accurate quantification of viral load [76]. High viral load tends to correlate with poor outcome; however, sufficient data to apply viral load testing to clinical practice are not available. Viral detection and high viral load in blood can be also a marker for severity of respiratory viral infections. Compared with an interventional procedure (e.g. bronchoscopy), blood testing would be more acceptable to HCT recipients; more prospective studies are needed to define the timing of viremia and the optimal blood component (plasma versus cells). Also, further prospective studies comparing viral load in respiratory secretions and blood with outcome are warranted. Once respiratory viral infection progresses to LRD, mortality rates are still high. Although some clinical risk factors have been identified (e.g. lymphopenia), disease progression can occur in patients without these factors. Therefore, research on biomarkers for progression to LRD is needed.

Only a few randomized treatment trials exist for RSV in the HCT setting. Randomized trials are the optimal way to evaluate novel interventions and are feasible for the treatment of RSV URI. However, such trials may be difficult or impossible to conduct for LRD owing to the low frequency of this event. The RSV LRD outcome study by Seo et al. re-emphasized that caution should be exercised in adopting treatments based on non-randomized data [6]. It took more than a decade to collect sufficient cases of patients who received palivizumab for a multivariate analysis at one of the largest HCT centers. Palivizumab treatment appeared to be ineffective while being enormously expensive, with no apparent adverse outcomes. Thus, the generation of evidence that exceeds the “expert opinion” state must be accelerated. The relatively low incidence of some of these infections requires innovative surveillance and intervention networks among HCT centers worldwide. Future approaches to improving the speed and quality of treatment strategies could include multicenter, web-based studies of rare complications using detailed case report forms that allow outcome studies similar to those recently published for respiratory viruses and other pathogens [6,38,182]. Efforts have started to establish such networks and web-based disease surveillance and outcome databases have the potential to become an invaluable tool to generate evidence that is of higher reliability than expert opinion.

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CHAPTER 92

HHV-6A, HHV-6B, HHV-7, and HHV-8 After Hematopoietic Cell Transplantation

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Introduction

This chapter deals with the four most recently discovered herpesviruses: the human herpesviruses HHV-6A, HHV-6B, HHV-7, and HHV-8 (also called Kaposi sarcoma-associated herpesvirus). Early studies did not distinguish between the two viruses HHV-6A and HHV-6B but we today know that HHV-6B is the virus that causes the majority of the disease associated with HHV-6. The most important clinical manifestation of HHV-6B is encephalitis frequently localized to the limbic system. This complication is more common in severely T-cell-suppressed patients, for example after cord blood (CB) transplantation. Other clinical associations such as bone marrow suppression, pneumonia, and skin rash mimicking acute graft-versus-host disease (GVHD) have been proposed. The studies have been hampered, however, by the possibility of chromosomally integrated HHV-6 that is found in 1–2% of the population. Monitoring for HHV-6 has been proposed but is currently not generally recommended since there is no intervention of proven effectiveness. The importance of HHV-6A, HHV-7, and HHV-8 is less in stem-cell transplant recipients, although cases with likely pathology due to these viruses have been documented.

Human herpesviruses 6A and 6B

Epidemiology and basic virology

Human herpesvirus 6 (HHV-6) was discovered by accident in 1986 in CB lymphocyte cultures designed for the isolation of HIV [1]; the virus was initially named human B-lymphotropic virus but was later found to infect and replicate in lymphocytes of the T-cell lineage, at which point the name was changed to its present form. Soon after the discovery of HHV-6, it was realized that viral isolates fell into two readily distinguishable groups, HHV-6A and HHV-6B, and that they differ in their epidemiology and association with disease. The virus genomes are double-stranded DNA, 160–162kb in size and colinear with an overall nucleotide sequence identity of 90%, but interspecies divergence of specific sequences is higher than 30%. There is no genetic gradient between the two viruses and recombinants have not been reported [2]. In 2011, in the face of the accumulating evidence, the International Committee on Taxonomy of Viruses declared that HHV-6A and HHV-6B meet the formal definition of separate species. In what follows, the general term HHV-6 will therefore be reserved for instances where it is not necessary to distinguish HHV-6A from HHV-6B or where the distinction has not been made.

The two viruses are members of the herpesvirus subfamily *Betaherpesvirinae*, and of the *Roseolovirus* genus. As with the other herpesviruses, HHV-6A and HHV-6B persist for life following primary infection with the potential for transmission from human to human, for reactivation, particularly in the immunocompromised, and for consequent disease. HHV-6A and HHV-6B can infect a variety of cultured cell types but grow best in CD4$^+$ T-lymphocytes. Although these cells are the most frequently infected, the host range includes not only lymphoid cells and monocytes but also those of epithelial and neuronal origin. The explanation lies in the nature of the virus receptor – CD46 (also known as membrane cofactor protein, a regulator of the complement cascade) [3] – which is expressed on all nucleated cells.

The study of HHV-6 has been confounded by two issues that many investigations have not taken into account. The first is that despite the evidence, the need to distinguish HHV-6A from HHV-6B has not been generally appreciated. The second is that HHV-6 has a unique feature whereby either of the two viruses' genomic sequences can sometimes persist integrated into the human chromosome in a minority of persons (see the section Chromosomal Integration of HHV-6).

On the scanty evidence available, HHV-6A is commonly encountered in Central Africa [4], but rarely in the United States, the United Kingdom, and Japan. It is noteworthy that in contrast to its prevalence in the general population, HHV-6A is markedly overrepresented in cases of chromosomal integration. The true prevalence of HHV-6A infection due to horizontal transmission as opposed to chromosomal integration remains to be determined.

Primary infection with HHV-6B is universal, almost always occurring in early life with most children infected by the age of 2 years [5,6]. The virus is commonly found in saliva [7–9] and this is the probable source of transmission within families.

Since HHV-6B replicates in T-lymphocytes and is thought to be latent in monocytes and bone marrow progenitor cells [10,11], transmission via blood transfusion and organ transplant is to be expected. The first report of this was of primary HHV-6 infection in a patient following liver transplantation from a seropositive donor [12]. There have been isolated case reports since then,

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including an infant who received a bone marrow transplant from his twin brother who had primary HHV-6B infection at the time of donation [13].

As regards disease, most children undergoing primary HHV-6B infection have at least some symptoms ranging from fever, sometimes accompanied by the rash of the childhood illness, exanthem subitum, to febrile convulsions, to status epilepticus with fever, and even to encephalitis [14–18]. After hematopoietic cell transplantation (HCT), HHV-6B reactivates and is the occasional cause of encephalitis and other manifestations (see below). No disease has been causally linked to HHV-6A although, intriguingly, there is a recent report of transmission of HHV-6A from a liver donor to recipient that was associated with syncytial giant cell hepatitis [19].

Chromosomal integration of HHV-6 (CIHHV-6)

This phenomenon, characterized by very high HHV-6 DNA loads in blood, is due to integration of full-length HHV-6A or HHV-6B DNA into host leukocyte chromosomes. Transmission of integrated viral DNA, occurring naturally in the germline, is unique amongst the human herpesviruses. CIHHV-6 was for many years considered an extremely rare occurrence, but it is now clear that it is uncommon rather than rare. Its frequency has been estimated as about 1% in UK blood donors [20] and in newborn children in the United States [21].

CIHHV-6 was first described in 1993 by Luppi et al. [22], who reported three individuals with viral DNA levels so high in leukocytes that they could be detected by Southern blotting and also by the very much more sensitive technique of polymerase chain reaction (PCR). Subsequently, fluorescence in situ hybridization (FISH) using HHV-6-specific probes identified integrated viral sequences in all three cases on chromosome 17 and close to, or in, the telomeres of the short arm [23]. Sequencing of the integration site has recently confirmed the telomeric location [24]. Since then, nine more sites have been found and always telomeric [25,26] (see the example in Figure 92.1), although on a seemingly random range of chromosomes and on either the short or long arms. The exact chromosome involved is unique to the individual.

Vertical transmission of CIHHV-6 was first reported in six different families in Japan [27–29] – in one family from both parents and in another through two generations. Since then, affected families have also been reported in Europe [30] and the United States [21], confirming that autosomal dominant inheritance of CIHHV-6 is a worldwide phenomenon. Since CIHHV-6 is in the germline, about one copy of HHV-6 DNA can be detected in every nucleated cell type in the body [31].

An important unresolved question is whether CIHHV-6 is associated with disease. This might occur in two ways, by virus reactivation or by disruption of telomere function. Considering the first possibility, of the very few studies conducted to date only one [32] has shown that integrated virus is capable of full replication in vivo. Regarding the second possibility, there are not nearly enough data available to unravel the possible long-term clinical consequences of HHV-6 integration at various telomeric chromosomal sites. In fact, no clinical consequences have been formally linked to CIHHV-6. Notably, CIHHV-6 has been found in a great many apparently healthy individuals, e.g. neonates [21], blood donors [20], and stem-cell donors [33].

In CIHHV-6, HHV-6 DNA can be found not only in whole blood but also in “cell-free” samples such as serum or plasma and cerebrospinal fluid (CSF), which contain human chromosomal DNA released from damaged cells during sample preparation. For most viruses, detection of significant levels of viral DNA in the blood, especially in serum or plasma, can be taken as evidence of active infection, but HHV-6 is an exception as such high viral DNA levels may be due to CIHHV-6. In the past, misunderstanding this has led to the inappropriate use of antiviral therapy [34]. Whereas multiple antiviral drug therapy has no effect on viral DNA levels in CIHHV-6 (Figure 92.2a), a good response was seen in a case of HHV-6-induced bone marrow suppression (Figure 92.2b). Diagnostic difficulties with CIHHV-6 also arise when the donor or recipient of HCT has CIHHV-6. If the donor has CIHHV-6, there will be elevation of HHV-6 DNA load in the recipient correlating with leukocyte engraftment and rising to characteristically and strikingly high levels [35]. On the other hand, if the stem-cell recipient rather than the donor has CIHHV-6, the converse will occur [36]. A comparison of the different possible findings regarding HHV-6 after HCT is given in Table 92.1.

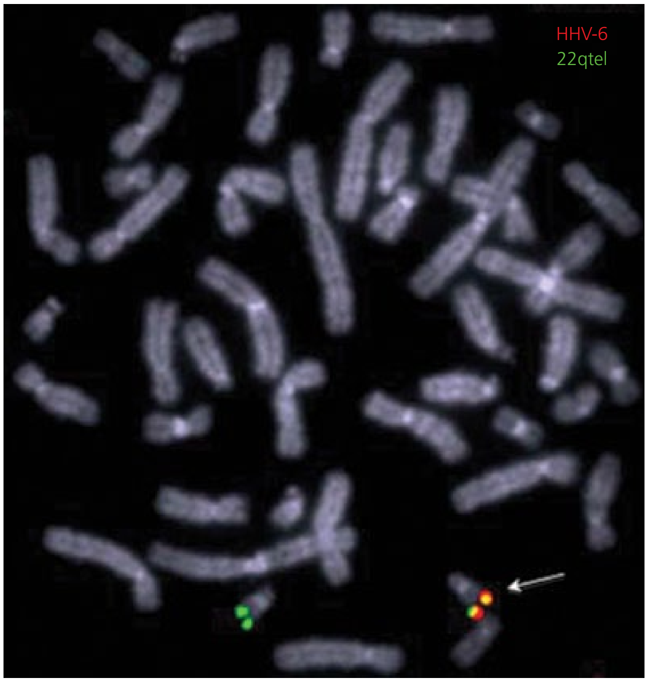

Figure 92.1 Fluorescent in situ hybridization (FISH) image of a mitotic cell from a patient with CIHHV-6 showing integration of HHV-6 (signal at bottom right) within the telomere sequences 22qtel (signal at bottom left and right). Note that these signals overlap at the 22qtel region (arrowed). Source: Lohi O. et al. *Pediatr Blood Cancer* 2010; **55**: 1236–1238. Reproduced with permission of John Wiley & Sons.

Diagnosis of HHV-6 infection

Diagnosis of primary HHV-6 infection in young children is based on antibody detection using indirect immunofluorescence [37], but such tests are difficult to interpret in older children and adults. Immunofluorescence testing cannot distinguish between antibodies to HHV-6A and HHV-6B but a recently developed immunoblotting assay [38] has this capacity and may prove useful for research purposes. However, serology has no place in the routine diagnosis of HHV-6 infection in HCT recipients, and detection of virus or parts thereof is required.

One diagnostic possibility is the use of antigenemia tests [39], but there is limited experience as their use has not been widespread. The gold standard is virus isolation in CB lymphocytes, but this is a

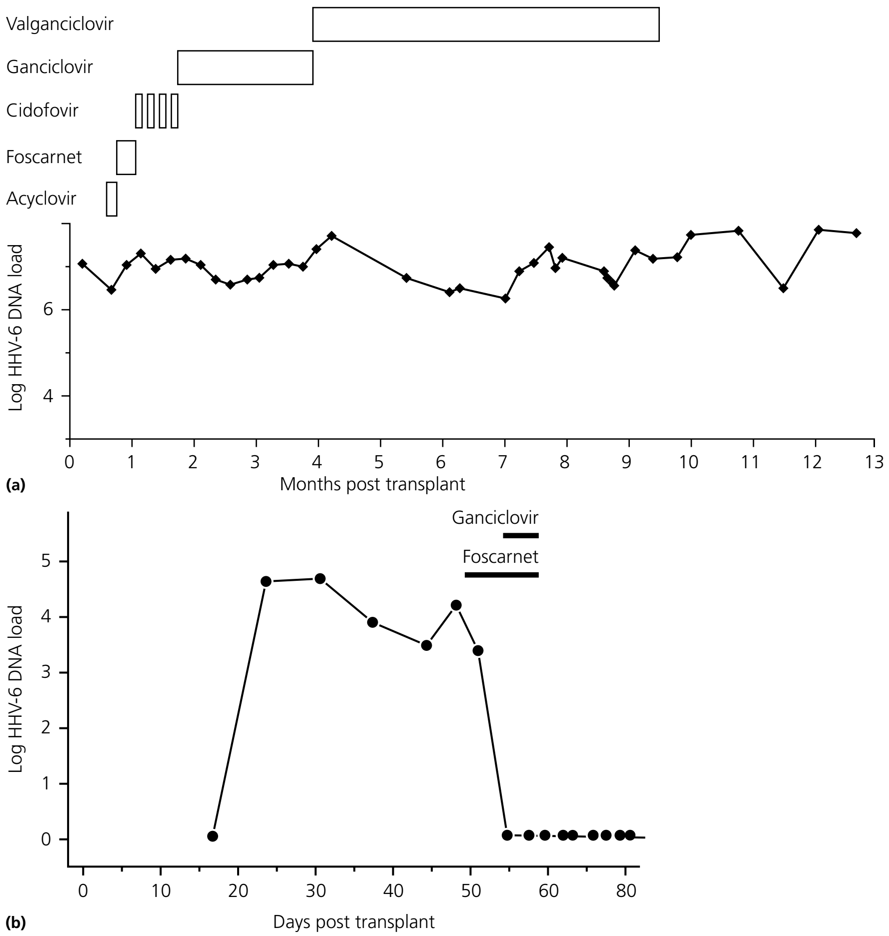

Figure 92.2 Comparison of effect of antiviral drugs on HHV-6 DNA load in blood (a) HHV-6 chromosomal integration and (b) active HHV-6 infection. (a) Failure of multiple antivirals to affect high HHV-6 DNAemia resulting from viral chromosomal integration in a case of severe aplastic anemia. Source: adapted from Hubacek et al. 2007 [34]. (b) Virologic response to antiviral therapy in a case of HHV-6-related graft failure following allogeneic bone marrow transplant. Source: adapted from Johnston et al. 1999 [133].

Table 92.1 Comparison of findings after HCT in various forms of HHV-6 infection

	HHV-6 status		
Clinical/laboratory observations	**Horizontal acquisition**	**Donor chromosomal integration**	**Recipient chromosomal integration**
≥1 HHV-6 copy/leukocyte	No	Yes[a]	No[c]
≥1 HHV-6 copy/hair follicle	No	No[b]	Yes[d]
HHV-6 species/ prevalence	B/>95%	A or B/~1%	A or B/~1%
Persistent HHV-6 DNA in blood	No	++++	+/–
Disease due to HHV-6	Yes, e.g. encephalitis	No?	No?
Response of HHV-6 to antiviral drugs	Yes: decrease in HHV-6 DNA level	No	No

[a] HHV-6 found persistently at extremely high level (chromosomal integration) in hematopoietic tissue, e.g. blood, bone marrow, spleen.

[b] Chromosomally integrated HHV-6 *not* found in cells and tissues of the body that are *not* involved in hematopoiesis.

[c] Chromosomally integrated HHV-6 *not* found in hematopoietic tissue.

[d] HHV-6 found persistently at extremely high level (chromosomal integration) in cells and tissues of the body that are *not* involved in hematopoiesis.

Source: Ward K.N. and Clark D.A. Roseoloviruses: human herpesviruses 6A, 6B and 7. In Zuckerman A.J. et al. (eds.) *Principles and Practice of Clinical Virology*, 6th edn. Wiley-Blackwell, 2009, pp. 223–244. Reproduced with permission of John Wiley & Sons.

specialized technique that is slow, labor-intensive, and not available to most diagnostic laboratories. The need is for rapid diagnosis and the norm is to use PCR to detect viral DNA in blood. The type of sample investigated varies between laboratories but the most commonly used are whole blood and plasma. In view of the differing natural histories of HHV-6A and HHV-6B (see above), it is advisable to use a quantitative PCR that distinguishes between the two [40], but this has rarely been attempted because the need to distinguish the two viral species was not recognized.

If CIHHV-6 is suspected, it can most easily be established by checking donor and recipient samples obtained prior to transplant for the characteristic high levels of HHV-6 DNA in blood using quantitative PCR. Viral DNA load in whole blood or leukocytes should be compared with the amount of cellular DNA, for example that of β-globin, so as to confirm that there is at least one copy of HHV-6 DNA per cell [41,42]. Further confirmation can be gained by testing hair follicles or nails [29,43].

Clinical manifestations in HCT patients

As already described, HHV-6 is now divided into two different viruses, HHV-6A and HHV-6B, and molecular epidemiological studies have demonstrated that HHV-6B, rather than HHV-6A, reactivation frequently occurs in HCT recipients [44]. Although

Table 92.2 Other symptoms and diseases that have been suggested to be associated with HHV-6 infection

Interstitial pneumonitis
Bone marrow suppression
Graft-versus-host disease
Skin rash
Induction of CMV reactivation
Indirect immunosuppression

several symptoms and diseases have been suggested to be associated with HHV-6 infection in HCT recipients, proof remains elusive except for the case of post-transplant HHV-6 encephalitis (see below). As HHV-6 is similar to cytomegalovirus (CMV), another betaherpesvirus, which causes disease in HCT recipients, similar symptoms or diseases have been anticipated as a result of HHV-6 infection (Table 92.2). Interstitial pneumonitis has been postulated to be associated with HHV-6 infection [45,46] because quantitative PCR analysis demonstrated large quantities of HHV-6 DNA in lung tissue obtained from such patients [45,46]. Additionally, HHV-6 antigens were also detected in the lung tissue obtained from interstitial pneumonitis patients, suggesting viral replication [45]. However, many other pathogens including CMV have to be eliminated to authenticate a positive correlation between HHV-6 and this disease. Bone marrow suppression is another possible important syndrome in which HHV-6 might be involved [47,48] since delayed platelet or monocyte engraftment has been found in HCT recipients with HHV-6 infection in several in vivo studies [49–54]. Furthermore, direct and indirect suppression of bone marrow progenitor cell proliferation have been demonstrated in vitro [55–57].

It is well known that HHV-6 reactivation generally occurs between 2 and 4 weeks after transplant, which coincides with the usual time period of acute GVHD. Hence an association between HHV-6 infection and acute GVHD, or skin rash resembling acute GVHD, has been suggested on the basis of numerous cohort studies [52,58–61]. Several investigators detected viral DNA in skin obtained from patients with acute GVHD [62,63]. Additionally, it was demonstrated that HHV-6 can upregulate surface molecules such as ICAM-1 on epidermal cells [64], which are considered to be important molecules for pathogenesis of acute GVHD. However, the major problem of determining the precise role of HHV-6 in the pathogenesis of acute GVHD has yet to be resolved. There are two possible relationships between HHV-6 and acute GVHD: HHV-6 could be a trigger for acute GVHD, because this virus can modulate the host immune responses, but on the other hand, acute GVHD could induce HHV-6 reactivation in HCT recipients. Hence future in-depth analysis is needed to elucidate an association between acute GVHD and HHV-6 infection. It has been shown that HHV-6 reactivation after HCT is associated with increased non-relapse mortality [52,65], and similar findings have also been reported in solid organ transplant recipients [66]. Secondary immune suppression induced by HHV-6 reactivation might be involved in this increase in patient mortality; however, it is difficult to determine a truly causal relationship between HHV-6 reactivation and the mortality rate in transplant recipients. In the absence of an intervention of proven effectiveness (see below), the best management of asymptomatic patients with HHV-6 DNAemia is currently unknown.

HHV-6 encephalitis

The most important and severe consequences of HHV-6 reactivation among post-transplant patients are caused by involvement of the central nervous system (CNS). HHV-6 can cause viral encephalitis but is also associated with other less overt neurologic symptoms [52,67]. In a large prospective study, Zerr et al. showed that HHV-6 detection in plasma was significantly associated with delirium and neurocognitive decline during the first months after HCT [68]. The long-term impact of these findings is still to be investigated and also whether any prophylactic intervention can reduce the risk.

In a recently published study, HHV-6 was the most common cause of viral encephalitis, being found in 28% of cases after allogeneic HCT [69]. It is of note that a number of patients in this series of cases with viral encephalitis had HHV-6 detected together with other viruses. In addition, several case reports and small case series have been published, although some of the early ones are most likely to have been hampered by not excluding CIHHV-6 [67,70–80]. The majority of HHV-6 encephalitis cases occur during the first month after HCT, matching the pattern of the HHV-6 peak viral load in plasma, which is also an early event [52,59,81].

Symptoms

The clinical picture of post-transplant acute limbic encephalitis (PALE) has been described as a typical feature of HHV-6 encephalitis. This fits with that the tropism of HHV-6 for astrocytes in the hippocampus [74,82]. Common symptoms are short-term memory loss, confusion, disorientation, and depressed consciousness [79,83]. Seizures are also fairly frequent whereas focal neurological deficits have been reported but seem to be uncommon. Another reported complication of HHV-6 CNS infection is the syndrome of inappropriate antidiuretic hormone secretion (SIADH) [83,84]. However, HHV-6 encephalitis can also affect other areas of the brain, although less frequently.

Risk factors

Although HHV-6 is a virus usually recognized as a pathogen in children, most published cases of HHV-6 encephalitis have occurred in adults. The large majority of such cases have been reported in allogeneic HCT recipients and only a few after autologous HCT. Acute GVHD grade II–IV is commonly reported in the different case series as are the use of human leukocyte antigen (HLA)-mismatched or unrelated donors [81,85]. Vu et al. reported an 11.6% incidence of HHV-6 encephalitis in patients receiving an alemtuzumab-containing regimen [86]. Patients undergoing HCT from CB donors seem to be at an increased risk for HHV-6 encephalitis [85,87]. Recently, a systematic review was performed which showed a risk of 8.3% in CB graft recipients compared with 0.5% in recipients of other graft sources [88]. It also showed that HHV-6 detection in plasma was twice as common in CB graft recipients (72% vs. 37%). Hill et al. recently reported a large retrospective cohort of 1344 patients and found that risk factors for HHV-6 PALE were unrelated CB HCT [adjusted hazard ratio (aHR) 20.0, $p < 0.001$], acute GVHD grades II–IV (aHR 7.5, $p < 0.001$), and adult mismatched donors (aHR 4.3, $p < 0.04$) [85]. All these risk factors support the importance of poor T-cell function as a component in the pathogenesis of HHV-6 encephalitis. It has also been reported that plasma interleukin (IL)-6 levels are increased before the development of HHV-6 encephalitis together with high HHV-6 viral load in plasma, suggesting that systemic inflammation is involved in the pathogenesis [89].

Findings

HHV-6 encephalitis is typically defined as the presence of CNS dysfunction, a positive PCR result for HHV-6 DNA in the CSF, and the absence of other identified causes of CNS dysfunction [80,90].

Other definitions have been used, such as high levels of HHV-6 DNA in blood instead of in CSF, or adding the requirement of limbic involvement shown by magnetic resonance imaging (MRI) of the temporal lobe. It has also been suggested that the likelihood is increased when the viral load is high in plasma [91]. Hill et al. produced receiver-operating characteristic (ROC) curves analyzing the sensitivity and specificity of plasma viral load and found that a level of $\geq 10^5$ copies/mL was 80% sensitive and 86% specific for HHV-6 PALE [85]. If the level was increased to $\geq 10^6$ copies/mL, the sensitivity decreased to 30% and the specificity increased to 97%. However, they did not exclude the possibility of CIHHV-6. These definitions without CSF verification can be used as a pragmatic definition if a lumbar puncture cannot be performed, but then CIHHV-6 must be excluded. Although detection of viral DNA in CSF supports the diagnosis of HHV-6 CNS involvement, it is not absolutely diagnostic. Wang et al. showed that HHV-6 DNA can be detected in CSF in a small proportion of non-symptomatic immunocompromised individuals [80], and this might well be due to chromosomal integration [92]. HHV-6B is the most commonly reported type, although HHV-6A detection has also been described [71,93]. There are no typical non-specific findings in CSF; many patients have a slight pleocytosis with mononuclear cell predominance but cell counts can also be normal. CSF protein levels can be increased.

At autopsy of patients dying from HHV-6 encephalitis, findings seen in affected areas of the brain were characterized by loss of neurons, reactive gliosis, demyelination, and lymphocyte infiltration [74,93,94].

A “typical” MRI finding has been described showing symmetric or non-symmetric inflammation within the medial temporal lobe, including the uncus, amygdala, entorhinal area, and hippocampus – “limbic encephalitis” – although other imaging patterns such as leptomeningitis are also seen (Figure 92.3) [83,95–97]. These changes of limbic encephalitis and edema can be found early after the onset of clinical signs of encephalitis. However, these are not pathognomonic for HHV-6 encephalitis since other organisms including HSV and VZV can give similar appearances on MRI [98]. A computed tomography (CT) scan is usually normal during the acute phase but can show hypodense lesions in the temporal lobe during follow-up. Electroencephalogram (EEG) abnormalities are also common in HHV-6 encephalitis.

Provenzale et al. compared clinical and MRI findings in nine patients positive for HHV-6 DNA in CSF and seven patients who were tested but found negative for HHV-6 DNA [99] and reported that hippocampal changes were similar in both HHV-6 CSF-positive and -negative patients whereas extrahippocampal involvement or abnormalities in the entorhinal cortex or amygdala were more common in HHV-6-positive patients [99].

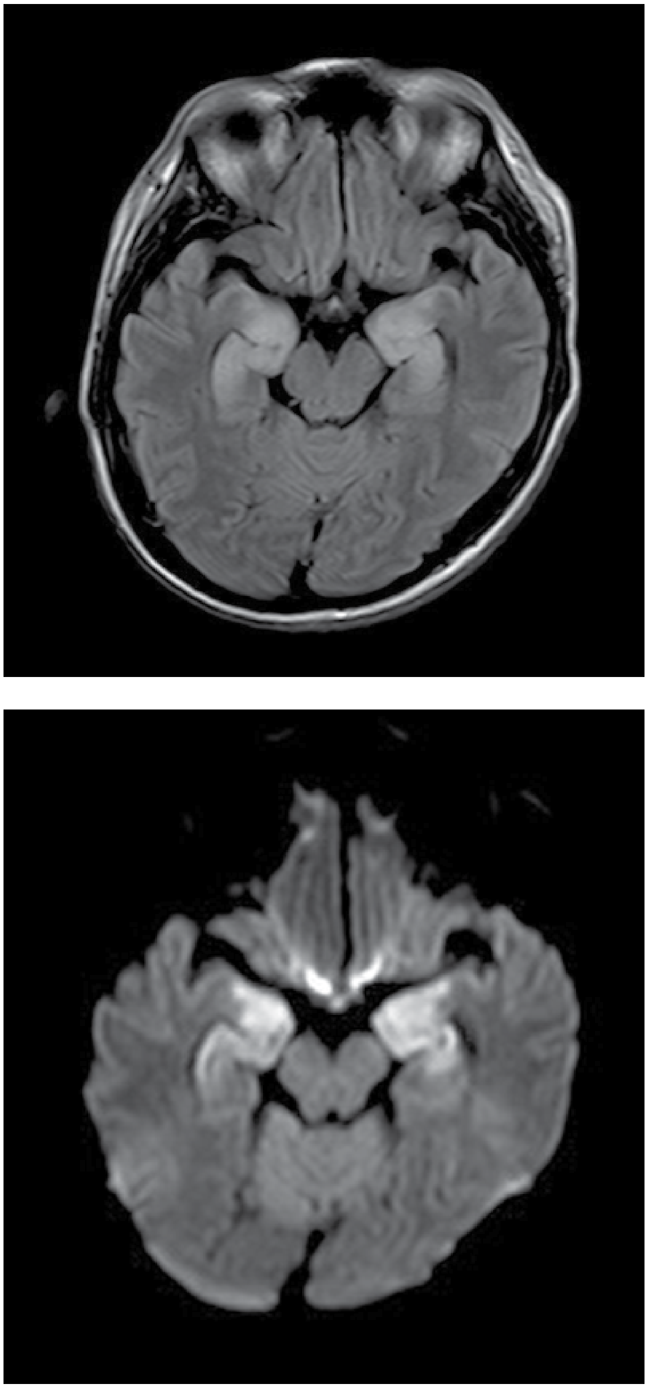

Figure 92.3 MR image of HHV-6 PALE. Source: Ogata et al. 2013 [104]. Reproduced with permission of Nature Publishing Group.

Outcome

HHV-6 encephalitis has been reported to be associated with a high rate of mortality and also significant late sequelae. Zerr reviewed the literature in 2006 and found among 44 cases an HHV-6-associated mortality of 25% whereas 14% died of other causes after showing some improvement [93]. Of the survivors, 43% made a complete recovery and 18% had long-term neurological deficits. In more recent series, Seeley et al. found that five out of nine (56%) adults with HHV-6-positive PALE died, and three out of four (75%) of the surviving patients maintained persistent cognitive impairment [83]. Hill et al. reported that death due to HHV-6 PALE occurred in 50% of CB recipients (5/10) compared with none (0/9) who received adult donor stem cells [85]. These two papers come from the same institution and the case collections overlap in time, so some cases presumably are the same. Muta et al. described 23 patients from Japan of whom two died, 11 (48%) recovered, and 10 (44%) had long-term memory loss [81]. In another survey of eight Japanese patients with HHV-6 encephalitis, three died and four of the five patients who survived suffered from permanent neurologic disability [87]. Vu et al. reported that four out of five adults developing HHV-6 encephalitis survived without neurologic consequences [86]. In a follow-up of four previously published cases from Karolinska University Hospital [80], all recovered without sequelae (P. Ljungman, unpublished results). Howell et al. reported the development of generalized epilepsy in three children 11–18 months after PALE, together with cognitive abnormalities and delayed intellectual development [100].

Management of HHV-6 infections

In vitro studies suggest that HHV-6 is susceptible to foscarnet, ganciclovir, and cidofovir, and moderately susceptible to acyclovir. No controlled study has been performed, however, with any antiviral drug. Zerr et al. reported decreases of HHV-6 viral loads in CSF or serum during ganciclovir or foscarnet therapy [101]. Ljungman et al. reported reductions in saliva viral load in asymptomatic patients receiving ganciclovir for preemptive therapy of CMV [102]. Hill et al. treated 18 patients with HHV-6 PALE with foscarnet 180mg/kg/day and reported improvements in the symptoms of most patients [85]. Schmidt-Hieber et al. reported a response rate of 63% with either foscarnet or ganciclovir therapy of HHV-6 encephalitis [69]. Vu et al. reported responses in four out of five patients treated with foscarnet [86]. Since the outcome of HHV-6 encephalits is poor, preemptive therapy with foscarnet or ganciclovir was investigated in two uncontrolled studies of patients with high HHV-6 viral loads in plasma [91,103] and in both there were cases of encephalitis that developed concomitantly with the detection of HHV-6 in plasma, suggesting only limited effectiveness of a preemptive strategy. There are no good published data regarding the treatment of other possible clinical manifestations of HHV-6.

Ideally, the primary step in high-risk patients should be to prevent HHV-6 reactivation. Unfortunately, there is no effective drug available that can be given prophylactically since valganciclovir is too toxic in the highest risk groups such as CB recipients and long-term foscarnet prophylaxis is impractical since it can only be given intravenously. Breakthrough cases have also been reported during prophylaxis with foscarnet [104], valaciclovir or acyclovir, and valganciclovir [86]. An interesting drug in development is CMX001, a lipid conjugate of cidofovir, that can be given orally and that has efficacy in vitro against HHV-6 and also against several other viruses of importance in high-risk HCT recipients [105]. Since HHV-6-associated complications occur in the presence of suppressed T-cell function, adoptive transfer of T cells could be a preventive or therapeutic strategy. Recently, it was reported that it is possible in vitro to generate T cells with multivirus specificity including HHV-6 activity [106]. However, there is so far no report regarding the clinical use of such cells and the effect on HHV-6-associated complications.

Human herpesvirus-7

Epidemiology and basic virology

HHV-7 is a T-lymphotropic virus belonging to the *Roseolovirus* genus of the betaherpesviruses together with the closely related viruses HHV-6A and HHV-6B. The HHV-7 genome is 153kb in size and over 90% of its genes are similar in amino acid sequence to those of HHV-6. There are only a small number of genes in HHV-7 where there is not a counterpart in HHV-6 and vice versa.

Similarities to HHV-6B include the following: HHV-7 is commonly found in saliva, which is the probable source of transmission within families [107,108]; infection is almost universal and occurs early in childhood, being gradually acquired over the first 5–6 years of life [109]; primary HHV-7 infection has been much less extensively investigated than HHV-6 infection but the pattern of disease is similar [15,110–113].

Features of HHV-7 that differ from HHV-6 include the following: the viral cellular receptor for the infection of T-lymphocytes is CD4, rather than CD46 [114]; tropism is restricted compared with HHV-6; HHV-7 has rarely been found in brain tissue but is found not only in blood and saliva but also in lungs, skin, and mammary glands [115] as the virus can infect cells that do not express detectable CD4; $CD4^+$ T cells have been proposed as a site of latency [116]; and HHV-7 is not vertically transmitted [117] and there is no evidence of viral chromosomal integration.

The general comments above on the diagnosis of HHV-6 infection apply equally to HHV-7, but in the latter case much less developmental work has been carried out on molecular and antibody tests [36,118].

Clinical manifestations in HCT patients

The impact of HHV-7 after HCT has been examined in longitudinal studies [119–121]. In contrast to HHV-6B, HHV-7 reactivation after HCT is relatively infrequent [122]. So far, it has not been possible to assign a clinical syndrome to HHV-7, with the exception of single case reports describing CNS infection with optic neuritis, encephalitis, or myelitis in stem-cell transplant recipients [122–125].

Human herpesvirus-8

Epidemiology and basic virology

HHV-8 (also known as Kaposi sarcoma-associated herpesvirus) is a gammaherpesvirus similar to Epstein–Barr virus (EBV). It is the cause of Kaposi sarcoma but has also been associated with primary effusion lymphoma and multicentric Castleman disease. The prevalence of HHV-8 infection is very high (>50%) in older children and adults in Africa and parts of the Amazon basin, intermediate (5–20%) in the Mediterranean, Middle Eastern countries, and the Caribbean, and low (<5%) in North America, northern Europe and Asia (<5%). The prevalence of infection is also very high in homosexual men in whom the virus is transmitted sexually. The virus is also transmitted non-sexually, probably via saliva, in areas of high endemicity. HHV-8 is latent in B-lymphocytes and may be transmitted by blood transfusion [126]. As regards diagnosis of HHV-8 infection, the general comments above on the diagnosis of HHV-6 and HHV-7 infection apply equally to HHV-8. Methods exist to detect HHV-8 DNA by quantitative PCR using whole blood, plasma or serum.

Clinical manifestations in HCT patients

Case reports suggest that HHV-8 can cause febrile syndrome and marrow failure in HCT recipients [127,128]. Although transmission from donor to recipient has been documented in the kidney transplant setting, there have been no reports of transmission via stem cells or CB to date [129]. In contrast to HIV-infected patients in whom HHV-8-associated Kaposi sarcoma develops after many years of severe T-cell dysfunction, only a few cases of have been reported in HCT recipients [130–132]. The reason why HHV-8 is not a significant pathogen in HCT recipients may be because very few patients until now coming to transplant are seropositive, and in those who are seropositive T-cell function is reconstituted too fast for the sarcoma to develop.

Conclusion and future development

HHV-6B is an important pathogen in HCT patients primarily through its CNS effects. The risk is highest in patients after CB transplantation and this is a group where it would be logical to implement preventive measures. However, there is no clearly documented effective strategy, for several reasons. We need in the future to differentiate clearly active viral replication from CIHHV-6. We also will need good controlled studies of either a prophylactic or preemptive strategy. There is currently no ideal antiviral available for testing such a strategy, although foscarnet has been best studied. CMX001 is a drug in clinical development that might be a future alternative. Adoptive transfer of HHV-6-specific T cells might also be a possible management strategy. We need more knowledge regarding the importance of HHV-6A as a pathogen in HCT recipients. HHV-7 and HHV-8 are rarely significant pathogens after HCT.

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CHAPTER 93

Human Adenovirus, Polyomavirus, and Parvovirus Infections in Patients Undergoing Hematopoietic Stem Cell Transplantation

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Introduction

The human adenovirus (HAdV), polyomavirus (HPyV), and parvovirus B19 (HPVB19] are small non-enveloped DNA viruses that differ in virion organization, epidemiology, and primary diseases in the human population. In hematopoietic cell transplantation (HCT) patients, however, HAdV, HPyV, and HPVB19 may cause some overlapping syndromes such as pneumonia, hemorrhagic cystitis, hepatitis, and central nervous system (CNS) disease. Specific laboratory tests such as the nucleic acid amplification testing (NAT) are most frequently used to differentiate one from another and from other etiologies. The propensity of these viruses to persist in an infected host or to establish latent infections limits the interpretation of NAT results when trying to distinguish a relevant cause of infectious disease from mere persistence or low-level reactivation. This can be partly addressed by applying increasingly accepted definitions distinguishing infection, replication and disease (Table 93.1). Moreover, these non-enveloped viral agents share a relative resistance to inactivation that has potential implications for healthcare-associated transmission through contaminated surfaces, instruments, and blood products. Only HAdV encodes a viral polymerase that could specifically be targeted by antivirals such as cidofovir, whereas the smaller HPyV and HPVB19 are dependent on host cell polymerase activity for efficient viral replication. Release of progeny virions is thought to occur in bursts of lysing host cells, thereby restricting the main protective effects of antibodies to neutralization and opsonization of extracellular virions and leaving little role for antibody-mediated cytotoxicity. Therefore, control of viral replication largely depends on recovering immune responses, and on virus-specific T-lymphocytes in particular.

Human adenovirus

Human adenovirus (HAdV) species can infect a variety of mucosal sites and cause conjunctivitis, upper respiratory tract infectious disease (RTID), gastroenteritis, and cystitis in immunocompetent individuals; organ-invasive and disseminated HAdV are rare in immunocompetent hosts. By contrast, HAdV replication is associated with life-threatening events in very young, presumably immunologically naïve children and in immunocompromised individuals [1,2]. HCT recipients, in particular children, are at increased risk for severe HAdV manifestations, including lower RTID, hemorrhagic cystitis (HC), nephritis, invasive gastroenteritis, and disseminated HAdV disease. Unfortunately, there are few treatment options, and mortality associated with HAdV disease is very high unless immunologic recovery, particularly of T-lymphocyte function, is observed.

Epidemiology

HAdV transmission occurs primarily through respiratory pathways in airborne droplets and through person-to-person contact. HAdV can also be transmitted through water sources, fomites, and even medical equipment, leading to outbreaks of disease [3]. HAdV shedding can occur persistently and intermittently for several months or years in healthy children, making acquisition by horizontal transmission a major mode of exposure [4].

Immunodeficient patients are at increased risk for complications from primary infection and also from reinfection from exogenous, or possibly endogenous, sources, although the sites and mechanisms of HAdV persistence/latency are not well characterized. It is thought that replication of a persistent strain is the principal mechanism for disease in the adult HCT population, whereas primary infection or reinfection with new HAdV strains to which the host has no prior immunity plays a key role in pediatric HCT recipients [5,6]. Accordingly, pediatric HCT recipients are more likely to develop HAdV DNAemia (20–26% vs. 5–9%) and invasive HAdV disease after transplantation [7]. The risk factors for HAdV DNAemia and associated organ-based disease are T-cell-depleted transplantation, cord blood grafts, graft-versus-host disease (GVHD), immunosuppressive therapy, and lymphopenia (<200–300μL) [8–11]. In HCT patients, the majority of HAdV events occur during the first 100 days after transplantation.

Virologic aspects

HAdV are large (90–100nm), non-enveloped icosohedral, double-stranded DNA viruses of the family Adenoviridae [1]. HAdV are highly infectious and their non-enveloped virologic structure allows them to remain stable in most environments [3]. They are categorized in seven subgroups A–G, and there are over 50 serotypes (Figure 93.1) [12]. After exposure of mucosal surfaces, HAdV infects epithelial cells and can then be detected in lymphoid

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tissues, such as the tonsils, adenoids, and/or the gastrointestinal (GI) tract [13]. The most common site of entry during primary infection uses the cocksackie and adenovirus receptor (CAR), except in HAdV subgroup B, which are thought to use CD46 [14]. The HAdV DNA polymerase is critical to intracellular replication, and can be inhibited by cidofovir. Viral proteins encoded by the viral E3 transcription unit likely help the virus avoid host immunologic responses [15]. The exact mechanism and clinical relevance of persistence versus latency are a subject of current research [15].

HAdV elicits both humoral and cellular immune responses. Neutralizing antibodies targeting hexon, penton base, and fiber proteins of the outer virus capsid convey some degree of protection against specific HAdV serotypes [16]. Since CD4$^+$ and CD8$^+$ T-cell responses are important to viral clearance after transplantation, delayed recovery and function of lymphocytes after HCT are associated with the development of prolonged HAdV replication and disease [11,17].

Table 93.1 Working definitions of infection, replication, and disease caused by human adenovirus, polyomavirus, and parvovirus in HCT patients

Virus infection – Evidence of virus exposure by detecting specific immune responses (epitope-specific antibody or T-cells) or by detecting viral antigens, nucleic acids
Asymptomatic virus replication – Evidence of viral replication by increasing viral loads, virus isolation by culture, or direct antigen detection in patient specimens without clinical symptoms or signs of organ disease
Probable virus disease – Evidence of viral replication plus compatible symptoms and signs of viral syndrome or organ disease, but without histological confirmation available and another etiology
Proven virus disease – Evidence virus replication plus corresponding specific histopathology

Diagnostic methods

There is currently no role for the determination of HAdV antibodies in HCT donors, recipient screening, post-transplant diagnosis, or follow-up, owing to the widespread exposure and serologic cross-reactivity of available assays, and the unreliable antibody responses in patients undergoing HCT. Similarly, the assessment of HAdV-specific cellular immune responses is not routine in clinical care, although increasing responses in peripheral blood mononuclear cell (PBMC) preparations have been associated with protection. Therefore, key diagnostic procedures focus on the demonstration of HAdV replication. Virus isolation by culture by conventional systems has a turnaround time of approximately 1–3 weeks, limiting its clinical use [1]. Culture by shell vial methods improves the sensitivity of detection and reduces the turnaround time to 2–3 days [18]. Direct antigen detection for HAdV is based on either particle agglutination assays, fluorescence-labeled monoclonal antibodies, or enzyme immunoassays combined with capillary chromatography. Direct antigen detection is typically

Subgroups	Serotypes
A	12, 18, 31
B1	3, 7, 16, 21, 50
B2	11, 14, 34, 35
C	1, 2, 5, 6
D	8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49, 51, 53, 54
E	4
F	40, 41
G	52

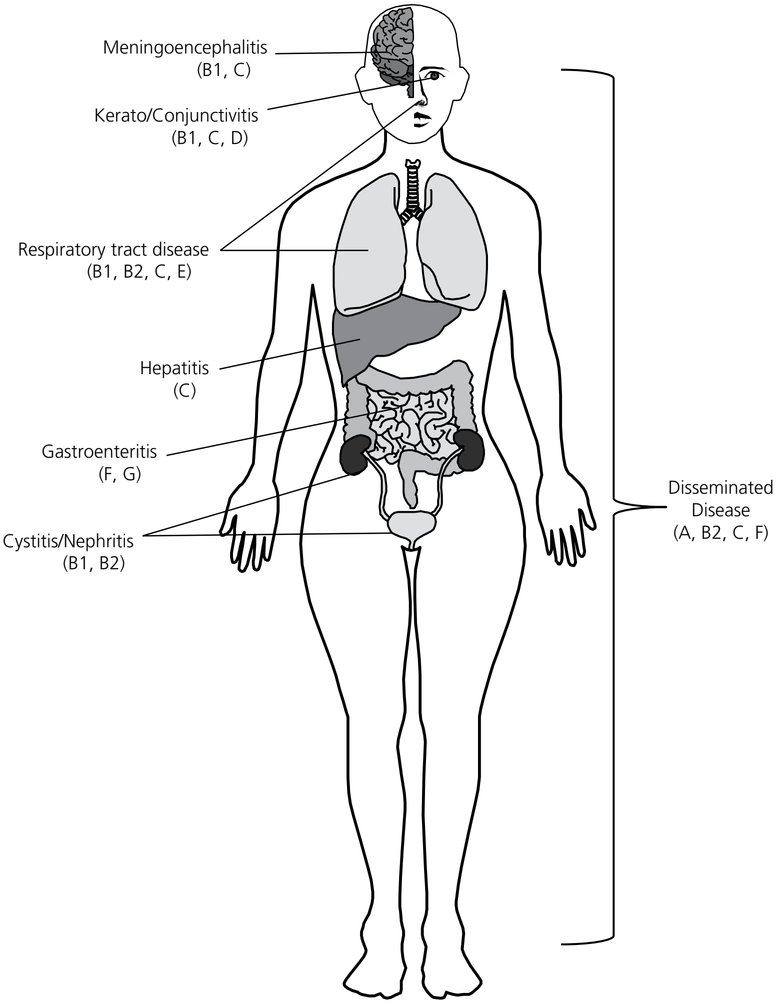
Meningoencephalitis (B1, C)
Kerato/Conjunctivitis (B1, C, D)
Respiratory tract disease (B1, B2, C, E)
Hepatitis (C)
Gastroenteritis (F, G)
Cystitis/Nephritis (B1, B2)
Disseminated Disease (A, B2, C, F)

Figure 93.1 Human adenovirus subtypes, serotypes and reported disease associations. Source: modified from Echavarria 2008 [1], Ison 2006 [2], and Harrach et al. 2011 [12]. Image by Kyoko Kurosawa. Reproduced with permission of Kyoko Kurosawa.

applied to stool suspensions using agglutination assays, and less frequently to nasopharyngeal aspirates, nasopharyngeal swabs suspended in virus transport medium, bronchoalveolar lavage, tracheal aspirates typically using other tests such as capillary immunoassays. Direct antigen detection usually requires only 15–30 minutes, thereby reducing the turnaround time to less than 2 hours, but is less sensitive than NAT, e.g. such as polymerase chain reaction (PCR) [18]. Therefore, direct antigen detection is often used as initial point-of-care testing of specimens in pediatric and severely immunocompromised patients who are more apt to have high viral loads.

In most centers, NAT has become the main method for HAdV detection in HCT patients as it can be performed on all routine specimens (blood, respiratory secretions, urine, stool, CSF) and also tissue samples [19,20,21]. NAT provides both high sensitivity and specificity, and can be performed in multiplex assays and (semi-) quantitation, which has been explored for predicting outcome and monitoring treatment. The detection of HAdV in the blood is usually a marker of severe disease and risk of dissemination, although asymptomatic low-level HAdV viremia has been described, particularly in children [22]. Detection of HAdV DNAemia occurs in approximately 6–28% of pediatric and 0–6% of adult patients undergoing HCT [23]; some centers report rates that are much higher, particularly in pediatric patients [24]. High serum HAdV viral loads of >10,000 copies/mL in most studies [25,26] have been linked to organ disease, dissemination, and subsequent mortality. NAT can also be used to detect specific HAdV genotypes, which can be helpful in evaluating nosocomial transmission or point source outbreaks [27]. Typing of HAdV has indicated that several different strains can replicate sequentially or simultaneously after HCT [27,28]

Clinical manifestations

Serotypes from all seven subgroups can cause disease in both immunocompetent and immunocompromised subjects (Figure 95.1) [1]. Definitions of HAdV replication and disease have varied in prior publications, but the recently updated European guidelines propose the combination of clinical data, virology results, and histopathology [20], similar to the definitions proposed for BKPyV [29] JCPyV [30] and other HPyVs in transplantation [31] (Table 95.1).

Respiratory HAdV disease

In the first 100 days after transplantation, HAdV makes up approximately 5–6% of all respiratory viral infections in HCT patients [32]. Typical symptoms are sore throat, fever, and cough, but not all patients with HAdV upper respiratory infections have symptoms [22]. Upwards of 25% will progress to lower tract disease (Figure 93.2) [33,34], followed by respiratory failure and ultimately death in 40–70% of patients (7,32). The most severe cases are characterized by HAdV DNAemia and dissemination.

Gastrointestinal HAdV disease

HAdV types can lead to acute gastroenteritis during primary infection, and can reemerge after HCT following re-exposure or from GI sites of persistence [13]. Patients with GI disease typically present with diarrhea, which is difficult to distinguish from other, occasionally simultaneously occurring, infectious and non-infectious causes, most notably GVHD; more severe GI manifestations such as hemorrhagic colitis can also occur [35]. Because patients can shed HAdV asymptomatically in stools, mere qualitative detection of HAdV may not be indicative of disease. However, HAdV loads in stools increasing to very high levels of >6 $\log_{10}$ copies/mL has been used for screening and identifying pediatric HCT patients at risk for HAdV viremia and disseminated disease [36].

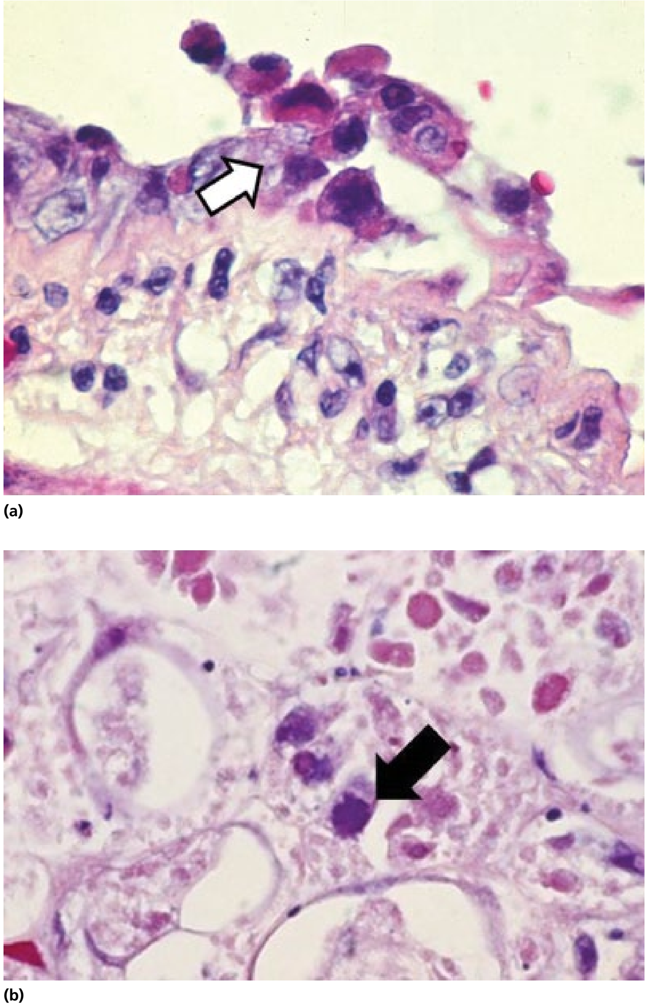

Figure 93.2 Adenovirus findings on histopathology. (a) Hematoxylin and eosin (H&E)-stained tracheal epithelial cells. This image demonstrates a cluster of dark-staining cells infected by adenovirus (white arrow). There is a progression of nuclear changes as the virus replicates and finally a dark-staining "smudge cell" is formed. (b) H&E-stained mucosal biopsy, demonstrating a smudge cell (black arrow) where basophilic inclusions have encompassed the entire nucleus. Source: reproduced with permission of Robert Hackman MD.

Hepatobiliary HAdV disease

Liver involvement after transplantation is less commonly diagnosed than GI HAdV disease. Liver enzyme elevations have been reported and are at times difficult to distinguish from other etiologies, including drug toxicity and GVHD. Fulminant hepatic failure from HAdV either in combination with viral dissemination or as the primary manifestation of the disease has been described in HCT patients [37,38]. In these cases, liver biopsies or post-mortem tissues demonstrate hepatic necrosis, cholestasis, and hepatocytes with intranuclear basophilic inclusions ("smudge cells") (Figure 93.2) [38]. Outcomes in patients with severe liver disease are very poor, despite aggressive treatment attempts [37–39].

Renourinary HAdV disease

HAdV shedding in the urine has been reported in 1–5% of HCT patients. HAdV is more readily associated with urologic symptoms and signs than JCPyV and BKPyV, suggesting a higher intrinsic pathogenic potential (Table 93.2). Most HCT patients present with self-limited dysuria and mild HC [40]. HAdV-associated HC is

Table 93.2 Diagnosis of virus-associated hemorrhagic cystitis

1. Clinical signs of cystitis	
Dysuria, urge, frequency, lower abdominal discomfort	
2. Hematuria grade II and higher	
Grade I	Microscopic hematuria (>100 erythrocytes per high-power field)
Grade II	Macrohematuria
Grade III	Macrohematuria with clots
Grade IV	Macrohematuria with urinary obstruction and postrenal failure
3. Viral replication in urine	
For BKPyV	Urine viral load >$7\log_{10}$geq/mL; plasma viral loads increasing above $4\log_{10}$geq/mL
For HAdV	Urine viral loads not defined (>$5\log_{10}$geq/mL?)
Plasma viral loads not defined (>$3\log_{10}$geq/mL?)	
4. Exclude major contribution of other etiology	
Infectious	Bacteria, other virus including cytomegalovirus, fungus, parasite
Urotoxic	Early onset <2 weeks of conditioning, cyclophosphamide, busulfan, total body irradiation, insufficient uroprotection with forced diuresis and mesna
Hematologic	Bleeding disorder, low platelets, graft-versus-host disease
Malignant	Urothelial carcinoma, other primary or metastasizing neoplasia
Mechanical	Catheter, ureteric stenosis, stenting or other procedure

characterized by macroscopic hematuria grade II–IV, clinical signs of cystitis with urgency, frequency, abdominal discomfort, immobilizing pain, and/or urinary obstruction. Progression to HAdV-associated tubulointerstitial nephritis and disseminated HAdV disease can occur [41], and HAdV-associated nephritis is primarily seen in conjunction with HC. HAdV nephritis often leads to progressive renal failure and is a strong predictor of HAdV-associated death [3,42]. Although less frequent than BKPyV-associated HC and nephropathy, HAdV is more likely to cause renourinary disease in patients with more profound immunosuppression, particularly those who have undergone T-cell-depleted transplants [43]; rates are much lower in autologous transplant recipients [44].

Neurologic HAdV disease

Neurologic complications, particularly encephalitis, are extremely rare HAdV complications in HCT [45,46]; pediatric patients make up the majority of cases. A large study of 11 German transplant centers detected HAdV in 3% of all encephalitis cases [47]. The affected patients can present with fever, headache, seizures, or mental status changes. Interestingly, not all patients who present with CNS disease have evidence of DNAemia and dissemination [46].

Disseminated HAdV disease

Systemic/disseminated HAdV replication is defined by detection of HAdV replication in ≥2 end organ sites or detection of HAdV in peripheral blood. Severe local disease may progress to disseminated disease [2,11]. Pediatric patients are at the highest risk for dissemination, and those who undergo cord blood transplantation or need immunosuppression for high-grade GVHD appear to dominate adult cases [8,9]. Mortality associated with dissemination is high at 60–80% [7,39].

Treatment

Patients with probable, proven, and disseminated HAdV disease in HCT should be considered for treatment. In line with the key risk factors of HAdV disease, improving HAdV-specific immune function is considered a cornerstone of treatment. Ideally, this involves improvement of immunologic function, but is often difficult or impossible with concurrently active GVHD. Moreover, recovery of HAdV-specific immunity may be impaired or prolonged as a result of the underlying disease, the conditioning regimen intensity, GVHD prophylaxis, corticosteroids, the administration of long-lasting lymphocyte-depleting agents such as alemtuzumab, or the use of a cord blood graft. Therefore, antiviral drugs has been widely used despite considerable limitations in efficacy and appropriate clinical studies.

Antiviral drugs

There are no approved antiviral drugs for the treatment of HAdV replication and disease. Ribavirin and cidofovir have been most frequently utilized for treatment, although neither of these agents has been evaluated in a randomized clinical trial for HAdV treatment. Cidofovir may be a more effective first-line therapy [4,9,48,49], but primarily renal, less so bone marrow toxicity, limit its routine use (Table 93.3). For ribavirin, some studies have reported no benefit [37], whereas others claim significant clinical improvements [50]. Data reported by Morfin et al. suggest that the susceptibilities of HAdV may differ for different subtypes, as ribavirin was only effective against non-C adenovirus infections, whereas cidofovir was effective in vitro against all subtypes [51]. A novel lipid conjugate of cidofovir brincidofovir (CMX001) is currently in randomized clinical trials, and early reports are encouraging [42], particularly since the drug has a less toxic profile than its parent drug. Ganciclovir and other nucleoside analogs have also been reported to have potential activity against HAdV (e.g. due to high concentrations in urine), but are not used as either first- or second-line therapy at most centers [5]. Current data purporting the major benefits of antiviral therapy must be interpreted with caution. Outcome data are confounded in the context of immune reconstitution, particularly since survival is affected by the kinetics of lymphoctye recovery [17,52].

Immunotherapy

In general, intravenous immunoglobulin (IVIg) can be considered as adjunctive therapy in patients with HAdV infections; however, the large number of serotypes may limit any major benefits. The use of adoptive immunotherapy is currently undergoing active trials, and initial data using expanded HAdV/viral-specific T cells have demonstrated excellent response rates [53,54]. Recent data from Leen et al. demonstrated that 14/18 (78%) of patients with severe HAdV disease had a complete or partial response to banked multiviral-specific T cells, showing the feasibility of such approaches [55]. However, costs and time requirements have limited their utility to only a few specialized centers. As newer rapid selection methods and "off-the-shelf" products are developed, these approaches may become more readily available for patients with severe disease [55,56].

Prevention

Screening and preemptive treatment

The limited available data suggest that weekly NAT screening and preemptive cidofovir therapy may reduce the development of HAdV disease in high-risk patients [49,57]. Some centers currently utilize this method for high-risk pediatric patients, but the toxicity of cidofovir therapy has led many to avoid this approach. A study evaluating preemptive therapy with CMX001 (brincidofovir) in children with asymptomatic DNAemia may provide future options in high-risk patients. However, studies are needed to determine the optimal screening procedure with respect to specimen (nasopharyngeal, oral, stool, blood), number of sites, and frequency, since these have not been defined in controlled trials. Routine screening is not warranted in patients at low or moderate risk for HAdV disease [58]. The role of pre-transplant screening is not established, although it may possibly be effective in predicting post-transplant HAdV complications [59].

Infection control

HAdV transmission can occur within inpatient and outpatient environments, owing to the hardy nature of its non-enveloped viral structure that is resistant to many cleaning agents. Small outbreaks have been reported in transplant and hematology units [27].

Table 93.3 Treatment options for probable and proven disease by human adenovirus, polyomavirus, and parvovirus B19 in HCT patients

Virus	First line	Alternative(s)	Comments
HAdV	Reduce immunosuppression if possible AND Cidofovir IV 5 mg/kg once weekly for 2–3 weeks, then every other week	1. Cidofovir IV 1 mg/kg three times weekly 2. Multiviral or HAdV-specific T cells (adoptive therapy), preferably within clinical trials	• Cidofovir has significant renal and marrow toxicity, so prophylactic measures need to be taken, such as hyperhydration, oral probenicid, and discontinuing other nephrotoxic co-medications, if possible • CMX001 may be an alternative after approval by health authorities
BKPyV	Hemorrhagic cystitis: Supportive care ↓ Immunosuppression if possible BKPyV-associated nephropathy: ↓ Immunosuppression if possible	Hemorrhagic cystitis: Cidofovir 0.25–5.0 mg/kg IV once weekly with or without probenicid IVIg 0.4 g/kg for 5 days BKPyV-associated nephropathy: Cidofovir 0.5–5 mg/kg IV once weekly	• Supportive care may include analgesics, spasmolytics, hydration, bladder irrigation, urologic treatment including local hemostasis, clot evacuation, ureteric stenting, nephrostoma. • Decreases in immunosuppression should be considered carefully in patients at risk for GVHD • Other not validated adjunct therapy includes levofloxacin, leflunomide, and possibly MTOR inhibitors • CMX001 may be an alternative after approval by health authorities
JCPyV	Progressive multifocal leukoencephalopathy: ↓ Immunosuppression if possible Rarely hemorrhagic cystitis: see BKPyV	None	• Therapy PML is limited, and no current antiviral options have shown any benefit, including cidofovir, IV immunoglobulins, serotonin uptake inhibitors, and/or mefloquine • CMX001 may be an alternative after approval by health authorities
MCPyV	Merkel cell carcinoma: Surgical resection	Chemotherapy	• No current antivirals are known to be effective against MCPyV carcinoma as a non-replicative pathology
HPVB19	Pure red cell aplasia: Intravenous immunoglobulins 0.4 mg/kg over a 5–10-day course OR Intravenous immunoglobulins 1–2 g/kg/day for 2–5 days	↓ Immunosuppression if possible	• Most IVIg formulations have sufficient antibodies to be effective against pure red cell aplasia • IVIg should be considered in other invasive illnesses associated with human parvovirus B19

HAdV, human adenovirus; PyV, polyomavirus; HPVB19, human parvovirus B19; CBT, cord transplant; GVHD, graft-versus-host disease; IV, intravenous; IVIg, intravenous immunoglobulin; TCD, T-cell-depleted transplantation;

Current recommendations require all patients with respiratory tract infection to be placed under contact and droplet precautions. HCT patients incontinent of stool and children wearing diapers should also be placed under contact precautions for the duration of their illness [60]. Inactivation of HAdV virions by routine cleaning procedures in HCT is largely undefined. In vitro studies comparing different surface-active substances suggest that povidone-iodine derivatives were more active, whereas low alcohol- or isopropanol-based disinfection methods were largely ineffective [61,62].

Human polyomaviruses

Human polyomavirus (HPyV) infections currently encompass more than 12 different species, all of which belong to the genus *Polyomavirus* within the family *Polyomaviridae* (Table 93.4) [63]. Serologic studies indicate that from 30% to more than 90% of the general population have been exposed to the different HPyVs, frequently during childhood [63]. It is thought that the lack of specific symptoms or signs linked to primary infection with any of the HPyV reflects either a subclinical course or an inconspicuous "flu-like" presentation shared with other viral infections [64]. The major mode of HPyV transmission is currently unresolved, but in addition to direct person-to-person contact, exposure to infectious body secretions, contaminated surfaces, sewage, or food items have been postulated as mechanisms for primary infection [65,66]. Approximately 5–40% of healthy blood donors asymptomatically shed BKPyV and JCPyV into the urine [67,68]. Similarly, other HPyVs such as Karolinska Institute PyV (KIPyV) and Washington University PyV (WUPyV) [69], Merkel cell PyV (MCPyV) [70], *Trichodysplasia spinulosa* PyV (TSPyV) [71], and several of the newer HPyVs [72] can be detected in asymptomatic individuals. The role of transfusion and organ transplantation is unresolved, although donor kidneys as the major site of BKPyV persistence are strongly implicated in PyV-associated nephropathy after kidney transplantation [73].

Epidemiology

HPyVs appear to be well adapted to the healthy human host seem to require significant cofactors for developing disease [64]. Indeed, where identified, HPyV-associated disease occurred almost exclusively in individuals with impaired immune functions, including those undergoing HCT (Table 93.4). In immunocompromised patients, the detection rates for most HPyVs seem to be higher than in immunocompetent individuals. Presumably, asymptomatic viral replication is a consequence of less stringent immune control, and is associated with an increased risk of progression to disease for some HPyVs. For BKPyV, the frequency of urinary shedding increases from <10% to 60–80% in patients undergoing HCT, typically occurring after Herpes simplex virus and before cytomegalovirus (CMV) reactivation [74]. In parallel, urine BKPyV loads increase substantially from <3 $\log_{10}$ copies (cp)/mL to >7 $\log_{10}$ cp/mL [75–78]. Interestingly, urinary JCPyV shedding is fairly high in immunocompetent individuals [67] and does not increase dramatically in most immunodeficient patients, suggesting significant differences between these two HPyVs [30,65,74]. KIPyV and WUPyV have been detected in 1–11% of respiratory secretions, and are generally higher in children and in HCT patients compared with respective controls [79–81]. KIPyV was significantly more frequent among HCT recipients (17.8% vs. 5.1%, $p = 0.01$) [82]. Overall, the epidemiologic data for the newer HPyVs in HCT are still incomplete.

Virologic aspects

HPyVs are small, non-enveloped virions of 45nm diameter that show remarkable resistance to inactivation. Their circular double-stranded DNA genome of only ~5100bp does not encode a viral DNA polymerase, nucleoside kinase, or protease, and hence lacks classic antiviral targets. HPyV genomes share a high degree of homology ranging from 50 to 80% at the nucleic acid level [72]. Such homology, and also genotype and strain variation, need to be taken into account when developing, interpreting, and revising diagnostic NAT and in situ hybridization assays for specific HPyVs [83–85].

Table 93.4 Human polyomavirus and disease

Polyomavirus [ref.]	**Abbreviation**	**Seroprevalence (%)**	**Persistent infection**	**Disease**		**Highest risk**
				Immunocompetent	**Immunocompromised**	
Patient 1 [68]	BKPyV	80–95	Kidney	Cystitis? Encephalititis? Prostate cancer? Ureteric stenosis	PyV-associated hemorrhagic cystitis PyV-associated nephropathy Ureteric stenosis Pneumonitis	Allogeneic HCT Kidney transplants
Patient 2 [30]	JCPyV	35–70	Kidney Central nervous system? Monocytes?	Tonsillitis? Encephalitis? Colon cancer?	Progressive multifocal encephalopathy PyV-associated nephropathy, cystitis	HIV-AIDS Heart and lung transplants Natalizumab-treated patients Allogeneic HCT Kidney transplants
Karolinska Institute [69]	KIPyV	60–80	Unknown	Tonsillitis? Bronchitis?	Pneumonia?	Allogeneic HCT
Washington University [69]	WUPyV	50–70	Unknown	Bronchitis?	Pneumonia?	Allogeneic HCT
Merkel cell carcinoma [68]	MCPyV	50–90	Skin, (monocytes?)	Unknown	Merkel cell carcinoma	Heart and lung transplants
Trichodysplasia spinulosa [71]	TSPyV	70	Skin	Unknown	*Trichodysplasia spinulosa*	Heart and lung transplants Leukemia
Human polyomavirus 6 [72]	HPyV6	37–98	Other? Skin?	Unknown	unknown	
Human polyomavirus 7 [72]	HPyV7	35–85	Skin?	Unknown	puritic keratinopathy plaques	Lung transplant patients
Human polyomavirus 9 [72]	HPyV9	20–69	Gastrointestinal tract? Blood? Kidney?	Unknown	unknown	kidney transplant patients
Human polyomavirus 10 [72]	HPyV10	Unknown	Gastrointestinal tract?	Diarrhea?		
(Malawi polyomavirus, MX polyomavirus) [72]	(MWPyV, MXPyV)				Unknown	
Saint Louis Polyomavirus (STLPyV)	HPyV11	Unknown	Gastrointestinal tract?	Diarrhea?	Unknown	
Human polyomavirus 12 [72]	HPyV12	12–33	Gastrointestinal tract?	Diarrhea?	Unknown	
Human Polyomavirus 13 [72a]	(New Jersey (HPyV13)	Unknown	Skin? Gastrointestinal tract?	Unknown	Proliferative skin disease, vasculopathy	Pancreas transplant patient

HPyV, human polyomavirus; PyV, polyomavirus.

HPyV-specific antibody responses are mainly directed against the VP1 protein forming the outer shell of virions and include binding and neutralizing antibody activities [86,87]. Increases in HPyV-specific antibody levels have been seen in individuals with impaired T-cell responses, increasing viral loads, and HPyV disease, suggesting that they are a marker of recent exposure and presumably derive from memory B cells [86–88]. Cellular immune responses against HPyVs have only been explored for BK-, JC-, and MCPyV [89–93]. Compared with T-cell responses directed against CMV or Epstein–Barr virus, BKPyV- and JCPyV-specific responses are at least 10–50-fold lower in blood, thereby limiting their routine clinical predictive use without prior expansion [92,93].

Diagnostic methods

Diagnosis of HPyV infection, replication, and disease is similar to that for other latent viruses (Table 93.1). HPyV replication is commonly diagnosed by quantitative NAT, whereas direct antigen detection (DAD) and virus isolation by cell culture (VIC) are not routinely used in clinical practice. HPyV NAT can be performed on urine, plasma/serum, blood, stool, CSF, and tissue samples. Qualitative NAT is not considered informative and is discouraged. (Semi-)quantitative NAT is the method of choice and HPyV loads in urine, blood, plasma or serum, and CSF have been proposed as a marker of risk, organ involvement, and outcome.

Proven HPyV disease is commonly diagnosed by immunohistochemistry for LTag as the key diagnostic technique. Staining for VP1 has also been used, but is typically negative in non-replicative HPyV pathology such as Merkel cell carcinoma. In situ hybridization is used in some centers, but determination of assay specificity is needed. Similar to solid organ transplantation [31], testing for HPyV-specific humoral or cellular immune responses currently has curently no role in the clinical evaluation and management of HCT patients.

Clinical manifestations

Proven HPyV disease has been defined for few species [64], namely BKPyV (nephropathy, hemorrhagic cystitis, ureteric stenosis) [68], JCPyV (progressive multifocal leukoencephalopathy, nephropathy) [30], MCPyV (Merkel cell carcinoma) [70], and TsPyV (*Trichodysplasia spinulosa*) [71]. For two, KIPyV and WUPyV, an association with respiratory tract infectious disease has been proposed, but histopathologic proof is only emerging [69].

Renourinary HPyV disease

PyV-associated hemorrhagic cystitis (PyVHC) occurs in 5–15% of allogeneic HCT patients, typically after engraftment, and is linked to high urine BKPyV loads [94]. Other HPyV have no role in PyVHC with the rare exception of JCPyV. As most adult and pediatric PyVHC patients are BKPyV seropositive prior to HCT [94,95], reactivation has been postulated as the major source BKPyV replication. However, some pediatric cases of PyVHC after HCT may result from (re)infection of children with low or undetectable BKPyV-IgG antibodies [78].

The pathogenesis of PyVHC has been suggested to result from a sequence of cooperating events [96,97] (Figure 93.3). First, subclinical injury of the bladder mucosa occurs as a result of urotoxic conditioning protocols used prior to HCT. Then, during the aplastic window, uncontrolled BKPyV replication in urothelial cells may cause denudation of the partly damaged bladder mucosa, and result in urine leakage, and hemorrhagic inflammation, which is exacerbated following engraftment of the allogeneic stem cells [64,97]. The inflammatory response may also be driven by innate immune signals to cytopathic denudation and failure [64], with or without contribution of overshooting responses of BKPyV-specific T cells [93].

The clinical diagnosis of virus-associated HC requires the triad of (1) clinical signs of cystitis, (2) macrohematuria (≥grade II), and (3) significant urine viral loads (Table 93.2). For BKPyV, urine loads are typically >7 $\log_{10}$ cp/mL, but may be lower for more pathogenic viruses such as HAdV [64,98] (see above). In addition, other etiologies should be excluded (Table 93.2). Detection of BKPyV in blood has been associated with a higher specificity of PyVHC after allogeneic HCT, especially when serum BKPyV loads exceed 4 $\log_{10}$ cp/mL [78,98–100].

High urine BKPyV loads, alone, even if together with macrohematuria, are not sufficient for a case definition of HC. Cystitis symptoms may range from low-grade urgency to completely immobilizing pain requiring hospitalization, intensive nursing efforts, and advanced pain management, with significant healthcare costs [101]. Low platelet counts and/or human herpes virus-6 viremia [102] have also been linked to PyVHC [100]. Imaging by ultrasound at the bedside is the most valuable diagnostic measure showing thickened bladder walls and occasionally ureteral involvement and hydronephrossis, whereas magnetic resonance imaging (MRI), or contrast-enhancing computed tomography (caution with renal toxicity of contrast) is less frequenty used. Biopsies are rarely taken because of the risk of excessive bleeding.

The risk factors of BKPyV-associated HC vary in different studies and eras of HCT, but most consistently, myeloablative conditioning, unrelated or mismatched donors, and the use of cyclophosphamide, busulfan, total body irradiation, and lymphocyte depletion have been reported [41,98,100,103–105]. More recently, cord blood HCT has emerged as a risk factor, suggesting that impaired T-cell immunity plays an important role [93,98]. GVHD is a risk factor due to immunosuppressive treatment, and infrequently its involvement of the bladder can be an alternative or concurrent diagnosis [43,106]. In addition to anemia and bacterial infection, BKPyV-associated HC can be complicated by obstructive renal failure with hydronephrosis and serum creatinine increases due to obstructive blot clots. In such cases, ultrasound evaluation may help to evaluate the patients for ureteral involvement and stenosis.

PyV-associated nephropathy (PyVAN) is rarely diagnosed after allogeneic HCT [92,107–111] compared with rates of 8% (95% confidence interval 1–15%) in kidney transplant patients [112]. Unlike HAdV-associated interstitial nephritis after HCT, HC may not be a precursor diagnosis to PyVAN [110,111]. The diagnosis of PyVAN should be suspected in all HCT patients with late-onset progressive renal failure [111]. Potential risk factors are insufficient T-cell responses or impaired recovery as the result of intense immunosuppression for GVHD, exposure to lymphocyte-depleting antibody therapies, and possibly cord blood HCT [109,110]. In pediatric patients, primary BKPyV exposure may be the cause of PyVAN after autologous HCT [108].

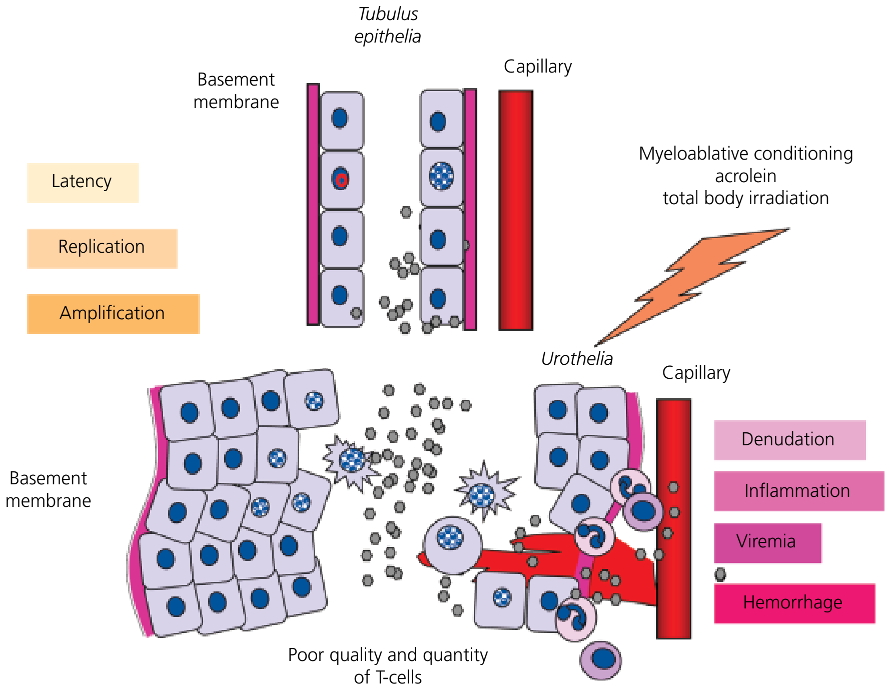

Figure 93.3 Polyomavirus-associated hemorrhagic cystitis after HCT. Source: adapted from Hirsch 2010 [64].

The differential diagnosis of PyVAN includes a variety of partly additive nephrotoxic insults from conditioning, immunosuppressive, antibiotic, antifungal, and antiviral drugs used during HCT. Calcineurin inhibitor toxicity is an important differential diagnosis of elevated creatinine and improvement of renal function following immunosuppression reduction has been interpreted as confirmative evidence. However, undiagnosed PyVAN is also likely to improve with reduced immunosuppression, suggesting that PyVAN frequency may be underestimated in HCT. Proven PyVAN requires renal biopsies demonstrating cytopathic changes of renal tubular epithelial cells with intranuclear inclusions and corresponding immunohistochemistry for large T-antigen [111]. When examined in patients with PyVAN, BKPyV in the blood is universally positive [109–111]. Therefore, in the absence of HC and other reasons for renal failure, plasma BKPyV loads may be a useful first indicator of PyVAN in allogeneic HCT patients. However, universal screening for BKPyV replication has not been evaluated in prospective trials and is currently not indicated in all HCT patients.

Ureteric stenosis after HCT is a rare complication after HCT and may be related to BKPyV-associated HC. This complication often presents as unilateral or bilateral hydronephrosis on ultrasound. In contrast to blood clot-mediated obstructive HC, proliferative changes of the ureteric ostium have been described [113,114], possibly subsequent to BKPyV infection as seen after kidney transplantation [115]. BKPyV viruria or viremia is not useful in this diagnosis, but histopathology of the resected tissues can be helpful in ruling out other diagnoses such as malignancies.

Neurologic HPyV disease

Progressive multifocal leukoencephalopathy (PML) is a frequently fatal demyelinating disease caused by JCPyV in immunodeficient patients [30]. Uncontrolled cytopathic JCPyV replication in myelin-producing oligodendrocytes impairs neuronal function and causes (multi)focal neurologic deficits. Recent studies suggest that adjacent astrocytes and gray matter neurons, and specifically granule cell neurons of the cerebellum, might also be affected [30]. PML was first recognized as a distinct pathologic entity in patients with hematologic malignancies such chronic lymphatic leukemia or diverse types of lymphomas long before the era of HCT, suggesting an important role of profound and chronic immune dysfunction affecting both T- and B-cell subsets [116]. PML is seen in other immunocompromised populations, such as HIV-AIDS patients [117] and solid organ transplant recipients [118], and has been reported as a complication of natalizumab, a monoclonal antibody used in multiple sclerosis, and rituximab [30]. The rates of PML in allogeneic HCT patients are not well defined and may be lower than the rate of 0.1% estimated in solid organ transplant patients [118]. When reported, PML occurred in the first year after HCT, ranging from 1 to 20 months, and was frequently fatal [119,120].

PML should be suspected in immunocompromised patients showing progressive focal neurologic deficits and compatible radiologic findings on MRI (possible PML). Neurologic findings associated with PML include motor and sensory deficits, ataxia, and alterations of memory, speech, and mental status. MRI demonstrates enhanced signals in fluid-attenuated inversion recovery and T2-weighted images, but hypointense signals in T1-weighted imaging [121]. Detection of JCPyV DNA by NAT of CSF is required for laboratory- or virologically confirmed PML (probable disease). The sensitivity of clinical JCPyV-NAT assays is critical and should reach <50 cp/mL [83]. In cases with undetectable JCPyV DNA in CSF, or other suspected etiologies, stereotactic brain biopsy is indicated, which permits the histopathologic diagnosis of proven disease showing typical cytopathic changes of oligodendrocytes and positive staining for LTag [30]. Unlike BKPyV-associated HC and nephropathy, JCPyV DNA may not be detectable in blood in patients with PML and cannot reliably be used for screening and monitoring [30]. The differential diagnosis includes calcineurin inhibitor-associated leukencephalopathy and viral infections of the CNS such as HPVB19, EBV, CMV, and HHV6. Other HPyVs are usually not detectable in CSF [122], but BKPyV has been implicated in a similar clinical presentation in non-HCT patients [68,123].

Other HPyV-associated diseases

Respiratory tract infectious disease (RTID) in HCT patients has been investigated for KIPyV and WUPyV as these viruses were first detected in respiratory specimens [69]. In a large prospective study of 222 HCT patients, the cumulative detection rate of KIPyV and WUPyV was estimated as 26 and 8%, respectively, in the first year after transplantation [79]. KIPyV- and WUPyV-positive patients were typically younger (age <20 years) and were more likely to have had sputum production and wheezing in the preceding 1–4 weeks. These viruses were not associated with GVHD, lymphopenia, hospitalization or death. However, co-infection of other respiratory and latent viral infections was common [124]. Semiquantitative KIPyV and WUPyV loads have been thought to help differentiate RTID from GI sites of replication [124], and higher KIPyV loads have been reported in bronchoalveolar lavage (BAL) of symptomatic children with leukemia and in those undergoing HCT [80]. The link between KIPyV and WUPyV severe RTID is largely missing, and to date histologic proof of invasive disease is only emerging in case reports [125,126]. In addition, BKPyV-associated pneumonitis has been described in single HCT case reports [127,128].

Gastrointestinal disease including colitis has not been proven by histopathology, but detection of BKPyV, JCPyV, KIPyV, WUPyV, HPyV9, HPyV10, and HPyV12 in fecal specimens has been suggested to indicate replication in this compartment [82,129,130]. However, contamination of fecal specimens from urine in patients with hemorrhagic cystitis [129] or from swallowed sputum confound the interpretation of HPyV detected from this site [124].

Skin diseases associated with HPyV include proliferative changes and non-melanoma skin cancer [64,131]. These entities are increased in immunodeficient patients, including in solid organ transplant and HCT recipients, compared with the general population [64]. Merkel cell carcinoma is a cutaneous malignancy that shows some histologic features of Merkel cells transmitting the sensation of touch [70]. In recent decades, Merkel cell carcinoma has increased from 0.1 to 0.9% in registry studies, and the risk factors for Merkel cell carcinoma are older age, exposure to sunlight, and immunodeficiency such as chronic lymphatic leukemia, HIV-AIDS, and solid organ transplantation [132]. There are few cases linked to hematologic diseases and HCT [133–135]. Clinically, patients develop exophytic growths on their skin (Figure 93.4), but aggressive spread to lymph nodes, visceral organs, bone, and marrow is linked to a high fatality rate of more than 50% within 1–2 years. MCPyV has been detected in more than 80% of Merkel cell carcinomas, with critical alterations of the viral genomes and small T-antigen as oncogenic driver [70]. Since MCPyV is widely detected on skin of asymptomatic healthy individuals, the pathogenesis presumably involves additional oncogenic events of virus, spreading, and host cell that lead to clinical disease in immunodeficient individuals. Serologic studies indicate higher antibody titers in patients with Merkel cell carcinoma compared with healthy individuals, suggesting that at some point, (re-)exposure to viral antigen must have occurred prior to the disease [87].

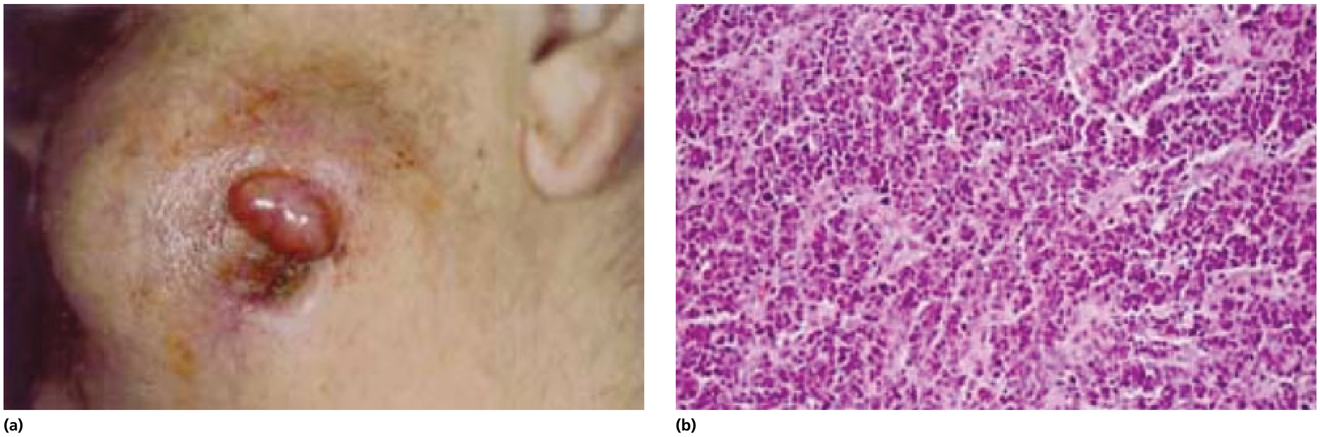

Figure 93.4 Merkel cell carcinoma: clinical and pathologic appearance. Merkel cell carcinoma in an HCT patient on left cheek (a) showing nests and cords of small cells with small cytoplasm in trabecular pattern on H&E staining (b). Source: Gilaberte et al. 2000 [134]. Reproduced with permission of Karger Publishers. (For a color version of this figure, please see the color plate section.)

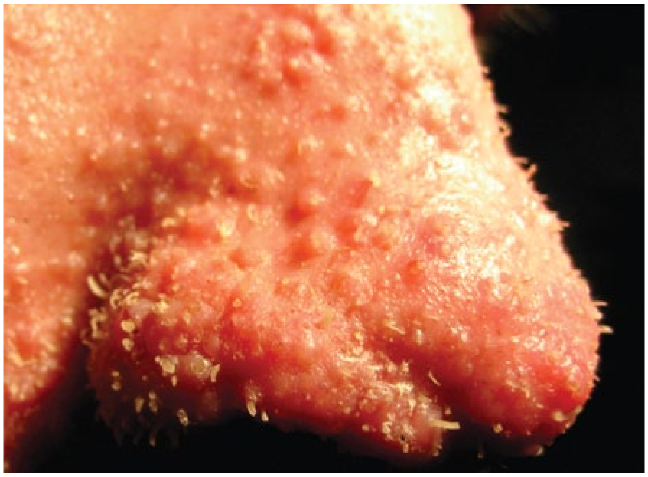

Figure 93.5 Trichodysplasia: clinical appearance. Close-up image of spiculae on the nose of an immunosuppressed patient. Source: van der Meijden et al. 2010 [137]. Reproduced with permission of PLoS in accordance with the Creative Commons Attribution Licence. (For a color version of this figure, please see the color plate section.)

Trichodysplasia spinulosa is proliferative skin disease of immunocompromised patients, which has led to the identification and cloning of TSPyV [136,137]. Other terms used have been *pilomatrix dysplasia* of immune suppression, or cyclosporine-induced folliculodystrophy. The clinical diagnosis is made by characteristic skin changes occurring in an immunocompromised patients. Typically, follicular papules are seen with outright spines in the face centering around the nose and the ears, and less frequently on the rest of the body (Figure 93.5). Non-scaring alopecia of the eyebrows may develop, replacing the hairs by hyperkeratotic protrusions. The affected skin areas thicken with the duration of the disease.

Histologically, the hair follicle is enlarged through proliferative changes replacing normal corneocytes. The changes are presumably caused by TSPyV causing proliferative alterations of the inner root sheath cells, in which immunohistochemistry for VP1 capsid is positive and PyV particles can be seen by electron microscopy [71,136,137]. In a thorough review, 25 trichodysplasia spinulosa cases were summarized and included eight cases with hematologic malignancies [71]. Trichodysplasia spinulosa is likely underdiagnosed in HST patients, especially those with chronic GVHD.

Treatment

The treatment of HPyV replication and disease represents a formidable challenge given the lack of specific antivirals (Table 93.3). To date, there have been no randomized controlled trials demonstrating clinical efficacy of any antiviral in the treatment of HPyV diseases. Although various case series suggest that intravenous cidofovir may be an option for therapy, the role of spontaneous HPyV-specific immune recovery cannot be excluded as the true etiology of improved outcomes. Given the limited in vitro efficacy of cidofovir, other agents such as CMX001, fluoroquinolones, leflunomide, and MTOR (mammalian target of rapamycin) inhibitors might have a beneficial effect in prophylaxis. Unfortunately, preliminary data with levofloxacin suggest only a limited role in prophylaxis, with little effect on HC symptoms [138]. CMX001 may provide a better ratio of antiviral activity over drug toxicity [139] and could be a potential future prophylaxis or treatment option, pending ongoing clinical studies [140].

Reducing immunosuppressive therapy needs to be considered as a primary treatment modality for all HPyV diseases in HCT patients. Unfortunately, increased risk for local and systemic immunologic damage from acute or chronic GVHD in HCT often hampers immunosuppression reduction. Adoptive T-cell transfer may be an interesting future option that is currently being explored [53].

BKPyV-associated HC is primarily treated symptomatically through pain management, spasmolytics, hydration and forced diuresis, and intravesical catheterization for bladder irrigation to reduce obstructive clots. More complicated cases require urologic clot evacuation, local hemostasis with coagulation factors, epsilon-aminocaproic acid, selective embolization, and even cystectomy. *Blood* products should be substituted as needed to counter anemia, low platelet counts, and possibly clotting factors. In a systematic review, the tremendous challenge of HC was acknowledged and the level of evidence for any single treatment was very low [141]. Some centers use intravenous cidofovir or intravesical cidofovir as off-label antiviral adjuncts, but results are mixed and overall disappointing with respect to response time and efficacy on viral loads [78,98,142–144].

BKPyV-associated nephropathy is primarily treated by reducing immunosuppression, but the role of low-dose intravenous cidofovir and switching immunosuppressive drugs to leflunomide and sirolimus is controversial [110,111].

Ureteric stenosis is primarily treated by urologic intervention following cystoscopy, and involves stenting or nephrostoma for the acute treatment and surgical resection for complicated cases.

Progressive multifocal leukoencephalopathy does not respond to currently available antiviral agents, and cidofovir therapy does not limit disease progression in other immunocompromised populations [30]. Suggestions that CMX001 might have some efficacy in vitro [145] and in vivo (H.H. Hirsch, and Chimerix Inc., personal communication), but need to be replicated in appropriate clinical studies.

Merkel cell carcinoma is primarily treated by early surgical resection and chemotherapy for metastizing cases with generally poor outcome. Trichodysplasia spinulos*a* can be treated by reduced immunosuppression and possibly topical 1%–3% cidofovir [137].

For HPyV-associated RTID, no recommendations can be made for severe cases; possibly intravenous immunoglobulins could be considered owing to their immunomodulating and neutralizing potential. In summary, specific antivirals would have an important role by extending the window until virus-specific immune responses have recovered.

Prevention

Prevention of HPyV replication and disease in HCT patients is a difficult task given the ubiquitous presence of HPyVs in healthy individuals and the lack of clinically validated antivirals. As HPyV detection rates and viral load levels increase in immunocompromised patients including those undergoing conditioning and HCT, exposure to endogenous HPyV infection cannot be prevented. However, exogenous exposure may be underestimated. The shedding of BKPyV and other HPyV at levels >7 $\log_{10}$ cp/mL urine or feces for several weeks and the fact that HPyVs are fairly resistant to inactivation similarly to other non-enveloped viruses [61,62,146,147] renders transmission from one immunocompromised patient to another via healthcare workers, nursing facilities, and toilet equipment a plausible scenario. Although most individuals are likely to have HPyV-specific antibodies, this may not be so for all HPyVs and their serotypes, particularly in younger children [78].

Screening of HCT patients for high-level BKPyV replication in urine and blood occurs at some centers, but generally is not recommended. Decisions regarding administration of preemptive cidofovir or other presumed antivirals such as leflunomide, fluoroquinolones, or intravenous immunoglobulins to patients with BKPyV viremia will requires careful randomized studies to determine the risk–benefit/efficacy ratio given significant concerns over toxicity issues, costs, and the development of resistance.

It is possible that cidofovir, leflunomide, and MTOR inhibitors might have a beneficial effect as prophylaxis, but none have been studied for this purpose. Data suggest that fluoroquinolones may have a role in prophylaxis, but randomized trials needed to confirm these results have failed in kidney transplant recipients [138,148,149]. However, a large prophylaxis study of lipid cidofovir conjugate CMX001 on CMV replication in HCT may provide a future option for either treatment or prevention [140,150].

Human parvovirus

The major human pathogen in the *Parvovirus* family is human parvovirus B19 (HPVB19). HPVB19 is the cause of erythema infectiosum (Fifth disease), a self-limited infection of childhood characterized by fever, fatigue, myalgias, a lace-like reticular rash, and a "slapped-cheek" appearance. The predominant manifestation in adults is acute symmetrical arthropathy that can mimic rheumatoid arthritis and other rheumatologic diseases. HPVB19 is also the cause of transient aplastic crisis in patients with hemolytic conditions, such as sickle cell disease [151]. HPVB19 replication is rarely detected in HCT recipients, but can lead to a number of clinically significant complications, particularly in cells of the erythroid lineage [152].

Epidemiology

HPVB19 is present throughout the world and studies demonstrate increasing seroprevalance with age, as approximately 5–20% of children, 30–60% of young adults, and over 75–90% of elderly persons have evidence of prior infection [153–155]. Individuals who work closely with children, have a history of intravenous drug abuse or HIV, or who are otherwise immunocompromised may be more likely to be seropositive [156,157]. It is thought that seronegativity may increase the risk of complications in HCT recipients, but incidence rates of HPVB19 DNAemia and disease are difficult to calculate owing to the rarity of the disease in this population [158,159].

HPVB19 is thought to be transmitted through three primary pathways: respiratory droplets, blood products, and from mother to fetus (vertical transmission) during acute maternal parvovirus infection. During primary DNAemia, HPVB19 leads to pulmonary shedding and respiratory droplets that can lead to transmission to close contacts. Transmission also occurs in susceptible patients through transfusion of blood components or transplantation. These are rare events with most single-component transfusions, as less than 1% of blood donors have detectable viral DNA [160]. Plasma derivatives, which are made from multiple units, are more likely to have detectable virus [161] and these plasma products undergo NAT routinely for HPVB19 DNA. Finally, in susceptible patients undergoing transplantation, the donor stem-cell product can also be the source of transmission [162].

Virologic aspects

Parvoviruses are small, non-enveloped, single-stranded DNA viruses of the family *Parvoviridae*. Although HPVB19 is the major human pathogen, two other *Parvoviridae*, parvovirus 4 (PAV4) and adeno-associated virus (AAV), have been detected in humans. To date, neither PAV4 nor AAV has been linked to disease manifestations in HCT [163,164].

HPVB19 primarily infects erythroid progenitor cells, which allow for viral propagation [165]. HPVB19 binds as its host receptor the common blood group P antigen [166]. The virus leads to cell death through apoptosis mediated by non-structural proteins such as NS1 [167]. Immunity to HPVB19 is thought to be primarily humoral; neutralizing antibodies to the capsid proteins play a major role in host defense [168]. Most immunocompromised patients have a marked decrement in antibody production, must contend with recurrent DNAemia, and are more likely to develop complications [159,168]. Seronegative HCT recipients may be at the highest risk for clinical disease, as experimental studies have shown that recipient, not donor, cells likely provide the majority of protection from post-transplant replication [169].

Diagnostics

The reliability of serology in the diagnosis of acute parvovirus infections in HCT recipients is limited owing to poor antibody production [168]. In a review of 24 HCT recipients with HPVB19, only 40% had evidence of serum IgM at the onset of disease and only 33% IgG [159]. Most cases of severe disease are defined in part by their lack of appropriate antibody responses.

Detection of viral DNA has become the most reliable way to confirm the diagnosis. High levels of virus can usually be found in peripheral blood and bone marrow samples of HCT recipients with disease [151]. The high level of sensitivity of PCR means that low-level HPVB19 DNA can be detected in those without evidence of clinical disease [170]. The mere presence of HPVB19 DNA in serum needs to be interpreted in conjunction with the clinical picture, particularly in pediatric transplant patients. In one study, for example, 30% of pediatric allogeneic transplant patients had detectable HPVB19 DNA, but none had clinical sequelae [164]. Evidence of PCR detection from biopsy samples may be needed to confirm tissue-invasive disease, but positive results, principally low-level values, do not by themselves define organ involvement [171].

Biopsy or other pathologic samples can increase the ability to make the diagnosis of HPVB19 disease. The most important site is the bone marrow, where classically there is an absence of erythroid precursors, and giant pronormoblasts with intranuclear inclusions and cytoplasmic vacuolation [172]. Biopsies from other sites including liver and cardiac tissue can also be evaluated for HPVB19 DNA in appropriate clinical situations.

Parvovirus B19 disease

Pure red cell aplasia

The true incidence of HPVB19-associated pure red cell aplasia is unknown among HCT recipients, but regardless, it is a rare post-transplant complication [159]. In these patients, recovery of B-cell function is often months after transplantation [173], and immunoglobulin concentrations can take up to 1 year to recover fully [174]. Antibody production may also be negatively affected by common post-transplant events, such as the use of immunosuppression, other infections [175], antithymocyte globulin (ATG) [176], and ongoing therapy for acute and chronic GVHD. Since antibody production is critical to clearance of DNAemia, HCT patients can develop chronic HPVB19 replication and pure red cell aplasia.

Patients with pure red cell aplasia may present initially with fever and rash, but others are asymptomatic and present only after developing anemia. Clinical signs of anemia, such as fatigue, pallor, and dyspnea, follow about 1 week after infection; rarely do patients present with classic arthropathy [159]. A high index of suspicion is needed to make the diagnosis as these symptoms and the presence of anemia are common during the post-transplant period. *Blood* smears in these patients will demonstrate normochromic, normocytic anemia, but classically with a reduction in reticulocytes. In some cases, patients may present with additional thrombocytopenia or neutropenia. Pathologic review of bone marrow demonstrates a lack of erythroid precursors and increased numbers of giant pronormoblasts that may have intranuclear inclusions and cytoplasmic vacuolation [172].

Other hematologic disorders

HPVB19 has been associated with thrombocytopenia and neutropenia in HCT recipients [152,159,162]. Although thrombocytopenia and granulocytopenia are rarely seen as isolated phenomenon in other populations, these cytopenias in HCT are universally reported with associated erythroid abnormalities. Rarely, HPVB19 has been implicated in the development of pancytopenia and marrow failure in HCT recipients [177]. Hemophagocytic syndrome has been described as a complication of HPVB19 in other immunocompromised populations [178].

Myocarditis

Myocarditis is an unusual complication of HPVB19 in transplant patients. However, in a large retrospective review of reported cases, myocarditis was seen more frequently in HCT patients (12.5%) than in other immunosuppressed populations [159]. It is thought that the target of the virus is endothelial cells, and that viral-mediated microcirculatory dysfunction leads to myocardial damage [179]. Although detection of HPVB19 viral genomes in patients with myocarditis suggests a direct link between HPBV19 replication and disease [180], it is unknown if the virus plays more of a bystander role in the development of myocarditis [171].

Other reported complications

HPVB19 pneumonia has been detailed [181], but viral shedding in the upper and lower respiratory tract during acute infection [182] means that direct associations must be considered cautiously and other pathogens ruled out before considering HPVB19 as the primary etiology. Patients with HPVB19 may have elevated liver enzymes during acute infection, and acute HPVB19 hepatitis has been described in HCT [183]. Cases of HPVB19 encephalopathy have also been reported sporadically [184,185].

Treatment

HCT recipients should be given therapy if HPVB19 is diagnosed as the causative agent for symptoms; treatment is often considered for patients with documented red cell aplasia while awaiting confirmatory testing. The mainstays of treatment are the use of IVIg and, if possible, modifications in immunosuppressive therapy (Table 93.3). A study of samples in the United States and Europe demonstrated that most IVIg preparations contain sufficient antibodies for HPVB19 neutralization [186]. Published data indicate that short-term IVIg can ameliorate anemia in pure red cell aplasia and has been shown to be effective as a therapeutic approach to patients with organ involvement [158,159]. Decreasing immunosuppression is an important adjunct to treatment with IVIg, but this is not always possible in HCT recipients.

Dosing of IVIg has been recommended from 0.4mg/kg body weight given over a 5–10-day course [187] to 1–2g/kg/day for 2–5 days [188]. In a retrospective study of immunocompromised patients [158], patients received a mean of 2.8 IVIg courses and a higher mean starting dose of 2.3g/kg; 38% had reductions in immunosuppression in conjunction with their treatment. Initial response was excellent with a total of over 90% recovering their counts after the first dose. Unfortunately, relapse occurred in 31% of patients at a mean of 4.3 months. A response to IVIg at 12 months was associated in multivariate analysis with an 89% reduction in mortality from HPVB19 disease [158].

Prevention

The relative rareness of HPVB19 disease in HCT limits the use of widespread strategies to prevent exposure beyond standard infection control measures. Baseline serologic testing is not currently recommended for patients undergoing HCT or their donors, and post-transplant screening is not currently recommended for prevention [189]. Although prophylaxis is not recommended, the routine use of IVIg in some populations during the post-transplant period has been hypothesized to decrease overall risk [190].

It is important to consider isolation for HCT recipients with ongoing HPVB19 replication, as HPVB19 has been associated with nosocomial outbreaks [191]. In addition to standard precautions, droplet precautions are recommended for HCT recipients diagnosed with HPVB19 [192]. Pregnant healthcare workers should be informed of potential risks of HPVB19 exposure, and seronegative persons should avoid taking care of these patients [192]. Recommendations are not clear for HCT patients who have improved symptoms but develop prolonged DNAemia, as it is unclear how long immunocompromised patients remain infectious. Such cases should be discussed with local infectious diseases and infection control professionals.

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CHAPTER 94

Gastrointestinal and Hepatic Complications

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Introduction

The frequency and severity of intestinal and liver complications of hematopoietic cell transplantation (HCT) have declined steeply over the last decade, with some complications disappearing completely, for example, herpes simplex virus (HSV) esophagitis, intramural hematomas, candidal liver abscesses, hyperammonemia, and gastric antral vascular ectasia [1,2]. Fewer patients have severe gut and liver damage from acute graft-versus-host disease (GVHD), as the development of more effective strategies to prevent GVHD has had a marked effect on its clinical presentation. Protracted jaundice is now far less common, as the frequency of sinusoidal liver injury caused by myeloablative regimens has fallen, and prophylaxis with ursodiol has been effective in mitigating cholestatic liver injury [1]. Prophylaxis with antiviral and antifungal drugs has greatly reduced the incidence of the most common gut and liver infections [2]. This chapter mentions now rare complications in an abbreviated way so that the lessons of the past are not completely forgotten. More emphasis is given to problems that are still common, for example, anorexia following conditioning therapy; acute GVHD, protracted acute GVHD, and liver presentations of GVHD; adenovirus, cytomegalovirus, and norovirus infections; and problems in long-term transplant survivors.

Evaluation of intestinal and liver problems before transplant

Ulcers, tumors, and infections in the gastrointestinal tract

In immunocompromised patients, mucosal ulcers may have an infectious etiology [e.g., cytomegalovirus (CMV), HSV, or fungal infection] that requires specific antimicrobial treatment. Thus, symptoms of esophageal pain, heartburn, dysphagia, abdominal pain, nausea, vomiting, and diarrhea should be investigated with endoscopy before HCT, and all intestinal ulcerations healed before the start of conditioning therapy, to avoid major bleeding when platelet counts fall. The goal of pre-HCT therapy in patients with ulcerative colitis or Crohn's disease is to minimize the extent of gross intestinal ulceration to lower the risk of bleeding after HCT and to rule out infections as a cause of colonic ulceration. CMV, *Entamoeba histolytica*, and clostridial infections are causes of colonic ulceration that may mimic inflammatory bowel disease. CMV disease before transplant is associated with a high risk for early CMV disease and death after HCT [3]. Selected patients with ulcerative colitis and Crohn's disease have undergone both allogeneic and autologous HCT without complications of bleeding, perforation, or dissemination of microorganisms [4–6]. Long-term resolution of Crohn's disease has been observed following myeloablative allogeneic HCT, and autologous HCT has also resulted in improvements.

Patients who are to undergo HCT for lymphoma, leukemia in relapse, myeloma, or metastatic breast cancer may have intestinal involvement with malignant cells. Cytoreductive therapy results in necrosis of these cells, which rarely can lead to large intestinal ulcers and perforation. Intra-abdominal abscesses and fistulae should be managed surgically before HCT. Patients over the age of 50 years are at risk for the development of colorectal polyps and cancer; the presence of occult blood in stool specimens from patients over 50 years old should prompt endoscopic evaluation, including colonoscopy.

Diarrhea

Patients with diarrhea should be investigated for organisms that may cause morbidity after HCT (*E. histolytica*, *Strongyloides*, *Giardia lamblia*, *Cryptosporidium,* CMV, rotavirus, norovirus, and adenovirus) [7–11]. Persistent eosinophilia in a patient from an area where parasites are endemic is also an indication for screening for parasitic disease, including helminths, and *Cystoisospora belli*. Specific therapy is available for amebiasis, giardiasis, strongyloidiasis and other helminths, and most coccidia and microsporidia. Many of these opportunistic infections can be refractory to treatment in immunosuppressed patients [12], while restoration of normal immunity after allogeneic HCT can result in clearance [10,13].

Other treatable causes of diarrhea in patients with hematologic malignancies include clostridial infections (*Clostridium difficile*, *C. perfringens*, and *C. septicum*) [14]. The patient who has had previous typhlitis (neutropenic enterocolitis) during neutropenia can present problems if there is persistent fever or tenderness over the cecum. Typhlitis is a syndrome of cecal edema, mucosal friability and ulceration, and fever often associated with polymicrobial sepsis; its cause is usually intestinal infection caused by *C. septicum*. After treatment, the risk of post-HCT typhlitis is no different from that of other patients. Pericolic phlegmons, abscesses, and persistent colitis caused by clostridial infections must be treated before the start of conditioning therapy.

As with Crohn's disease and ulcerative colitis, gut inflammation in patients with immune disorders has been noted to disappear after

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successful allografts; examples include refractory celiac disease and a spectrum of pediatric disorders of immune dysregulation — severe combined immunodeficiency (SCID); immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome; interleukin (IL)-10-receptor defect or IL-10 deficiency; chronic granulomatous disease; Wiskott Aldrich syndrome; common variable immune deficiency (CVID) with severe autoimmune problems; X-linked lymphoproliferative disease; X-linked agammaglobulinemia; trisomy 8 mosaicism (Behcet's like); nuclear factor-kappa B (NFκB) essential modulator (NEMO) deficiency; and STAT1 gain-of-function mutations [15,16].

Perianal pain

Transplant candidates are not spared common causes of perianal pain (anal fissure, thrombosed external hemorrhoid), but if a patient is neutropenic, bacterial infection should be suspected until proven otherwise. Most anorectal infections begin in the anal glands deep to the crypts at the dentate line. Inflammation of anal crypts may cause pain in the absence of an abscess. Abscesses tend to originate in the intersphincteric space and may extend into the perianal, ischiorectal, or supralevator space. Extensive supralevator and intersphincteric abscesses may be present without being apparent on external examination, but can be diagnosed by axial computed tomography (CT), magnetic resonance imaging (MRI), or transperineal sonography. Perianal infections must be dealt with before HCT, as extensive tissue necrosis and septicemia may result from uncontrolled infection [17]. Administration of broad-spectrum antibiotics, including anaerobic coverage, is adequate treatment in most cases, with surgical incision and drainage reserved for progressive infections [18]. Proctitis and genital ulcers due to HSV infection, perianal CMV ulcers, and rarely fungal infections may also lead to perianal pain [19].

Liver disease in donors and recipients

Viral hepatitis in allogeneic HCT donors

Hepatitis B and C will be transmitted from viremic donors to transplant recipients [1]. When equally human leukocyte antigen (HLA)-matched donors are available, a donor who is not infected by hepatitis viruses should be selected. If the most suitable donor has chronic hepatitis B or C, however, preventing passage of virus and mitigating the effects of infection in the recipient become priorities. Oral nucleos(t)ide analogs such as entecavir and tenofovir result in rapid hepatitis B virus (HBV) suppression with negligible risk of antiviral resistance and, where possible, should be used to reduce viral load prior to donor hematopoietic cell harvest. However, HBV may persist in donor peripheral blood mononuclear cells despite clearance from serum [20], and the recipient transplanted from an HBsAg-positive, serum HBV DNA-negative donor should receive prophylactic oral antiviral therapy for at least 1 year. Hepatitis B vaccination of HBsAg-negative recipients prior to the start of conditioning therapy may also be beneficial if there is risk of passage of virus from an infected donor. When both donor and recipient are infected by HBV, the recipient should be treated with oral nucleos(t)ide therapy starting prior to conditioning therapy. In this setting, there may also be theoretical reasons to lower the viral load in the donor, for example, if the recipient's HBV is in a latent or occult phase or if donor and recipient HBV differ in genotype.

Occult hepatitis B is the presence of detectable serum HBV DNA in HBsAg-negative individuals. The prevalence of occult hepatitis B varies according to the HBV prevalence in the population, being higher in HBV-endemic countries where rates up to 6–15% in HBsAg negative/anti-HBc-positive blood donors have been reported [21]. Individuals with isolated anti-HBc positivity are more likely than those with both anti-HBc and anti-HBs to have occult hepatitis B. Donors with occult hepatitis B can be used, but if possible the recipient should be vaccinated prior to conditioning therapy and given oral antiviral prophylaxis for up to 1 year after transplant. A donor who is naturally anti-HBc positive may be the preferred donor if the recipient is HBsAg positive or anti-HBc positive, as adoptive transfer of immunity can effect clearance of virus [22].

If a preferred donor is hepatitis C virus (HCV) antibody and RNA positive in serum, HCV will usually be transmitted, and the rate of spontaneous viral clearance from the recipient is likely to be low. If time permits, treatment of the donor with antiviral therapy prior to harvest of donor cells may render cells and serum non-viremic and much less likely to transmit infection. Direct Acting Antiviral (DAA) drugs can clear extrahepatic HCV from donors more quickly than interferon and ribavirin without significant toxic effects on the donor marrow [23,24]. If time does not permit treatment of an HCV-infected donor, the use of HCV-infected hematopoietic cells for an HCV-uninfected recipient is not absolutely contraindicated because new DAAs can provide a virologic cure in the majority of transplant survivors after recovery and immune reconstitution. Therapeutics for HCV treatment are rapidly evolving; we recommend this website for continuously updated information: http://www.hcvguidelines.org. As of 2015, for HCV infection with genotype 1a, 1b, or 4, treatment with one of the following DAA combinations is recommended: Daily fixed-dose combination of ledipasvir and sofosbuvir, or twice daily fixed-dose combination of ombitasvir, paritaprevir, ritonavir and dasabuvir, or daily sofosbuvir plus simeprevir. For infection with genotypes 2, 3, or 5, the preferred regimen is sofosbuvir plus weight-based ribavirin. Monotherapy with a DAA is not recommended regardless of HCV genotype. If HCV is transmitted, the acute phase of HCV infection may cause elevated liver enzymes at 2–3 months post-HCT, after recovery of T-cell function [25]. However, severe hepatitis and fibrosing cholestatic hepatitis have rarely been described after HCT [26]. Overall, outcomes in HCV-infected recipients in 10 years of follow-up are no different from those in transplant recipients without hepatitis C infection [25,27]. In the long term, antiviral treatment should be offered to the recipient who remains with active hepatitis C, as such a patient is at risk for development of cirrhosis and hepatocellular carcinoma [1].

Chronic liver disease in candidates for HCT

Patients with active liver disease, particularly those with severe hepatic fibrosis or cirrhosis, are at increased risk for fatal sinusoidal obstructive syndrome (SOS) (formerly known as venocclusive disease of the liver) following hepatotoxic myeloablative regimens [1]. The presence of cirrhosis may provide a contraindication to any high-dose conditioning regimen because such patients are at risk for fatal sepsis or hepatic decompensation after HCT even if given a reduced-intensity conditioning regimen [28]. Liver biopsy should be considered if there is a clinical suspicion of cirrhosis or extensive fibrosis (for example, in patients with myelofibrosis [29]). There are alternatives to biopsy: 1) Analysis of blood test panels are not recommended because their components are often affected by underlying hematologic disease; and 2) Vibration-controlled transient elastography can be useful in pre-HCT screening for fibrotic liver disease [30,30a,30b]. Patients with fibrotic and necroinflammatory chronic liver disease should be considered for either reduced-intensity or "liver-friendly" myeloablative regimens (see the section Sinusoidal obstruction syndrome).

Hepatitis B-infected HCT recipients are at risk for severe hepatitis flares and fulminant liver failure. In the absence of antiviral prophylaxis, HBV reactivation is common, and fatal fulminant hepatitis develops in approximately 15% [1]. Prophylaxis with oral nucleos(t)ide therapy starting prior to conditioning therapy is therefore recommended for all HBsAg-positive recipients. Occult hepatitis B may also be associated with HBV reactivation, with rates up to 35% seen in recipients with isolated anti-HBc antibodies, particularly during treatment for acute GVHD [31]. Patients born in countries where hepatitis B is endemic need close monitoring for HBV reactivation after HCT, even when pre-HCT blood tests were negative for prior HBV infection [32].

Recent liver dysfunction in candidates for HCT

Patients who come to HCT following recent chemotherapy or radiation therapy may be at risk for unexpected toxicities following myeloablative conditioning regimens, particularly if these therapies have resulted in liver dysfunction. Many chemotherapy drugs cause liver dysfunction either directly (cholestatic injury, hepatocyte necrosis, or sinusoidal damage) or indirectly (cholestasis caused by sepsis or endotoxemia, i.e., cholangitis lenta) [33]. Severe liver injury from recent chemotherapy is uncommon, however. Recent exposure to standard chemotherapy has not been associated with an increased risk of fatal SOS after cyclophosphamide (CY)-based myeloablative regimens, but patients presenting for HCT with persistent jaundice and elevations of aminotransferase enzymes are likely at risk. Liver injury has been associated with prolonged exposure to antimetabolites such as 6-thioguanine and also agents such as imatinib (Gleevec) and newer tyrosine kinase inhibitors. Gemtuzumab ozogamicin (Mylotarg) causes sinusoidal liver injury in 3–15% of patients, thus predisposing to SOS after hepatotoxic myeloablative regimens, particularly if the patient is transplanted within 3 months of high-dose gemtuzumab exposure [1,34].

Fungal liver infections

A search for hepatic fungal infection should be undertaken in patients with tender hepatomegaly, fevers, and abnormal liver enzymes. Diagnosis may be established by sensitive liver imaging (high-resolution CT or MRI) in conjunction with fungal biomarkers such as serology for fungal antigens (galactomannan and glucan assays) and polymerase chain reaction (PCR) or culture of liver biopsy material [35]. Fungal liver infection should be controlled with systemic antifungal agents, with newer agents such as the echinocandins offering reduced toxicity, fewer drug–drug interactions, and a broader range of coverage. Therapy should be continued through HCT until engraftment is established, which can effect the resolution of intractable fungal liver abscesses.

Gall bladder and bile duct stones

HCT candidates with asymptomatic gallstones (incidentally discovered during a CT scan or ultrasound) do not require operative intervention. Patients with symptomatic gall bladder or common duct stones are at significant risk of sepsis after HCT, and consideration should be given to pre-transplant cholecystectomy or an endoscopic biliary procedure.

Iron overload

HCT candidates with diseases such as thalassemia, aplastic anemia, myelodysplastic syndrome, and chronic leukemia or lymphoma may come to HCT with marked hepatic iron overload caused by transfusions, gut hyperabsorption of dietary and medicinal iron, and uncommonly from genetic hemochromatosis. Extreme liver iron content (>12,500 μg per gram dry weight), more accurately assessed by T2* MRI than serum ferritin or transfusion history, has been associated with increased non-relapse mortality post-HCT from cardiac arrhythmias and mold infections [36]. In patients with extreme iron overload, effective pre-HCT chelation therapy improves post-HCT survival. While some studies suggest an association between excess tissue iron stores and regimen-related toxicity, others have failed to demonstrate this. After HCT, severe iron overload has been associated with non-specific liver dysfunction that may respond to erythropoietin-assisted phlebotomy or chelation. In most patients, the quantitation of tissue iron stores and consideration of iron removal can be deferred until after recovery from HCT [37].

Problems from transplant through the first year

Nausea, vomiting, and anorexia

Studies of gastric emptying and myeloelectric activity in HCT patients have shown that symptoms of nausea and vomiting are frequently accompanied by retention of radionuclide meals and disordered electrical activity [38–40]. Promotility agents such as metoclopramide, domperidone, and low-dose erythromycin are occasionally useful, but in patients with persistent symptoms, endoscopic biopsies to look for the underlying cause of symptoms should precede protracted anti-emetic and promotility therapy.

Conditioning therapy

Mucositis caused by conditioning therapy leads to oral mucosal swelling, pain, and, in severe cases, sloughing of pharyngeal and esophageal epithelium, intense gagging, an inability to swallow, vomiting, retrosternal pain, and airway obstruction. Opioid therapy is effective in relieving pain but can lead to gastric stasis and intestinal pseudo-obstruction. Some regimens, notably those that contain cytarabine, etoposide, high-dose melphalan, or multiple alkylating agents, may cause unusually severe intestinal mucosal necrosis and further delay the return of eating. Oral cryotherapy has been shown to decrease the severity and duration of oral mucositis after high-dose melphalan [41]. Myeloablative conditioning therapy makes most patients nauseated and anorexic, leading to delayed gastric emptying [38] and poor oral intake, with a nadir at day 10–12 post-transplant but often extending to day 20 [42]. Conditioning therapy can lead to activation of vagal afferents by serotonin released from enterochromaffin cells [43]. Serotonin-antagonist drugs are very effective in relieving acute symptoms during conditioning therapy, with the second-generation 5-HT$_3$ receptor antagonist palonosetron also being helpful in treating delayed nausea and vomiting (day 2–5) [44,45]. Addition of dexamethasone and/or NK1 antagonists that block the action of substance P may prevent breakthrough nausea and vomiting [46]. Protracted anorexia may be related to circulating IL-1, IL-6, and tumor necrosis factor-alpha (TNF-α), cytokines known to affect appetite centers [42].

Upper gut GVHD

Early signs of acute GVHD include loss of appetite, absence of pleasure from eating, and satiety, often followed by nausea and vomiting, especially when more subtle signs are not investigated. When the onset of GVHD is before day 15, following a peripheral blood allograft, for example, the histologic features of GVHD may be indistinguishable from those resulting from conditioning therapy. Some patients never experience improvement in appetite before the onset of GVHD, but rather have a seamless persistence of anorexia and nausea. After day 20, over 80% of patients with intractable anorexia, nausea, or vomiting will have gastric and duodenal GVHD as the sole explanation [47]. Endoscopy shows edema of the gastric antral and duodenal mucosa, patchy erythema, and bilious gastric fluid [48]. Histology may show epithelial cell apoptosis and dropout, often with localized lymphocytic infiltrates [48,49]. However, the predictive value of negative histology for GVHD is not high, as what the endoscopist sees as mucosal edema is not what the pathologist relies upon to make a diagnosis of GVHD (apoptosis of crypt cells) [50]. Hence the gamut of clinical presentation, endoscopic appearance, absence of infection, and histology needs to be integrated to arrive at a diagnosis when histology is equivocal [48,49,51]. Immunosuppressive therapy using a 10-day induction course of prednisone 1 mg/kg/day plus oral beclomethasone dipropionate 8 mg/day is an effective therapy for upper gut-predominant GVHD that avoids prolonged prednisone exposure [52,53]. In patients whose symptoms have responded to this 10-day regimen, tapering prednisone over 1 week but continuing topical glucocorticoid therapy is adequate for most patients with upper gut GVHD. Recipients of autologous grafts may also develop a syndrome of anorexia, nausea, and vomiting that is associated with diffuse gastric edema and erythema [54]. Gastric histology shows typical GVHD, and symptoms respond to a 10-day course of prednisone 1 mg/kg/day.

Infection

Endoscopy also serves to rule out intestinal infection with herpesviruses, bacteria, and fungi. CMV infection of the esophagus and upper intestine accounted for one-third of patients with unexplained nausea and vomiting during the pre-ganciclovir era, but CMV infection is now an uncommon cause of these symptoms [47]. CMV infections in the gastrointestinal tract are usually diagnosed between 50 and 150 days after HCT [55], but can appear earlier if patients have CMV infection before HCT or receive cord blood transplants [3,56]. CMV infections can be detected in the gastrointestinal tract in the absence of CMV antigen or DNA in peripheral blood. HSV esophagitis may present in patients not receiving prophylactic acyclovir. Fungal esophagitis may cause anorexia but not the incessant vomiting often seen with GVHD or CMV infection. Nausea and vomiting may be prominent symptoms of some parasitic infection (if missed during pre-HCT screening) [9,10] and infections caused by non-culturable viruses (norovirus, rotavirus, astrovirus) [11]. Anorexia and vomiting may also be manifestations of increased intracranial pressure related to CNS infection; other neurologic signs and symptoms usually dominate the clinical picture with these disorders. Non-infectious causes of increased intracranial pressure may also present with nausea and vomiting, for example, cyclosporine-related pseudotumor cerebri and subdural hematomas.

Medications

Oral non-absorbable antibiotics (particularly nystatin), cyclosporine, mycophenolate mofetil (MMF), trimethoprim–sulfamethoxazole, voriconazole, posaconazole, itraconazole, intravenous amphotericin, and high-dose opioids also cause nausea and occasionally vomiting. Infusions of fat, glucose, and amino acids in total parenteral nutrition reduce appetite, slow gastric emptying, and cause nausea. Even after total parenteral nutrition has been stopped, appetite suppression may linger for 1–3 weeks [57].

Jaundice, hepatomegaly, and abnormal liver tests

Development of jaundice following HCT is an ominous prognostic sign, with increased non-relapse mortality in patients whose total serum bilirubin exceeds 4 mg/dL [58,59]. In patients with acute GVHD, jaundice is an independent predictor of mortality [60,61]. Because treatment of severe liver dysfunction is often futile, transplant physicians must recognize risk factors for hepatobiliary problems before the transplant process starts, implement measures to prevent liver damage, and identify post-transplant liver disorders that have specific treatments [1,59]. Over the last decade, this approach has resulted in a marked reduction in the incidence of liver injury and clinical jaundice, resulting from prevention of hepatic sinusoidal injury, fungal and viral prophylaxis, and the introduction of prophylactic ursodiol for all patients undergoing transplant [2]. Nonetheless, there remain multiple hepatic causes of jaundice and elevations of serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) [1,59]. Additional processes can contribute to jaundice following HCT while not usually being important causes of jaundice in isolation, for example, hemolysis and renal insufficiency.

Sinusoidal obstruction syndrome (SOS)

Definition

SOS is a syndrome of tender hepatomegaly, fluid retention and weight gain, and elevated serum bilirubin that follows some high-dose myeloablative conditioning regimens. This form of liver injury is initiated by damage to hepatic sinusoids; occlusion of hepatic venules is not essential to development of signs and symptoms, hence the older term “venocclusive disease” (VOD) is inaccurate.

Incidence

The frequency and severity of SOS have fallen dramatically because: (1) doses of total body irradiation (TBI) >14 Gy are seldom used; (2) fludarabine is replacing CY; (3) patients at risk for SOS are now given liver-friendly regimens that do not contain either CY or TBI >12 Gy; (4) the prevalence of chronic hepatitis C in transplant candidates, formerly a common risk factor for SOS, is now very low; and (5) therapeutic drug monitoring allows personalized dosing of chemotherapy drugs that have variable metabolism [1]. SOS is caused by toxins in certain conditioning regimens; important contributors to variability in the incidence of SOS are (1) variations in the metabolism of CY from patient to patient in CY/TBI regimens [62]; (2) underlying fibro-inflammatory liver diseases; (3) the order of delivery of drugs in multidrug conditioning regimens [63,64]; (4) concomitant use of drugs during and after conditioning therapy that either affect the metabolism of myeloablative drugs (e.g., itraconazole) or cause concomitant liver injury (e.g., methotrexate, sirolimus, norethisterone). Although fatal SOS has nearly disappeared from most adult transplant units [2,65], pediatric patients receiving busulfan/melphalan conditioning regimens remain at risk [66]. Many past patients diagnosed as having SOS on the basis of jaundice probably had mostly cholestatic and not sinusoidal liver injury, a conclusion based on data from ursodiol prophylaxis studies [2,67,68].

Clinical presentation and diagnosis

The onset of SOS is heralded by an increase in liver size, right upper quadrant tenderness, renal sodium retention, and weight gain, occurring 10–20 days after the start of CY and later after other myeloablative regimens [1]. Hyperbilirubinemia follows these signs of portal hypertension by 4–10 days. Concomitant renal and lung dysfunction and refractory thrombocytopenia strongly suggest SOS [69,70]. Measurement of total serum bilirubin is a sensitive test for SOS but not a specific one. Elevations of serum aspartate aminotransferases (AST/ALT) weeks after the clinical onset of SOS reflect ischemic hepatocyte necrosis from sinusoidal fibrosis [71]. Several plasma proteins have been reported to be abnormally high in patients with SOS (endothelial cell markers, thrombopoietin, proinflammatory cytokines, vascular endothelial growth factor, procollagen peptides and a panel of biomarkers); some laboratory tests are abnormally low in patients with SOS (protein C, antithrombin III, and platelet counts). These tests do not appear to have diagnostic or prognostic utility beyond the clinical criteria of weight gain, jaundice, and hepatomegaly. Doppler ultrasound is useful for demonstrating hepatomegaly, ascites, periportal edema, attenuated hepatic venous flow, and gall bladder wall edema consistent with SOS, in addition to excluding other causes of hepatomegaly and jaundice. Abnormal findings later in the course of SOS may include an enlarged portal vein diameter, slow or reversed flow in the portal vein or its segmental branches, high congestion index, portal vein thrombosis, and increased resistive index to hepatic artery flow. Unfortunately, ultrasound findings very early in the course of SOS – when there is often diagnostic uncertainty – do not appear to add to the information provided by clinical criteria.

A transvenous approach that allows both biopsy and hepatic venous pressure measurements is the most accurate diagnostic test. A hepatic venous pressure gradient above 10 mmHg is highly specific for SOS [72,73]. Initial histologic changes are dilation of sinusoids, extravasation of red cells through the space of Disse, necrosis of perivenular hepatocytes, and widening of the subendothelial zone in central veins [74,75]. A finding of “hemorrhage” in zones 2 and 3 of the liver acinus is the result of destruction of sinusoidal endothelium – the initiating injury in SOS. In severe SOS, fragmented hepatocyte cords can be seen, with dislodgement of hepatocytes into both portal and hepatic venules. The later stages of SOS are characterized by activation and proliferation of stellate cells, extensive collagenization of sinusoids, and a variable degree of obstruction of venular lumens leading to obliteration of sinusoidal blood flow [74,75]. In severe SOS – if patients survive beyond day 50 post-transplant – a pattern of reverse cirrhosis may develop, with extensive linkage between obliterated central venules by fibrous bridges, collapse, and acinar extinction. Intensity of collagenization of sinusoids and central veins and the degree of hepatocyte necrosis as reflected in serum ALT levels are correlated with outcome [71,75].

Clinical course and prognosis

Complete recovery from SOS occurs in over 70% of patients with just supportive care. Patients with severe SOS seldom die of liver failure, but rather from renal and cardiopulmonary failure [69,70]. For research purposes, a retrospective scoring system classifies SOS as mild (clinically obvious, requires no treatment, resolves completely), moderate (signs and symptoms requiring treatment such as diuretics or pain medications, resolves completely), or severe (requires treatment but does not resolve before death or day +100). Useful prognostic findings include the rapidity with which weight is gained and serum bilirubin rises, serum AST/ALT levels over 1500 U/L, development of ascites, renal insufficiency, and hypoxemia [69–71,76].

Pathogenesis of SOS (reviewed in [74,77])

Toxic damage to hepatic sinusoidal endothelial cells, not venular damage and not thrombosis within the liver vasculature, is the proximate cause of SOS. The most common causes of sinusoidal damage are regimens that contain CY and regimens that include TBI, with the combination of CY and TBI doses over 12 Gy being the most common cause of fatal SOS [69]. The metabolism of CY is highly variable and unpredictable; patients who generate a greater quantity of toxic CY metabolites are more likely to develop fatal SOS [62]. Accurate methods to target the dose of CY to a metabolic endpoint allow personalized CY dosing, significantly reducing liver and kidney injury [78]. These methods are not widely available, but data suggest that a total CY dose of 100–110 mg/kg, instead of 120 mg/kg, will lower the frequency of organ toxicity in the CY/TBI regimen [78]. In combination with CY 120 mg/kg, there is a clear relationship between the total dose of TBI and the frequency of severe SOS – approximately 1% after CY/TBI 10 Gy, 4–7% after CY/TBI 12–14 Gy, and 20% after CY/TBI >14 Gy. Busulfan (BU) is another component of regimens with a high frequency of SOS, but BU itself does not appear to be hepatotoxic [79,80]. BU may contribute to liver injury by inducing oxidative stress, reducing glutathione levels in hepatocytes and sinusoidal endothelial cells, and altering CY metabolism [64,79]. Giving CY before intravenous targeted BU, particularly in patients with myelofibrosis [29], leads to less liver toxicity than with targeted BU/CY or i.v. BU/CY [80a,64] [64]. Melphalan pharmacokinetics, also widely variable from patient to patient, can be normalized using therapeutic drug monitoring [81]. Gemtuzumab ozogamicin causes sinusoidal liver injury in about 10% of patients with acute myeloid leukemia (AML) [34]. The risk of SOS increases when high-dose gemtuzumab ozogamicin given in proximity to myeloablative regimens that contain sinusoidal toxins. Gemtuzumab may also cause SOS when given for relapsed AML after HCT.

Procollagen peptides appear in the serum of patients who develop more severe SOS, along with inhibitors of fibrolysis, consistent with the intense fibrosis in sinusoids and venular walls that is common in fatal SOS. Immunohistology for α-actin in liver specimens from patients with SOS shows intense staining in activated stellate cells, the source of collagen deposition into sinusoids [74].

Genetically determined differences in drug metabolism or susceptibility to toxic injury might explain some of the variability in the frequency of SOS, but there is no current method of altering drug dosing or determining the susceptibility of individual patients to sinusoidal injury based on genomic data [82].

Prevention of SOS in patients receiving myeloablative therapy

The only certain way to prevent fatal SOS is to avoid damaging hepatic sinusoidal endothelial cells, especially in patients at risk. The challenge for transplant oncologists is to remove drugs that are toxic to sinusoidal endothelial cells from conditioning regimens without sacrificing engraftment or malignancy relapse rates. Prevention of severe sinusoidal liver injury begins with an assessment of the risk in patients with underlying liver disease, for a given myeloablative conditioning regimen (Table 94.1). These strategies have had a striking effect on the frequency of SOS as a complication of HCT [2,65].

Patients at risk for fatal SOS have several options: (1) conventional therapy that does not involve HCT; (2) a reduced-intensity conditioning regimen; (3) a myeloablative regimen that does not contain CY, for example, targeted BU–fludarabine for allogeneic [83,84] or BEAM for autologous [85] HCT; (4) modification of CY-based regimens with either a change in the order (CY/targeted BU [64], for example) or reduction in CY dose to 100–110 mg/kg [78]; (5) use of pharmacologic approaches to prevent sinusoidal liver injury. Of the proposed methods for more accurate, personalized dosing of chemotherapy

Table 94.1 Factors that increase the risk of severe sinusoidal liver injury resulting from the conditioning regimen

Liver diseases at baseline	Specific conditioning regimens	Concomitant drugs during conditioning therapy
Inflammatory diseases	***Cyclophosphamide-based***	Itraconazole
Chronic hepatitis B or C	CY 120 mg/kg + TBI (greater risk with higher TBI dosing)	Sirolimus (rapamycin)
Non-alcoholic steatohepatitis	BCV (BCNU + CY + VP-16)	Norethisterone
Alcoholic hepatitis	BU + CY (greater risk without therapeutic drug monitoring of BU)	
Fibrotic diseases		
Cirrhosis	***Melphalan-based***	
Lobular fibrosis	BU + MEL + thioTEPA	
Myelofibrosis (extramedullary hematopoiesis with sinusoidal fibrosis)	BU + MEL	
Cholestatic disorders	***Other regimens***	
Jaundice caused by intrahepatic cholestasis	BU + TBI (greater risk with higher TBI dosing)	
Past history	Gemtuzumab ozogamicin-containing myeloablative regimens	
Prior SOS from conventional chemotherapy	High-dose radiolabeled antibody myeloablative regimens	
Recent exposure to gemtuzumab ozogamicin		
Prior liver irradiation		
Prior myeloablative hematopoietic cell transplant		

BCNU, bis(chloroethyl)nitrosourea; BU, busulfan; CY, cyclophosphamide; MEL, melphalan; thioTEPA, *N*-triethylenethiophosphoramide; TBI, total body irradiation.

agents, only therapeutic drug monitoring has been studied in the HCT setting [78,86]. If a CY/TBI regimen must be used for a patient at risk for fatal SOS, modifications should be considered for both CY (100–110 mg/kg) and TBI (≤12 Gy) dosing. Shielding the liver during TBI will lessen liver injury but leads to relapse of underlying hematologic disease. If a BU/CY regimen must be used for a patient at risk for fatal SOS, liver toxicity is less frequent if CY is given before targeted BU [64] or if dosing of CY is delayed for 1–2 days after completion of BU. The metabolism of intravenous BU is variable, with a several-fold range in AUC_{BU}, a problem that can be addressed by therapeutic drug monitoring [84]. Use of therapeutic drug monitoring to normalize exposure to melphalan is also feasible [81]. Another approach to decreasing the toxicity of regimens that contain BU and melphalan is to give them in reverse order (MEL/BU).

Pharmaceutical prevention of SOS has been achieved in animal models of sinusoidal injury, but these strategies (repletion of intracellular GSH or inhibition of matrix metalloproteinase enzymes) have not been studied in the clinical setting. Infusion of defibrotide has been reported to be effective as prophylaxis; results from a large randomized trial in children reported less liver disease and marginally better outcomes in those receiving defibrotide [66]. Prospective studies have shown no benefit from the use of prophylactic heparin, antithrombin III, or *N*-acetylcysteine in preventing fatal SOS. A meta-analysis suggested that ursodiol may prevent SOS [68], but SOS was not differentiated from cholestatic liver disease in these studies and a large randomized trial showed no effect of ursodiol on the frequency of SOS but a large effect on the frequency of cholestatic jaundice [67].

Treatment of patients with SOS

For >70% of patients with SOS who will recover spontaneously, treatment involves management of sodium and water balance, preservation of renal blood flow, and repeated paracenteses for ascites that is associated with discomfort or pulmonary compromise. Patients with a poor prognosis can be recognized soon after disease onset by steep rises in total serum bilirubin and body weight, serum ALT values >1500 U/L, portal pressures over 20 mmHg, development of portal vein thrombosis, and especially by multiorgan failure requiring dialysis, hemofiltration, or mechanical ventilation [69–71,76]. There are no satisfactory therapies for severe SOS; the best current results (46% complete response rate, defined as total serum bilirubin <2 mg/dL and resolution of multiorgan failure) are with intravenous defibrotide [87]. Defibrotide, a mixture of porcine oligodeoxyribonucleotides, has antithrombotic and profibrinolytic effects in vitro and in vivo. However, its mechanism of action in the treatment of SOS is not known. The complete recovery of some patients with severe SOS and multiorgan failure suggests that the drug has biologic effects in man; defibrotide appears to be safe when given after HCT [88]. There have been no randomized trials.

Numerous other approaches to the treatment of severe SOS have been reported (tissue plasminogen activator, intravenous *N*-acetylcysteine, human antithrombin III concentrate, activated protein C, prostaglandin E_1, prednisone, topical nitrate, vitamin E plus glutamine, and use of a liver assist device), but none can be currently recommended. Transhepatic shunts have been placed in patients with SOS to reduce portal pressure and mobilize ascites, but neither serum bilirubin levels nor patient outcomes were improved. Patients with persistent ascites and normal serum bilirubin have undergone successful portosystemic shunts. Peritoneovenous shunts for intractable ascites have been unsuccessful. Successful liver transplants for severe SOS have been reported [89]. However, in most centers, patients at risk for recurrent malignancy are low-priority candidates for a cadaveric liver. Prevention of sinusoidal injury has proven to be a more effective strategy for improving transplant outcomes than treatment of patients with severe sinusoidal damage [2].

Cholestatic disorders: cholangitis lenta, acute GVHD, and drug-induced liver injury

Prophylactic ursodeoxycholic acid reduces the frequency of cholestasis in general and GVHD-related cholestasis specifically and improves outcomes, compared with placebo [2,67]. Now that the frequency of SOS has declined greatly, the leading cause of post-HCT jaundice is cholestatic liver injury [1,59].

Cholangitis lenta

Hyperbilirubinemia is common when patients are neutropenic and febrile and have gut mucosal injury from the conditioning regimen. Hepatocyte retention of conjugated bilirubin is mediated by endotoxins, IL-6, and TNF-α [90]. Although this disorder is often referred to as "cholestasis of sepsis," it occurs in patients with fever alone and in the presence of localized infection in the lungs and soft tissues.

Acute GVHD

Acute GVHD develops in up to 70% of allograft recipients. Prophylaxis with ursodiol has greatly decreased the frequency of jaundice after transplant and has altered the clinical phenotype of GVHD [2,67]. In retrospect, what had been called hepatic GVHD is a mélange of three processes. The first process is jaundice developing in a patient with cutaneous and intestinal GVHD. A blinded study could identify no histologic features characteristic of GVHD when liver biopsies were performed within several weeks of the onset of GVHD [91], suggesting that jaundice occurring early in GVHD is related to cholestasis caused by cytokines such as IL-6 [42]. The second process is characterized by increases in bilirubin, AP, and γ-glutamyl transferase (GGT), usually in patients with gastrointestinal GVHD, in whom liver biopsies show lymphocytic infiltration of small bile ducts with nuclear pleomorphism, epithelial cell dropout, and cholestasis in zone 3 of the liver acinus [91]. Although the bile duct lesions of GVHD resemble those of primary biliary cirrhosis and some patients have positive antimitochondrial antibodies (AMAs), all AMA-positive samples are false positives and the histology differs (no granulomata or large bile duct lesions are seen in GVHD) [92]. Inflammatory infiltrates may be minimal because of immune suppression. Persistent hepatic GVHD and worsening jaundice are associated with ductopenia. The third process in hepatic GVHD is most commonly seen in allograft recipients on minimal immunosuppression or after donor lymphocyte infusion, in whom GVHD presents as an acute hepatitis with marked elevation of serum ALT [93,94]. Prognosis in patients with GVHD is not related to peak severity of signs and symptoms, but rather to the area under a disease activity curve where persistent jaundice is an independent predictor of mortality [60]. Although anecdotal evidence suggests that some immune suppressive regimens result in resolution of cholestatic liver injury more frequently that with other regimens, data from controlled trials are lacking, for example, pulse CY [95] or extracorporeal photopheresis or switching from one calcineurin inhibitor (CNI) to another or sirolimus or oral budesonide.

Drug-induced liver injury

Cyclosporine inhibits canalicular bile transport and commonly causes mild increases in serum bilirubin without an effect on serum ALT or AP. Tacrolimus less commonly causes cholestasis, except in the setting of toxic blood levels. Many other drugs used after HCT have been associated with liver dysfunction (e.g. trimethoprim–sulfamethoxazole, itraconazole, voriconazole, fluconazole, posaconazole), although drugs are usually not responsible for severe liver injury in this setting [33,71].

Acute hepatitis

The incidence of severe hepatocellular injury after HCT has declined remarkably from the 1990s to the current time, most likely due to declining incidence of severe SOS [2,71]. A sudden rise in serum aminotransferase enzymes (AST and ALT) following HCT is usually due to a non-infective cause such as zone 3 hepatocyte necrosis in SOS (peaking around day 20), hypoxic liver injury (as in septic or cardiac shock or respiratory failure), acute biliary obstruction (choledocholithiasis), drug-induced liver injury, or the acute hepatitic presentation of GVHD [1]. If a likely cause is not apparent, acute viral hepatitis should be suspected; early diagnosis and treatment may prevent a fatal outcome.

Acute hepatitis caused by HSV, varicella zoster virus (VZV), adenovirus, and HBV can lead to fatal fulminant hepatic failure after HCT, whereas hepatic infections caused by CMV and HCV are seldom severe. With routine use of prophylactic acyclovir/valacyclovir, acute hepatitis due to HSV and VZV is now rare; however, human herpesvirus-6 (HHV-6) and HHV-8 reactivation and hepatitis E virus (HEV) as causes of hepatitis have been reported after HCT [96,97]. When there is uncertainty about the cause of rising serum ALT and AST levels, serum PCR tests for herpesviruses, adenovirus, HCV, HBV, and, in some circumstances, HEV should be performed. Blood tests for viruses have largely replaced the need to obtain liver tissue for viral diagnosis, but transvenous measurement of the wedged hepatic venous pressure gradient and transvenous liver biopsy can be useful in the diagnosis of unsuspected SOS or another cause of hepatiocyte necrosis. If acyclovir is not being given, it should be started empirically, particularly if the patient presents with abdominal complaints typical of VZV infection [98]. Adenovirus hepatitis should be suspected if the patient has concomitant pulmonary, renal, bladder, or intestinal symptoms but may present just with fevers, raised liver enzymes, and hypodense regions in the liver on abdominal CT scan along with features of bone marrow suppression [99]; the most effective treatment is cidofovir when given early in the course of infection, but many cases are fatal [100,101]. New agents are in development.

Fulminant hepatitis B may develop during immune reconstitution in patients at risk, but can be prevented with prophylactic antiviral agents [102]. If severe hepatitis B reactivation does occur, usually because a diagnosis of HBV was not made prior to HCT, antiviral therapy with the most potent antiviral drug available (entecavir or tenofovir) should be initiated immediately; however, progression to fatal liver failure is not uncommon [103]. Fulminant hepatitis B has also been reported following discontinuation of prophylactic antiviral therapy, and all patients, particularly those with high pre-transplant HBV DNA levels, should be monitored following antiviral drug withdrawal [104,105].

Chronic hepatitis C in HCT recipients usually results in asymptomatic elevation of ALT from days 60 to 120, coinciding with the tapering of immunosuppressive drugs [25]. DAA therapy after HCT should be considered for patients with fibrosing cholestatic hepatitis C [26]; those with cirrhosis whose condition is deteriorating [28]; and patients who underwent HCT for HCV-related lymphoproliferative disorders [105a]. HCT recipients are characteristically receiving multiple drugs that could have substantial pharmacologic interactions with DAAs (including, but not limited to, cyclophosphamide, etoposide, tacrolimus, cyclosporine, sirolimus, posaconazole, and voriconazole) [105b, 105c, 105d, 105e]. Future DAA regimens are likely to be pangenotypic, of short duration and well tolerated without significant drug drug interactions. Therapy directed at chronic HCV infection should be considered once the patient has ceased all immunosuppressive drugs and has no evidence of active GVHD [106], as discussed in the later section Problems in long-term transplant survivors.

Fungal and bacterial infections

Antifungal prophylaxis has significantly reduced the incidence of hepatic fungal disease in HCT recipients, particularly in those requiring ongoing immunosuppression for treatment of GVHD [107]. If invasive fungal disease involving the liver does occur, presenting features include fever, tender hepatomegaly, and increased serum AP levels; resistant *Candida* species or molds should be suspected [107]. High-resolution CT scan or MRI may demonstrate multiple fungal abscesses, and serologic tests for fungal antigens may be useful for diagnosis [35]. Treatment courses may need to be protracted in immunosuppressed patients with visceral fungal infection, but return of neutrophil function after HCT can effect resolution of previously treatment-refractory *Aspergillus* infection [108]. Bacterial liver abscesses are rare in HCT recipients, probably because systemic antibiotics are so widely used; however, latent mycobacterial infection may reactivate within the liver with prolonged immunosuppressive therapy. Disseminated Bacille Calmette–Guérin infection with liver involvement has been reported. Disseminated clostridial infection and gall bladder infection with gas-producing organisms may lead to air in the liver and biliary system.

Gall bladder and biliary disease

Biliary sludge (calcium bilirubinate and crystals of CNIs) is very common in HCT recipients [109]. Biliary sludge is usually asymptomatic; however, passage down the bile duct may cause epigastric pain, nausea, and abnormal serum liver enzymes. Biliary sludge may be a cause of acute "acalculous" cholecystitis, acute pancreatitis, and bacterial cholangitis [110,111]. Acute cholecystitis is uncommonly seen in HCT recipients and is frequently without larger gallstones [112]. Cholecystitis in this setting may also be due to leukemic relapse with gall bladder involvement or infection by CMV, fungi, or rarely by other organisms such as *Hemophilus influenzae*. GVHD may affect gallbladder mucosa.

Diagnosis of cholecystitis after HCT is difficult because of the high frequency of gall bladder abnormalities on ultrasound or CT in asymptomatic patients. Pericholecystic fluid, gall bladder wall necrosis or localized tenderness suggest cholecystitis. Non-visualization of the gall bladder after a radionuclide bile excretion study with morphine infusion suggests cholecystitis. Biliary obstruction is a rare event, caused by a variety of disorders [e.g., common bile duct calculi or inspissated biliary sludge; GVHD of the ampullary mucosa; lymphoblastic infiltration of the common bile duct, gall bladder, and ampulla of Vater in Epstein–Barr virus (EBV) lymphoproliferative disease; CMV-related biliary disease; dissecting duodenal hematoma complicating endoscopic biopsy; and leukemic relapse (chloroma) in the head of the pancreas] [111,112]. In patients undergoing autologous transplantation, biliary strictures are commonly due to recurrent malignancy [113]. Non-invasive imaging (ultrasound, magnetic resonance cholangiopancreatography, CT cholangiography) or endoscopic ultrasound is usually adequate to demonstrate biliary pathology. Use of ursodiol as a stimulant of bile flow in patients with biliary sludge after HCT is ineffective. Endoscopic retrograde cholangiopancreatography is indicated only in patients with clinical evidence of cholangitis and radiologic evidence of biliary obstruction and allows for biliary stenting or dilatation with acceptable risk [112,113].

Malignant disorders

EBV lymphoproliferative disease is now an infrequent complication of HCT, largely because of EBV DNA surveillance and preemptive treatment with rituximab. The highest incidence is in recipients of HLA-mismatched, T-cell-depleted grafts and those receiving potent anti-T-cell therapies for GVHD. Symptoms include fever, sweats, generalized malaise, enlarged tonsils, and cervical lymphadenopathy, often with liver involvement (abnormal serum ALP and hepatosplenomegaly) and gastrointestinal invasion. Recurrent cancer in patients transplanted for leukemia, lymphoma, or solid tumors may present with abnormal liver enzymes, hepatomegaly, or abnormal imaging studies within the first year after HCT.

Idiopathic hyperammonemia and coma

A syndrome of hyperammonemia and coma has been described in patients who received high-dose chemotherapy, including conditioning for HCT [114]. Patients present with progressive lethargy, confusion, weakness, incoordination, vomiting, and hyperventilation with respiratory alkalosis. The diagnosis is confirmed when the plasma ammonia exceeds 200 μmol/L and there is no evidence of liver failure. This syndrome is rare, but is associated with high mortality. Its pathogenesis likely involves the unmasking of a latent genetic disorder similar to ornithine transcarbamylase deficiency in children [115]. If available, urinary metabolic screen should be performed.

Gastrointestinal bleeding

Bleeding that does not require transfusion is very common, particularly when platelet counts are below 50×10^9/L. Causes include retching trauma to esophageal or gastric mucosa, mucosal injury from conditioning therapy, peptic esophagitis, *C. difficile* colitis, anal fissures, internal hemorrhoids, and acute GVHD. The incidence of severe gastrointestinal bleeding after HCT is 1–2% – lower than in the past because of effective prophylaxis against viruses, fungi, and acute GVHD [116]. Mortality from severe intestinal bleeding, however, remains at 40% [116,117].

The most common cause of severe bleeding is refractory acute GVHD, with bleeding from extensive ulceration in the small intestine and cecum. In some patients with GVHD, bleeding may appear to be coming from specific areas of the mucosa, but when such patients are operated on or come to autopsy, diffuse rather than focal mucosal ulceration is the rule [118]. Bleeding is an independent predictor of mortality in patients with severe gut GVHD [61]. Ulcers in the stomach or duodenum that develop after HCT are usually caused by acute GVHD or CMV infection, but with ganciclovir prophylaxis and preemptive therapy, bleeding CMV ulcers have become rare [116]. Gastric ulcerations may also be caused by infection by VZV, bacteria (phlegmonous gastritis) or EBV (lymphoproliferative disease). Although now rare after HCT, EBV lymphoproliferative disorder involves the gastrointestinal tract in 20–25% of cases and can present as gastrointestinal bleeding. Gastric antral vascular ectasia is also a cause of severe upper intestinal bleeding in HCT recipients, particularly those who have received oral BU [119]. Diffuse areas of hemorrhage are seen in the gastric antrum and occasionally small and large intestine, but the underlying mucosa is intact [119–121]. Histology is diagnostic, revealing abnormal dilated capillaries, thromboses, and fibromuscular hyperplasia in the lamina propria. Patients who are transplanted for advanced systemic sclerosis may bleed from similar vascular lesions. Endoscopic laser therapy and argon plasma coagulation are treatments of choice to control bleeding from vascular ectasia, but multiple treatments may be required to obliterate ectatic lesions [119]. Other rare causes of bleeding post-HCT include ulcers caused by molds, yeasts, Dieulafoy lesions, Curling (stress) ulcers, duodenal biopsy sites, mycophenolate-induced enteritis, adenovirus enterocolitis, and *C. septicum* infection (typhlitis). Patients with severe amyloidosis who undergo autologous HCT may bleed from multiple ischemic ulcers throughout the intestine [122].

There is no effective therapy for mucosa that is diffusely oozing blood other than raising the platelet count to $>50 \times 10^9$/L and treating the underlying condition. Continuous infusion of octreotide at 25 μg/hour has resulted in cessation of bleeding from GVHD, but recurrent bleeding developed when infusion was decreased [123]. In GVHD, re-epithelialization of ulcerated intestinal mucosa is very slow. Focal lesions, especially mucosal infection, can be treated with endoscopic cautery, heater probe, or epinephrine injection provided that platelet counts are adequate, but these therapies are futile in mucosa that is oozing blood diffusely. Unless the underlying disease process is eliminated, endoscopic methods will not cure the bleeding problem. Attempts to resect large segments of diffusely bleeding intestine involved with GVHD have not been successful [118].

Dysphagia, painful swallowing, and esophageal pain

Oral and hypopharyngeal mucositis, acid–peptic esophagitis, and pill esophagitis are currently the leading causes of dysphagia and esophageal pain. Infections of the esophagus (fungal, viral, bacterial) have largely disappeared because of antimicrobial prophylaxis; when fungal esophagitis is discovered in a patient receiving fluconazole, the organism is likely to be a resistant candidal species or a mold [124]. Rarely, fungal esophagitis can lead to perforation [125].

Mucositis caused by conditioning therapy may lead to pain on initiating a swallow and inability to move a bolus past the cricopharyngeus, symptoms that can also be seen with oropharyngeal GVHD or HSV infection. Rarely, non-healing esophageal ulcerations, strictures, and dysphagia result from conditioning therapy [126]. In patients with gastric stasis related to acute GVHD, painful esophagitis is common, caused by reflux of both gastric acid and bilious fluid. The use of proton pump inhibitor therapy, although effective in treating acid–peptic reflux, may lead to colonization of the aerodigestive system with bacteria and fungi, and increased risk of *C. difficile*-associated colitis [127,128]. Symptom relief can be obtained by treating the cause of gastric stasis and placing the patient in the reverse Trendelenburg position when in bed. The abrupt onset of severe retrosternal pain, hematemesis, and painful swallowing suggests a hematoma in the wall of the esophagus, a result of retching when platelet counts are very low [129]. Endoscopy is relatively contraindicated, as many intramural hematomas represent contained perforations. CT is the diagnostic test of choice [130]. The course of intramural hematomas is one of slow resolution over 1–2 weeks. In patients with severe GVHD, esophageal edema, erythema, and a peeling epithelium lead to ulcerations [131]. Pill esophagitis occurs after ingestion of some medications that might be used after HCT, for example phenytoin (dilantin), foscarnet, captopril, oral bisphosphonates, ascorbic acid, ciprofloxacin, clindamycin, and oral potassium chloride. Most cases resolve within a few days without specific therapy beyond discontinuance of the offending medication.

Diarrhea

Conditioning therapy

Diarrhea caused by mucosal damage from conditioning therapy is seldom severe, usually resolving by day 12–15, with some exceptions. Cytarabine-containing regimens, high-dose melphalan (≥ 200 mg/m^2), and regimens containing multiple alkylating agents may cause more severe, protracted diarrhea. Intravenous infusion of octreotide and oral loperamide (at 4 mg orally every 6 hours) may be effective for severe diarrhea associated with conditioning therapy [132]. There is a correlation between the amount of diarrhea in the week following completion of conditioning therapy, serum levels of TNF-α and IL-6, and the risk of subsequent GVHD [133,134].

GVHD

Acute GVHD is the most common cause of diarrhea after day 15 [135]. However, GVHD may not be the sole cause of diarrhea in an individual patient, especially in patients receiving prolonged immune suppression, in whom restaging the cause of diarrhea may detect infections not present at baseline [136]. The onset of GVHD diarrhea can be sudden, with volumes in excess of 2 L daily in severe cases. GVHD in patients who were grafted after reduced-intensity conditioning regimens may have its presentation delayed, occurring after day 100 in many patients [137]. The diarrheal fluid of GVHD is watery, green in color, with ropy strands of mucoid material that reflect transmucosal protein loss. In an allografted patient with skin and liver abnormalities typical of acute GVHD, this diarrheal syndrome is almost diagnostic of intestinal GVHD, particularly when accompanied by decreasing serum albumin and negative stool studies for infection [138]. In atypical cases where GVHD is suspected, abdominal imaging with CT may reveal intestinal edema and mucosal enhancement, particularly in the ileum and right colon, although the appearances of acute GVHD and CMV infection overlap [139,140]. Ultrasound can also demonstrate bowel wall thickening and hypervascularity; contrast-enhanced ultrasound may provide sufficient specificity for diagnosis of GVHD [141,142]. Similar information can be obtained with positron emission tomography (PET) imaging. Pneumatosis intestinalis, which may be associated with GVHD or CMV enteritis, may be seen by plain X-ray, CT, or MRI, and in the absence of ancillary evidence of clinical severity, managed conservatively [143].

A definitive diagnosis of GVHD in problematic cases requires endoscopic views of mucosa, histology of mucosal tissue, and exclusion of infection. What the endoscopist sees should carry equal diagnostic weight to what the pathologist sees through the microscope [48]. In mild cases, gastroduodenal and rectosigmoid mucosa are grossly normal, but moderately severe GVHD causes diffusely edematous and erythematous mucosa throughout the gastrointestinal tract. Severe GVHD may lead to ulcerations and large areas of mucosal sloughing in the stomach, small intestine, and colon [144]. Even when the appearance is normal, biopsies from the gastric antrum or rectosigmoid colon often reveal intestinal crypt necrosis and apoptotic bodies diagnostic of acute GVHD [145]. The diagnostic yield of mucosal biopsy is best when biopsies are obtained either from the stomach and distal colon or from the colon and ileum [135,144,145]. Reports by endoscopist and pathologist can be discordant, as mucosal edema and erythema are not criteria that are used for the histologic diagnosis of GVHD; visual inspection of the mucosa should be viewed as complementary to histologic findings. Other histologic findings that support the diagnosis of GVHD include pericapillary hemorrhage, infiltrating neutrophils or eosinophils, and evidence of endothelial damage. The use of capsule endoscopy for diagnosis of GVHD has been described; this method does not allow biopsy, but can provide visual inspection of the small intestine that cannot be seen with routine endoscopy [146]. The predictive value of a negative capsule endoscopy examination of the small intestine appears to be high, and therefore useful for excluding more severe acute GVHD.

In severe cases of GVHD, whole crypts are destroyed, then adjacent crypts, and finally whole segments of intestinal mucosa. Bleeding often accompanies diarrhea in patients with mucosal ulceration [116]. Successful treatment of acute GVHD with immunosuppressive therapy results in a dramatic reduction in stool volume, with resolution of accompanying symptoms of abdominal pain, nausea, and vomiting. The management of patients whose diarrhea and other symptoms of intestinal GVHD persist after 5 days of immunosuppressive therapy is unsatisfactory, as the rate of failure of secondary therapy is high [147]. Mortality in patients with high-volume diarrhea caused by GVHD can be predicted by a panel of clinical features within 14 days of onset: jaundice, age over 18 years, lack of response to prednisone, and gastrointestinal bleeding [61]. Other methods of predicting outcome in patients with GVHD include calculation of the Acute GVHD Activity Index, fecal calprotectin level, GVHD Risk Score measurement of decline of serum albumin from baseline, and panels of serum biomarkers [60,138,148,149a,b,c]. Plasma biomarkers that appear to be useful in predicting more severe GVHD and non-relapse mortality include TIM3, sTNFR1, ST2, Reg3a, IL6, and angiogenic factors [149d, 149e, 149f, 149g, 149h]. It is not clear whether early predictors of mortality in patients with severe GVHD have any utility in preventing death in the absence of curative therapy.

Infection

In allograft recipients, infectious causes of diarrhea are far less common than GVHD, accounting for only 10–15% of diarrheal episodes [117,135,150]. Common bacterial pathogens are extremely rare after HCT [150]. except in geographic areas where such infections are prevalent [7]. There are several infections that can closely mimic intestinal GVHD or, more commonly, coexist with GVHD, including non-culturable enteric viruses (norovirus, rotavirus, astrovirus), mycobacteria, fungi, and parasites [124]. *C. difficile* colitis is usually a relatively mild, treatable disease when diagnosed at the onset of diarrhea [135,151]. The recent emergence of more virulent strains of *C. difficile* has changed the natural history of this infection, and prevention of nosocomial *C. difficile* infection in both hospital and outpatient settings has therefore become even more important. One often overlooked factor is the inappropriate use of proton-pump inhibitors for marginal indications, which increases the risk of *C. difficile* colitis twofold [128]. The most common cause of infectious diarrhea in one prospective study was astrovirus (a small, round virus similar to norovirus); diagnosis can be made by PCR of stool specimens or by electron microscopy of small intestinal biopsies [135,152]. Norovirus may also spread within hospitals as a nosocomial infection in HCT patients, with devastating consequences [11]. Some serotypes of adenovirus cause necrotizing enteritis and rapidly fatal multiorgan failure involving the gut, liver, lungs, and kidneys [124,153]. There should be a sense of urgency in identifying adenovirus as a cause of enteritis, as delayed treatment is often ineffective [154,155]. However, not all patients in whom adenovirus is found in diarrheal stools have adenovirus enteritis.

CMV is the only common infectious cause of enteritis after HCT that requires an intestinal biopsy for diagnosis [135]. As with adenovirus, CMV DNA can be detected in diarrheal stool specimens from patients who do not have demonstrable mucosal CMV disease. CMV can be found in mucosal biopsies in patients whose blood is negative for CMV antigen or DNA [3,156]. Otherwise, the predictive value of a negative stool examination for other viruses, bacteria, fungi, and parasites is high, particularly if molecular methods are used [157]. After HCT, watery diarrhea secondary to intestinal parasite infection (*Cryptosporidium*, *Giardia lamblia*, *Entamoeba histolytica*, helminths) is rare among patients who come to transplant without diarrhea, but sporadic cases are seen that can be confused with GVHD [9,10,158]. *Strongyloides* infection and hyperinfection syndrome have been described after HCT; patients from endemic areas should be screened before HCT [159]. Non-tuberculous mycobacterial infection of the small intestine has been described post-HCT [160,161].

Other causes of diarrhea

In addition to infection, several other conditions may mimic GVHD or may contribute to diarrhea that might otherwise be attributed to GVHD, for example, brush border disaccharidase deficiency, bile salt malabsorption, pancreatic insufficiency, mucosal toxicity from MMF, and intestinal thrombotic microangiopathy (Table 94.2).

Intestinal inflammation often results in downregulation of brush border disaccharidases such as lactase and sucrase/isomaltase, leading to diarrhea if lactose or sucrose is ingested. Failure of bile salt absorption in the small intestine leads to inefficient water transport in the colon, a mechanism of diarrhea in patients with gut GVHD that can be treated with bile acid sequestrants such as colesevelam [162]. Pancreatitis and pancreatic insufficiency have been associated with CNI therapy [163]. Transient pancreatic insufficiency has been described as a consequence of mucosal edema at the ampulla of Vater [164]. Japanese investigators have described intestinal microangiopathy related to CNIs as a disorder that mimics GVHD [165]. MMF causes intestinal ulcerations and apoptotic crypt cells indistinguishable from the histology of acute GVHD [166]. In a patient with intestinal symptoms whose immunosuppressive drug regimen includes MMF, it may be difficult to make a diagnosis of GVHD until MMF has been discontinued. Fewer gastrointestinal side effects have been reported after conversion to enteric-coated mycophenolic acid in solid organ transplant recipients [167]. Diarrhea may also result from carbohydrate malabsorption (particularly in patients on antibiotics that affect the colon flora's ability to salvage carbohydrate), oral magnesium salts, tacrolimus (a motilin agonist), plerixafor (an immunostimulant) and brincidofovir (an oral antiviral drug). The multiple causes of diarrhea not directly caused by GVHD in an allograft recipient may explain why diarrhea is not a component of the Acute GVHD Activity Index that predicts day 200 non-relapse mortality [60]. A syndrome of antibiotic-responsive, culture-negative diarrhea has been reported in cord blood recipients with *Bradyrhizobium enterica* as a futative cause. Pathology of endoscopic biopsies shows chronic active colitis with granulomas, and patients have responded to antibiotics, albeit with a high recurrence rate [168]. This syndrome has not been found in other transplant centers [168a].

Table 94.2 Difficult-to-diagnose disorders that can mimic or complicate gastrointestinal acute GVHD

Disorder	Comments
Mycophenolate mofetil toxicity	A wide range of gastrointestinal pathology has been described in MMF-treated organ transplant patients. Very difficult to differentiate from GVHD in the setting of HCT. Far less gut toxicity is seen with enteric-coated mycophenolic acid
Cytomegalovirus or adenovirus infection	Diagnosis may be difficult when virus in present only in gastrointestinal mucosa (blood can be virus free). Differentiating infection from gut mucosal disease can be difficult
Brincidofovir toxicity	Diarrhea caused by gut mucosal damage is a dose-limiting toxicity of this anti-viral drug.
Cryptosporidial infection	Rare and treatable only by withdrawing immunosuppression. Molecular diagnosis (PCR) is far more accurate than microscopic methods of diagnosis
Non-culturable virus infections (astrovirus, norovirus, rotavirus)	The most common post-HCT infection(s) in a prospective study, but seldom diagnosed because of lack of commercial diagnostic tests. PCR is now widely available for diagnosis of norovirus infection
Tacrolimus-related thrombotic microangiography	The hypothesis is that tacrolimus-related vascular injury leads to ischemic damage to mucosa. Withdrawal of tacrolimus has been followed by improvement in some cases
Non-tuberculous mycobacterial infections	Granulomatous mucosal involvement is part of the spectrum of mycobacterial infections in transplant patients
Diffuse fungal infection	Mold infection leading to granulomatous enteritis has been described
Osmotic diarrhea related to carbohydrate malabsorption	Lactose, sucrose, isomaltose in the diet may cause diarrhea if gut inflammation has led to downregulation of brush-border disaccharidases. Antibiotics that depress anaerobic colon flora will impact the ability of anaerobes to metabolize malabsorbed carbohydrate
Bile acid malabsorption	Bile acids not retrieved in the ileum will inhibit salt and water transport in the colon. Potentially treatable with colesevelam but at the risk of binding oral medications

Abdominal pain

It is extremely important to distinguish abdominal pain as an indicator of a rapidly progressive, fatal illness from illnesses with a benign natural history that require only conservative management. The illnesses that may be rapidly fatal include intestinal perforation, some infections (e.g. typhlitis caused by *C. septicum*, adenovirus enteritis, visceral VZV infection, *Aspergillus* vasculitis), gall bladder necrosis, and bacterial liver abscess. Fortunately, these disorders are far less common than intestinal pseudo-obstruction (dilation of the bowel in the absence of a mechanical obstruction), narcotic bowel syndrome (non-propulsive segmental contractions), acute GVHD, liver pain related to SOS, and hemorrhagic cystitis – causes of severe pain to be sure, but not imminent death. A systematic approach in evaluating abdominal pain in these difficult patients is important in excluding rapidly fatal diseases.

Does the patient need urgent surgery?

Surgical intervention for pain after HCT is a rare event, but is indicated for intestinal perforation, acute cholecystitis, drainage of abscesses, appendicitis, and in some patients with intestinal or biliary obstruction, typhlitis, and dissecting hematomas [169]. Deciding who has one of these conditions as a cause of pain and who does not depends mostly on the clinical situation, abdominal examination, and judicious use of imaging. Modern imaging tests (ultrasound, MRI, and CT scan) and careful examination should allow the surgeon to be highly selective in choosing candidates for operation, Clinical judgment may lead to surgical exploration or laparoscopy in problematic cases when one cannot exclude the possibility of a surgical disease, for example, pneumoperitoneum of unknown cause or possible gall bladder necrosis. Intestinal perforation may develop in the setting of lysis of a transmural lymphoma or metastatic carcinoma shortly after conditioning therapy, or later from CMV ulcers or diverticular perforation. Perforation may present with only mild-to-moderate abdominal pain and pneumoperitoneum on abdominal CT, especially in granulocytopenic patients. Pneumoperitoneum can also be a manifestation of pneumatosis intestinalis, a much more benign process than free perforation, usually caused by CMV or GVHD [170]. Recognition of acute cholecystitis is more difficult, as right upper quadrant pain (usually from SOS) and fever are common in the early post-HCT period, and imaging studies frequently show gall bladder wall thickening and luminal sludge in completely asymptomatic patients. A radionuclide study with morphine that shows filling of the gall bladder suggests that surgical cholecystitis is not present, but false-positive results (i.e. lack of gall bladder filling) do occur in severely ill patients on total parenteral nutrition [171]. The diagnosis of SOS as a cause of pain can usually be resolved by its timing of onset in relation to conditioning therapy, by liver tenderness on examination, and by consistent findings on Doppler ultrasound [1]. When pain is localized to the right lower quadrant, typhlitis (*C. septicum* infection) and appendicitis are diagnoses that can be made by imaging, along with exclusion of other causes of right lower quadrant pain (renal, ovarian, abdominal wall). The most common cause of moderate-to-severe abdominal pain, intestinal pseudo-obstruction with bowel distention, does not usually require abdominal imaging, as the finding of distention and tympany on percussion in the setting of opioid or anticholinergic drug exposure is virtually diagnostic. Most patients with pseudo-obstruction have an underlying intestinal disease, such as enteritis from conditioning therapy, GVHD, or infection. Pseudo-obstruction is more frequent among patients transplanted for lymphoma or myeloma, as a result of intestinal neuropathy from past use of vincristine. In visceral VZV infection, abdominal distention, severe pain, fever, and rising serum ALT levels may precede cutaneous manifestations by up to 10 days – a presentation that occurs only among patients not receiving acyclovir prophylaxis [98,172]. Acyclovir should be started on clinical suspicion while serum is analyzed by PCR for VZV DNA. Clinical pancreatitis is an uncommon cause of abdominal pain in HCT patients; subclinical pancreatitis does occur, with symptoms masked by immunosuppressive drugs [110]. Patients with low platelet counts or prolongation of blood clotting may rarely bleed into the retroperitoneum, abdominal wall, or intra-abdominal viscera, particularly after duodenal biopsy, causing significant pain.

Is the pain a manifestation of acute GVHD?

Acute gastrointestinal GVHD can present with just abdominal pain, but more commonly, a skin rash, nausea, vomiting, anorexia, or diarrhea accompany pain. GVHD causes pain by two mechanisms: gut wall edema and distention with malabsorbed fluid. The presentation can include a rigid abdomen with rebound tenderness, but more commonly crampy and periumbilical pain are present. With gastric mucosal edema, pain is localized to the epigastrium. The decision to treat a patient empirically when definitive evidence of GVHD is not at hand is difficult, but when the pretest probability of GVHD is high and that of perforation or infection low, prednisone therapy at 2 mg/kg/day should be started. CT abdominal imaging or abdominal ultrasound (particularly with sonographic contrast) shows intestinal wall thickening in acute GVHD, a non-specific but highly suggestive finding, particularly when CMV infection is unlikely [139,141,173]. Although one might withhold prednisone therapy until a definitive biopsy diagnosis has been made, delays in therapy may lead to more extensive mucosal necrosis and morbidity. If the pain later proves to be due to causes other than GVHD, prednisone can be discontinued.

Is the pain due to a treatable infection?

Recent advances in antiviral and antifungal prophylaxis have made intestinal and liver infections rare causes of abdominal pain [2]. Disseminated VZV may present with difficult-to-explain abdominal pain 2–6 months after HCT in someone not currently receiving acyclovir or ganciclovir therapy. Importantly, abdominal pain may precede typical VZV skin lesions by several days [98]. Marked hyponatremia due to the syndrome of inappropriate antidiuretic hormone secretion and elevated serum ALT (from VZV hepatitis) have also been observed in this setting [174]. Patients should be started on intravenous acyclovir while a definitive diagnosis is sought (VZV DNA in serum, gastric mucosal or liver biopsy). Pain from visceral VZV infection may be poorly localized, and the patient may not look particularly unwell at presentation, but if not treated promptly, the disease can follow a rapidly fatal course. Other intestinal infections that may present with significant pain include adenovirus, CMV, cryptosporidia, invasive molds such as *Rhizopus*, *C. difficile* colitis, and *C. septicum* cecitis (typhlitis). Symptoms of typhlitis include fever, right lower quadrant pain, nausea and vomiting, diarrhea, occult blood in the stool, and shock; the diagnosis of typhlitis is usually made by imaging studies showing cecal edema [175]. Surgical resection for typhlitis is rarely necessary provided that imipenem and oral vancomycin therapy for *C. septicum* are started along with systemic coverage for luminal bacteria and fungi in febrile patients [176].

Is the pain caused by intestinal pseudo-obstruction?

In patients with intestinal pseudo-obstruction and gaseous distention, medical management is almost always effective and perforation rare. Although there are some infections of gut neurons (VZV and CMV) that lead to pseudo-obstruction, opioid and anticholinergic medications are the usual causes, particularly in patients with gut inflammation. There are three approaches to opioid-related pseudo-obstruction: (1) decreasing the opioid dose to low levels; (2) peripheral μ-opioid receptor blockade with methylnaltrexone [177]; or (3) switching to a κ-opioid agonist (e.g., butorphanol).

Anticholinergic medications are contraindicated in this setting, and drugs with anticholinergic side effects should be discontinued. In patients who are on a respirator, gaseous distention can be prevented by use of a nasoesophageal sump tube connected to suction, thus eliminating swallowed air from entering the gut. Neostigmine (2 mg intravenously) has been successfully used in patients with acute colonic pseudo-obstruction after HCT [178]. If gut distention and pain are caused by fluid, as in severe acute GVHD, or by true obstruction, a rare event, the approach is to place a nasogastric or nasoenteric sump tube to prevent further accumulation, and then address the underlying cause. Octreotide is useful for treating gut distention with fluid related to obstruction.

Identification of other causes of abdominal pain

Once the diseases that need urgent surgical or medical care have been ruled out, one can perform directed diagnostic tests to identify specific causes of pain. Cystitis is usually seen after CY exposure but also occurs with adenovirus or polyomavirus infection; it is always accompanied by hematuria. Hematomas of the abdominal wall or intestine should be suspected in patients with a rapidly expanding anterior abdominal wall mass or obstructive symptoms after intestinal biopsy in the setting of profound thrombocytopenia.

Perianal pain

Perianal pain after HCT can be caused by an anal fissure, a thrombosed external hemorrhoid, cellulitis related to tissue maceration, and infections. In patients with granulocytopenia, infections in the perineum or perianal spaces are usually polymicrobial, arising either from anal glands or from tears in the anal canal. After HCT, these infections can be difficult to recognize because they may not produce abscesses but rather a spreading cellulitis. Extensive supralevator and intersphincteric abscesses may be present without being apparent on external examination [17]. CT or MRI scans or endoscopic or transperineal ultrasound can give a clear view of the anatomy involved, particularly if there is pus present [179]. When antibiotics covering both anaerobic and aerobic bacteria are given to patients with "cryptitis" (incipient perianal infection), far fewer patients require surgical drainage than in the past [18]. Rarely, surgical drainage will be needed for patients with abscesses and rapidly progressive tissue necrosis. Unusual causes of perianal pain include giant condyloma acuminata (genital warts) caused by human papillomavirus, HSV infection, fungal infection, and CMV-induced cutaneous vasculopathy with perineal ulcers [19,180].

Problems in long-term transplant survivors

Gastrointestinal and hepatobiliary problems in the second year after allogeneic HCT are usually a continuation of symptoms of protracted acute GVHD, chronic graft-versus-host disease (cGVHD), medication side effects, and infection related to immune suppression [1,37,181]. As time passes, as tolerance develops, and as immunity improves, the frequency and severity of these problems wane – but new problems involving the gut and liver may arise, sometimes decades later.

Gastrointestinal problems

Esophageal symptoms: heartburn, dysphagia, and painful swallowing

Heartburn may be worse if cGVHD is present because salivary gland destruction reduces bicarbonate production, which normally neutralizes refluxed gastric acid. cGVHD may also involve the esophagus, leading to dysmotility and failure to clear refluxed acid [182]. Protracted acute GVHD involving the stomach leads to gastric stasis and reflux of both bile and acid. To minimize esophageal injury, patients should elevate the head of their bed by 4–6 inches, avoid all protein intake within 4 hours of bedtime, and take a proton pump inhibitor. Less common causes of esophagitis in survivors include fungal and viral infection and inflammation caused by retained pills (pill esophagitis). There is a long list of pills that cause esophageal injury, including non-steroidal anti-inflammatory drugs (NSAIDs) and oral bisphosphonates. Pill esophagitis presents with dysphagia and painful swallowing, and can lead to perforation and difficult-to-dilate strictures if offending pills are continued.

Dysphagia can be a particularly difficult symptom; common causes include esophageal and oral cGVHD, infection, xerostomia, and poor dentition [181]. Tongue and hypopharyngeal inflammation can make initiating a swallow difficult. Myasthenia gravis, a rare complication of cGVHD, is an extreme example of hypopharyngeal dysmotility [183]. Patients with extensive cGVHD often complain of dysphagia, for solid food and pills. Some patients, especially children, present with insidious weight loss, retrosternal pain, and aspiration of gastric contents, leading to pulmonary disease that can be mistaken for GVHD-related bronchiolitis obliterans syndromes. The diagnosis of esophageal cGVHD is made by barium contrast X-ray and endoscopy, which should be done with caution, as perforations have been reported. Radiologic findings include bullae, webs, concentric rings, narrowings, strictures, and aperistalsis [181]. Sloughed mucosa may hang in shreds into the lumen, visible to the radiologist as a web or a fibrous shelf at the level of the cricopharyngeus. Long, tapering strictures in the proximal and midesophagus are better seen by X-ray than by endoscopy. Endosopic findings include bullae, desquamation of the epithelium, upper esophageal webs, ring-like narrowings, and tapering strictures [181]. Histologic findings include infiltration of the esophageal mucosa with lymphocytes, neutrophils and eosinophils, necrosis of individual squamous cells in the basal layer, and desquamation of the superficial epithelium. Patients with esophageal cGVHD need systemic immunosuppressive therapy. However, even when the inflammatory component is under control, submucosal esophageal fibrosis may lead to abnormal peristalsis, luminal narrowing, and persistent symptoms. Esophageal strictures and webs may require endoscopic dilation to avoid progressive luminal narrowing, but dilations must be performed with extreme care, as the perforation risk is higher than with peptic strictures. Failure to dilate strictures may lead to progressive esophageal narrowing and require a feeding gastrostomy. A fibrotic, stenotic esophagus that cannot be dilated may require esophagectomy and interposition surgery. Topical glucocorticoid therapy can be effective for esophageal inflammation [184,185]. The risk of squamous cell carcinoma of the esophagus is 10-fold higher after HCT than among controls; cGVHD is the major risk factor [186].

Upper gut symptoms: anorexia, nausea, vomiting, satiety

Gastroduodenal GVHD in long-term survivors does not fall under the heading "chronic GVHD" but rather "protracted acute GVHD" because symptoms, endoscopic appearance, and histology are identical whether they occur before day 100 or years after transplant [181]. Gastrointestinal symptoms of protracted acute GVHD can appear in the absence of cGVHD elsewhere. Long-term use of oral topical glucocorticoid can be effective in patients with protracted upper gut GVHD [187,188]. However, both prednisone and topical glucocorticoids can lead to suppression of the hypothalamic–pituitary–adrenal axis, particularly in children, and symptoms of adrenal insufficiency (anorexia, nausea) may mimic those of upper gut GVHD. Now rare, a syndrome of diarrhea, abdominal distention, and severe malabsorption has been described in patients with untreated cGVHD who had extensive submucosal and subserosal fibrosis [181]. Focal intestinal strictures, however, can be seen as sequellae of gut GVHD.

Herpesviruses (HSV, CMV, VZV) may cause nausea, vomiting, and satiety in survivors who have not fully reconstituted immunity, especially if prophylactic antiviral medications and viral surveillance have been discontinued. When upper gut symptoms appear along with abdominal distention and elevated serum ALT levels, visceral VZV infection should be at the top of the list of possible diagnoses; the test of choice is PCR for VZV DNA in blood [124]. Gut infection with enteric viruses (e.g. rotavirus and norovirus) and parasites (*Giardia lamblia*, cryptosporidia) can present with anorexia and nausea with minimal diarrhea [10]. In areas where *Helicobacter pylori* is prevalent, it may be difficult to discern the cause of upper gut symptoms when both infection and GVHD are present [189].

Mid-gut and colonic symptoms: diarrhea and abdominal pain

Protracted acute GVHD can cause gastrointestinal inflammation decades after transplant, with symptoms that wax and wane. Diarrheal symptoms usually respond to opioids but can require a short course of immune suppressive medications or topical glucocorticoids. Histologic findings are similar to those of microscopic enterocolitis (lymphocytic inflammation with occasional apoptotic crypt cells) [49]. Some patients who have had more severe GVHD in the past present later with enigmatic abdominal pain, often with a history that suggests intestinal obstruction (pain in the interdigestive period, distention, relief with diarrheal stools). Some will have an identifiable area of obstruction or inflammation on CT enterography, MRI, barium contrast, or capsule endoscopy, but in others, no discrete abnormality can be found [190]. This presentation can also be seen with narcotic bowel syndrome, in patients who still require opioids for pain. Vague abdominal symptoms may be a manifestation of colon or ovarian cancer, particularly in patients over 50 years old.

Sporadic cases of gut infection may occur (*C. difficile*, norovirus, CMV, and rarely *Giardia* and cryptosporidia) [124]. Rarely, chronic intestinal viral infection can be seen in patients who remain on immunosuppressive drugs, for example, rotavirus, norovirus, and adenovirus. These infections may mimic GVHD [10]. MMF may cause gastrointestinal toxicity, usually manifest as diarrhea and colon ulcerations, with apoptotic crypt cells indistinguishable from the histology of GVHD [166]. In a patient with intestinal symptoms whose immunosuppressive drug regimen includes MMF, it may be difficult to make a diagnosis of GVHD until MMF has been discontinued or changed to enteric coated mycophenolic acid [167]. Bile acid malabsorption in the ileum, leading to inhibition of salt and water resorption in the colon, may contribute to persistent lower volume diarrhea; bile acid-sequestering agents such as colesevelam may be helpful [162]. Rare cases of "transmission" of intestinal disease via donor T cells have been reported, for example, inflammatory bowel disease and celiac sprue [191].

Liver diseases

GVHD

There are three presentations of liver GVHD in long-term survivors [1,37,181]: (1) Asymptomatic elevation of serum ALT, AP, and GGT as isolated laboratory abnormalities, in the absence of jaundice; (2) slowly progressive cholestatic jaundice, usually associated with elevated serum AP, a result of damage to small bile ducts; (3) acute hepatocellular injury (hepatitic GVHD), that is, abrupt elevations of serum ALT to over 500 U/L without preceding hepatic dysfunction. Hepatitic GVHD tends to occur especially in the setting of tapering immunosuppressive therapy or after donor lymphocyte infusion [93,94]. Diagnosis of liver GVHD may require a liver biopsy, particularly in patients who develop hyperbilirubinemia. Patients with jaundice secondary to destruction of small bile ducts by GVHD or those with an acute hepatitic presentation of GVHD may die if there is delay in starting immunosuppressive therapy.

In long-term survivors, the differential diagnosis of jaundice is narrower than it is before day 100, and there is an imperative for immediate treatment according to cause. The differential diagnosis of cholestatic jaundice includes GVHD, biliary obstruction, drug-induced liver injury (DILI), and fibrosing cholestatic hepatitis B or C [1]. Iron overload has been reported to mimic mild GVHD [192]. In contrast, patients with asymptomatic elevations of serum ALT, AP, and GGT may have subclinical liver GVHD, DILI, ethanol effect, biliary disease, infiltrative liver disease, or inflammation related to fat accumulation (non-alcoholic steatohepatitis). If a cause of elevated liver enzymes is not apparent, a biopsy may be indicated to identify GVHD as a cause. While elevated ALT, AP, and GGT may presage destructive damage to small bile ducts and ductules, immunosuppressive therapy (and thus liver biopsy) are not usually indicated when stable, minor elevation of serum enzymes is the only finding. The addition of ursodiol (12–15 mg/kg/day) to the immunosuppressive regimen results in significant biochemical improvement in liver GVHD [193]. The long-term use of ursodiol is safe and well tolerated and should be prescribed for survivors with cholestatic liver injury, along with standard immune suppressive drugs used to treat cGVHD [194].

A major issue is how best to treat survivors whose liver GVHD has not responded to standard therapies. Avoiding precipitous decreases in immune suppression and recognizing laboratory signs of impending destruction of small bile ducts caused by GVHD should prevent deep, prolonged jaundice in most. However, some patients reach the point of ductopenia and deep jaundice because of discontinuation of immune suppression to achieve a graft-versus-leukemia (GVL) effect, or because of donor lymphocyte infusion (DLI). Hepatologists have long recognized that recovery from small bile duct injury is highly variable, even when the cause is a drug that can be discontinued, and GVHD is no exception to this variability in outcomes. Some patients whose total serum bilirubin levels over 25 mg/dL have reverted to normal over the course of several months of therapy with prednisone, CNI, and ursodiol therapy [93], but in most such cases, prednisone-sparing immune suppression is sought because of glucocorticoid side effects and infection risk. Several case series, but no randomized trials, have touted as effective treatments pulse cyclophosphamide [95] or extracorporeal photopheresis or switching from one CNI to another or sirolimus. Oral beclomethasone dipropionate or budesonide could be used for hepatic cGVHD, as ~30% of these topical glucocorticoids reach the portal circulation [195] and the liver; this approach to other inflammatory liver diseases has been effective. Liver transplantation, including living-donor transplantation from the original hematopoietic cell donor, has been performed patients with refractory cholestasis or end-stage liver disease due to GVHD [89,196–198].

Patients who present with an acute hepatitis, that is, steeply rising serum ALT with or without jaundice, present a differential diagnosis of viral infection, DILI, and hepatitic GVHD. These patients require urgent diagnosis and treatment. Hepatic histology is usually needed to confirm the diagnosis of GVHD; histological findings include hepatocellular injury, lobular inflammation, lymphocytic infiltration in and around small bile ducts, extensive damage to, and loss of, small bile duct epithelial cells, cholestasis, portal fibrosis, and piecemeal necrosis [93]. A serum autoantibody test for CYP1A2 may prove useful in the diagnosis of hepatic GVHD, as this enzyme appears to be a target antigen in GVHD [199]. Blood tests for viral antigen or DNA/RNA will exclude acute hepatitis due to a herpesvirus (HSV or VZV), a hepatitis virus (hepatitis A–E), or adenovirus. Acyclovir should be started pending results of tests for herpesviruses; if viral studies are negative, a CNI and prednisone 1–2 mg/kg/day should be begun while histology is pending to prevent extensive ductular damage from GVHD [93].

Chronic viral hepatitis and cirrhosis

HCV infection in HCT survivors almost always results in chronic hepatitis. In the first 10 years of HCV infection after HCT, there is little liver-related morbidity except for rare cases of fibrosing cholestatic hepatitis [27]. However, cirrhosis of the liver related to chronic HCV infection is rising in frequency among patients transplanted before the 1990s. In a cohort of 195 HCV-infected HCT recipients followed prospectively, the cumulative incidence by 20 years of severe liver complications, including cirrhosis, liver failure, and liver transplantation, was 11.7% [200]. The rate of progression of HCV is higher in HCT recipients than in matched controls, with reports of cirrhosis in up to 24% by 20 years of follow-up and 35% after 40 years [201]. The reasons for more rapid fibrosis progression after HCT are unclear, but may be related to concomitant liver involvement with GVHD, iron overload [202], and immunosuppression. Clues to the presence of cirrhosis include a switch in the normal ratio of serum aminotransferases so that AST is higher than ALT, thrombocytopenia, and hepatic nodularity and splenomegaly on imaging, although thrombocytopenia may be unrelated to liver disease in HCT survivors. Confirmation of cirrhosis using either liver biopsy or non-invasive methods such as transient elastography [30] is crucial so that antiviral treatment can be prioritized and patients can be monitored for complications including esophageal varices and hepatocellular carcinoma. Chronic hepatitis C may also be a risk factor for development of lymphoma and other lymphoproliferative disorders after transplant [105a,203,204].

All patients with chronic HCV, including those with compensated cirrhosis, should be considered for DAA antiviral therapy unless contraindications exist. Therapeutics for HCV treatment are rapidly evolving; we recommend consulting with a hepatologist or infectious disease specialist and this website for continuously updated information on genotype-specific drugs of choice: http://www.hcvguidelines.org. Future DAA regimens are likely to be pangenotypic, of shorter duration, and without significant drug-drug interactions. Liver transplantation should be considered in any HCT survivor with hepatic decompensation or early hepatocellular carcinoma. Living-donor transplantation from the original stem-cell donor is an attractive consideration in this situation, as minimal immunosuppression would likely be required in the long term [196,205].

The prevalence of chronic HBV infection among HCT survivors varies widely depending on the country. The serologic pattern of HBV infection may be atypical in HCT survivors, probably as a consequence of immunosuppression. Clearance of surface antigenemia may be observed, and is particularly likely if the donor was anti-HBs positive because of prior HBV infection [22]. Patients who remain HBsAg positive after HCT are at risk of flares of hepatitis activity, particularly at times of reduction of immunosuppression, such as during taper or cessation of treatment for chronic GVHD, and there should be a low threshold for having these patients on oral antiviral agents such as tenofovir or entecavir. All long-term survivors with chronic hepatitis B should be regularly monitored to assess virologic and disease status, and the need for antiviral therapy. Hepatitis B DNA levels and liver enzymes should be monitored every 6–12 months regardless of whether the patient is on antiviral treatment. International guidelines should be followed with regard to making decisions about antiviral treatment [206–208]. The need for antiviral treatment with an oral nucleoside or nucleotide analog (such as entecavir or tenofovir) is based on the ALT and HBV DNA levels and the severity of hepatic fibrosis [209], and may change over time, hence the need continually to reassess patients who are not on treatment. Although pegylated interferon is used as first-line therapy in non-transplant patients with chronic HBV, it cannot be recommended in allogeneic HCT recipients because of the concern for flares of cGVHD. Non-invasive assessments of hepatic fibrosis using methods such as transient elastography are proving very useful in monitoring patients with chronic hepatitis B and making treatment decisions [210]. Long-term survivors with chronic hepatitis B do not seem to have an increased rate of progression to cirrhosis compared with non-HCT patients, and effective antiviral treatment should essentially prevent any disease progression. As HCT survivors are at increased risk of second malignancy or recurrent malignancy, care should be taken to reassess HBV viral status prior to reintroduction of chemotherapy [33]. Biologic agents, such as rituximab used in the treatment of B-cell malignancy, have a particularly high risk of reactivation of occult hepatitis B (HBsAg negative and anti-HBc positive), and prophylactic antiviral therapy is recommended. As in the peri-transplant period, patients with positive HBsAg should receive an antiviral agent whenever they receive immunosuppressive or cytotoxic therapy [211].

It is now recognized that HEV may result in chronic infection in immunosuppressed individuals, including HCT recipients. In contrast to water-borne HEV due to genotype 1 and 2 found in the developing world, HEV genotype 3 (HEV3) is found in mammalian reservoirs including domestic pigs, wild boar, and deer in Europe, North America, and China [96]. Infection from ingestion of undercooked meat from HEV3-infected animals may result in acute or chronic hepatitis, and reactivation may occur after HCT [97]. Diagnosis is by detection of anti-HEV antibodies and HEV RNA. Oral ribavirin has been shown to be effective therapy.

Ascites

The most common cause of new ascites in HCT survivors is cirrhosis, either the progression of cirrhosis present before transplant or a result of accelerated development of cirrhosis in patients with chronic hepatitis C [200,201,212] and rarely in patients with hepatic GVHD [198]. There are other, non-cirrhotic causes of enigmatic ascites: constrictive pericarditis, nodular regenerative hyperplasia, pancreatic ascites, peritoneal carcinomatosis, and a serositis syndrome related to cGVHD. The differentiation between intrahepatic sinusoidal hypertension and peritoneal causes of ascites is best made by the combination of the serum-ascites albumin gradient (SAAG) and cytology (to detect malignant cells).

Drug-induced liver injury

DILI may be related to antihypertensive drugs, lipid-lowering agents, hypoglycemic agents, NSAIDs, antidepressants, antibiotics, antifungal agents, or herbal preparations that are prescribed for the complications of HCT [33]. Some drug reactions result in chronic liver disease.

Acute hepatocellular injury

The differential diagnosis of extreme elevations of serum ALT in an HCT survivor includes acute viral hepatitis (including VZV hepatitis), a hepatitic presentation of chronic GVHD, flares of chronic hepatitis B or C, and drug-induced liver injury [1].

Hepatic iron overload

Iron overload may be an important cofactor in liver disease in long-term survivors, but cardiac, endocrine, and cancer-related effects of iron excess often outweigh the clinical impact of excess hepatic iron, as discussed below [37]. Iron overload is rarely, if ever, the sole cause of serious liver disease in HCT survivors. Iron overload is particularly severe in thalassemic patients who have undergone HCT [202]. Iron overload may rarely be a cause of liver enzyme elevation after HCT that responds to iron mobilization [192].

Hepatocellular carcinoma

Compared with the general population, patients who survive over 10 years post-HCT have an eightfold risk of developing a new solid malignancy. Because of the increased rate of chronic viral hepatitis, mostly hepatitis C, the risk of hepatocellular carcinoma is particularly elevated. Transplant survivors with risk factors for hepatocellular carcinoma (HCV cirrhosis, chronic HBV infection, or cirrhosis of any cause) should undergo surveillance with 6-monthly liver ultrasound scans according to international guidelines [213].

Nodular regenerative hyperplasia

Rarely, patients who receive high-dose chemotherapy in either the oncology or HCT setting will develop hepatic nodules (not separated by fibrosis) that compress portal venules [214]. This process is usually clinically silent unless ascites and esophageal varices develop; liver tests are normal. The diagnosis can be challenging, as both liver imaging and wedged hepatic venous pressure measurements can be misleadingly normal. A transjugular intrahepatic portosystemic shunt may be required for management of complications [215].

Focal nodular hyperplasia

In one series of HCT survivors undergoing liver MRI, incidental focal nodular hyperplasia (FNH) lesions were present in 12% [216]. The likely cause is sinusoidal injury caused by myeloablative conditioning regimens. FNH lesions have characteristic central scars that differentiate them from hepatocellular carcinoma and fungal lesions. Because their appearance is so characteristic on MRI [217], liver biopsy is often not necessary. However, when serum α-fetoprotein levels are elevated in patients with FNH, biopsy may be needed to exclude a hepatocellular carcinoma.

Fungal and bacterial infections

These liver infections are now rare late complications. Fungal or mycobacterial infection in the immediate aftermath of HCT can become quiescent, only to flare when exposed to prednisone. Nonsterile herbal remedies contaminated by molds and ingested may also lead to liver abscesses in survivors [218].

Gallbladder and biliary diseases

Long-term survivors have an increased incidence of gallstones and gallstone complications related to the formation of calcium bilirubinate microliths (biliary sludge) following myeloablative conditioning therapy [109]. These microliths serve as nucleating material for development of gallstones whose subsequent accretions are mostly cholesterol, yielding mixed pigment–cholesterol stones. Biliary sludge, gallstones, and crystals of CNI medications may cause cystic duct obstruction, common bile duct obstruction, and acute pancreatitis [111].

Pancreatic disease

Acute pancreatitis is very uncommon in long-term survivors, with most cases related either to biliary stone passage or, rarely, to tacrolimus-related pancreatic damage [219]. Pancreatic insufficiency has been noted in a small number of transplant survivors, usually diagnosed because of steatorrhea and weight loss despite adequate caloric intake. The etiology of pancreatic atrophy after HCT is not known; speculation centers around repeated subclinical episodes of pancreatitis, toxicity from drugs (prednisone, tacrolimus), stone or sludge passage, glandular destruction by GVHD, and infection by viruses with tropism for the pancreas [220,221]. Fat malabsorption caused by pancreatic insufficiency is diagnosed by a finding excessive fat in stool sample with a qualitative Sudan stain plus a dramatic response to pancreatic replacement therapy. Useful screening tests include measurement of serum trypsinogen (levels <20 ng/mL) and measurement of fecal elastase-1 and chymotrypsin. Pancreatic atrophy is detected by CT or MRI imaging [181,221]. Oral pancreatic enzymes are the only treatment for pancreatic insufficiency unless the cause is duct obstruction in the head of the pancreas.

Systemic iron overload

Secondary iron overload is caused by both repeated transfusions and increased gastrointestinal iron absorption in the setting of ineffective erythropoiesis and inflammatory conditions, including GVHD. Morbidity from iron overload in transplant survivors comes mostly from cardiac iron accumulation, which is only marginally correlated with liver iron content [222].Serum ferritin is commonly used as an indirect measure of body iron stores, but specificity is problematic as serum ferritin can be elevated in a variety of infectious and inflammatory states [36]. Iron-specific MRI methods can accurately measure not only liver iron, but also iron accumulation in the heart, pituitary, pancreas, and thyroid [223].

The effects of persistent iron overload on long-term HCT outcomes have not been fully investigated except in patients transplanted for thalassemia, in whom very high iron burdens translate to increased mortality from cardiac causes. However, recent studies suggest that iron overload could be a risk factor for increased non-relapse mortality among HCT survivors with less extreme iron burdens than in thalassemia patients [37]. Patients who died before day 100 after allogeneic HCT had levels of liver iron in the hemochromatosis range (1832–13,120 μg/g dry weight) [224]. The fate of excessive iron stores after successful HCT has been studied using serum ferritin, showing that serum ferritin levels fall slowly over time, reaching normal levels five or more years after HCT; the greater the iron loading at transplant, the longer it takes for normalization of serum ferritin [225,226]. In heavily iron-overloaded survivors, iron reduction therapy may significantly improve transplantation outcomes [227] and cardiac function, but few data are available for transplant survivors from underlying diseases other than thalassemia major.

Potential clinical consequences of iron overload in transplant survivors include cardiac, endocrine, and neurological disorders and development of secondary malignancy [37]. The risk of fatal cardiac ischemia, cardiomyopathy, and arrhythmias in HCT survivors is increased 3.6-fold over controls [228]. Although these late cardiac complications have been attributed to anthracycline therapy, myeloablative doses of cyclophosphamide, and chest radiation, elevated cardiac iron may play a role, as suggested by epidemiologic data from non-transplant patients with iron overload. In one study, some patients continued to accumulate cardiac iron despite a negative liver iron balance, suggesting that heart iron accumulation and cardiac dysfunction can occur despite good control of liver iron. Below a myocardial T2* of 20 ms, a progressive and significant decline in left ventricular ejection fraction has been noted. Data on cardiac iron loading in candidates for HCT and transplant survivors are limited. A study of 11 extensively transfused patients with myelodysplastic syndrome showed only one patient with T2* myocardial iron excess; this patient was without cardiac dysfunction [229]. Among 48 patients with early-stage hematologic malignancy, 85% had hepatic iron overload at the time of HCT (median 3100 μg/g dry weight), while only one patient had cardiac iron overload, defined as cardiac T2* <20 ms [230]. One study of 16 HCT survivors with liver iron concentrations of >5000 μg/g dry weight reported only one patient with cardiac dysfunction [231]. These results suggest that while significant hepatic iron overload is common in patients with hematologic malignancy before and after HCT, cardiac iron overload might be quantitatively less in contrast to patients with thalassemia major. At what point HCT survivors with liver iron excess develop cardiac iron deposition, at what point cardiac dysfunction develops after iron deposition, and the natural history of cardiac iron excess after transplant recovery are unknown.

Iron as a cancer-promoting factor has long been suggested on the basis of epidemiologic studies in both the population at large and in patients with genetic hemochromatosis [37]. The role of iron as a risk factor for cancer may not require iron excess, as two studies suggest that even in patients with iron levels in the normal range, iron mobilization may result in a lower incidence of cancer [232,233]. An increased risk (1.85–34-fold) of secondary malignancy in HCT survivors has been well documented and the cumulative incidence continues to increase over time [234,235]. No data are available on iron overload in long-term HCT survivors as a risk factor for secondary solid malignancy. If such a correlation exists, there is opportunity for cancer prevention through mobilization of iron stores.

Endocrine dysfunction can result from iron deposition in the anterior pituitary, pancreas, and thyroid; in patients with extreme iron overload such as thalassemia, these abnormalities may cause significant clinical effects [37]. Whether similar effects are related to iron excess in transplant survivors has not been studied.

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CHAPTER 95

Lung Injury Following Hematopoietic Cell Transplantation

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Introduction

Lung injury occurs frequently following hematopoietic cell transplantation (HCT) and significantly contributes to morbidity and mortality in the immediate post-transplant period and in the months and years that follow [1–4]. Historically, approximately half of all pneumonias seen after HCT have been secondary to infection, but the judicious use of broad-spectrum antimicrobial agents has tipped the balance towards non-infectious causes [5]. However, infectious lung injury remains a significant problem, particularly in patients with acute or chronic graft-versus-host disease (GVHD) or poor/delayed immune reconstitution. Non-infectious lung injury can be either acute or chronic depending upon the time of onset after HCT and the tempo of disease progression. Chronic non-infectious lung injury is further subdivided into two types: obstructive and restrictive [6]. Although non-infectious lung injury can be observed following autologous HCT, the allogeneic setting significantly enhances the severity of disease in both the acute and chronic time frames. This chapter reviews the definitions, risk factors, and pathogeneses of lung injury occurring both early and late after allogeneic HCT.

Diagnostic considerations and role of bronchoalveolar lavage

The approach to HCT patients with pulmonary dysfunction is complex as both pulmonary and non-pulmonary causes are possible. Because symptoms of respiratory distress can progress rapidly once established, the timely coordination of care is essential to optimize outcomes (Figure 95.1). A meticulous history and physical examination are paramount and will help dictate which diagnostic tests and possible consultation with experts in the fields of pulmonology, cardiology, nephrology, radiology, and critical care medicine may be necessary. Determination of the severity of respiratory dysfunction, including an assessment of supplemental oxygen requirement, overall fluid balance, renal function, and cardiac output should be followed by radiographic imaging. An initial chest X-ray or computed tomography (CT) scan may identify the presence of lobar, multilobar, or diffuse pulmonary infiltrates. Although such findings may impact the decision-making process, they are non-diagnostic in and of themselves.

In the absence of obvious left-sided heart failure or iatrogenic fluid overload, bronchoscopy with bronchoalveolar lavage (BAL) or surgical lung biopsy should be considered when multilobar or diffuse infiltrates are present in order to distinguish optimally between infectious and non-infectious causes. BAL samples should be sent for a variety of diagnostic tests to determine the presence of community-acquired and/or hospital-acquired opportunistic infections. In addition to bacterial, fungal, and cytologic stains, quantitative cultures, direct fluorescent antibody stains, and centrifugation (shell vial) cultures should be performed. The emergence of a battery of polymerase chain reaction (PCR) assays may also be very useful, particularly when interpreted in the context of other supportive and correlative data. *Pneumocystis jiroveci (carinii)* pneumonia (PJP) may be identified through a number of techniques, including cytologic studies, special stains, or PCR-based assays.

Despite a large number of published reports, the need to complete BAL in HCT recipients with respiratory compromise remains a matter of debate, particularly in those patients who are critically ill. The diagnostic yield from BAL fluid has ranged from 31 to 67% [7]. Patients are often referred for BAL after several days of symptoms, and often after empiric antibiotic therapy has been initiated. Unfortunately, this approach provides inadequate coverage in over 40% of patients who ultimately are found to have infection [8], and may limit the diagnostic utility of subsequent bronchoscopic procedures. In a large retrospective study of 598 patients who underwent BAL within the first 100 days after HCT at the M.D. Anderson Cancer Center, the overall diagnostic yield of BAL was 55%, with the yield 2.5 times greater amongst patients in which a BAL was performed within the initial 4 days of clinical presentation. In addition, pneumonia-associated deaths were three times higher (18% versus 6%) in those patients undergoing bronchoscopy at least 4 days after onset of clinical symptoms [9]. Yanik et al. examined 444 BAL procedures completed on 300 patients (20% of all patients) who received HCT at the University of Michigan from 2001 to 2007 [10]. It was found that 13% of BAL specimens collected in the first 30 days of HCT were positive for infection, which increased to 33% between days 31 and 100. Hence, although the majority of BAL fluid samples collected within the first 100 days may be negative, a significant number of individuals had evidence for infection. To this end, BAL data resulted in changes in medical management in approximately 60% of cases, and modifications in antimicrobial therapy in just under half. Since the medical management for non-infectious and infectious pneumonia is quite different, making the appropriate diagnosis is important. In both the M.D. Anderson and

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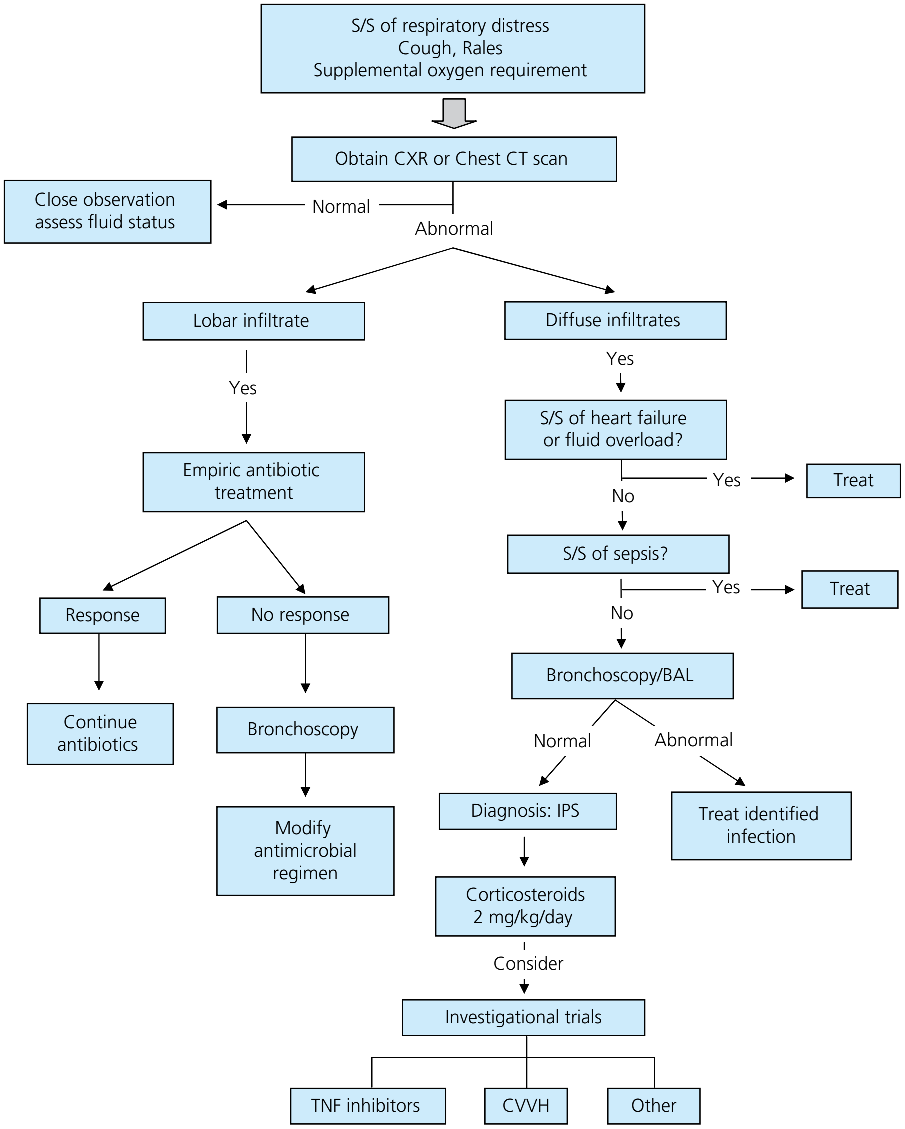
S/S of respiratory distress
Cough, Rales
Supplemental oxygen requirement
Obtain CXR or Chest CT scan
Close observation
assess fluid status
Normal
Abnormal
Lobar infiltrate
Diffuse infiltrates
Yes
Yes
S/S of heart failure
or fluid overload?
Yes
Treat
No
Empiric antibiotic
treatment
S/S of sepsis?
Yes
Treat
No
Response
No response
Bronchoscopy/BAL
Continue
antibiotics
Bronchoscopy
Normal
Abnormal
Diagnosis: IPS
Treat identified
infection
Modify
antimicrobial
regimen
Corticosteroids
2 mg/kg/day
Consider
Investigational trials
TNF inhibitors
CVVH
Other

Figure 95.1 Approach to HCT patients with acute respiratory dysfunction. The approach to HCT patients with acute pulmonary dysfunction is complex. The timely determination of the severity of respiratory dysfunction, including an assessment of the need for supplemental oxygen support, overall fluid balance, renal function, and cardiac output should be followed by radiographic imaging and consideration for bronchoscopy and bronchoalveolar lavage. CXR, chest X-ray; S/S, signs and symptoms.

University of Michigan series, the bronchoscopy-related complication rates were less than 2.0%.

Infectious lung injury

Major factors contributing to the development of infectious lung injury following HCT include the suppression of laryngeal or cough reflexes, impaired removal of respiratory secretions due to decreased mucociliary clearance or airway obstruction, and dysfunctional or absent humoral and cellular defense mechanisms. Quantitative and/or qualitative defects in neutrophil or lymphocyte function can allow non-pathogenic organisms ultimately to become both invasive and pathogenic. A fine balance exists between those organisms which become true pathogens and those which remain commensurate, with the scales capable of tipping in either direction based on the quantity of inoculum received and the host-defense factors outlined above. Infectious pneumonias may be subdivided into those associated with either interstitial or parenchymal involvement. Common pathogens that cause interstitial pneumonitis include community-acquired respiratory viruses [e.g., parainfluenza, respiratory syncytial virus (RSV), influenza, metapneumonia], mycoplasma, and opportunistic pathogens such a *P. jiroveci*, whereas bacterial and fungal pathogens are more frequently associated with parenchymal changes (Table 95.1). While advancements in molecular techniques and the development of improved anti-microbial therapies have helped improve early detection and successful management of pneumonia caused by several opportunistic organisms, infectious lung injury remains a significant problem following HCT particularly in patients with GVHD or delayed immune reconstitution. The epidemiology, clinical spectrum of disease and overall impact on HCT outcomes of opportunistic bacterial, fungal and viral (including CMV) respiratory infections are described in Chapters 82, 85, and 92, respectively.

Table 95.1 Common infectious pathogens of diffuse interstitial pneumonia

Viral:
- Cytomegalovirus (CMV)
- Adenovirus
- Human herpes virus 6 (HHV-6)
- Varicella virus
- Respiratory syncytial virus
- Parainfluenza type 1 and 2
- Influenza A and B
- Rhinovirus
- Meta-pneumovirus

Protozoan:
- *Pneumocystis jirovecii* pneumonia (PCP)
- *Toxoplasma gondii*
- *Mycoplasma*
- *Chlamydia*

Mycobacterial infections:
- Miliary tuberculosis

Non-infectious, acute lung injury

Non-infectious lung injury following HCT may be mediated by either immune or non-immune mechanisms. Common alloimmune lung complications include idiopathic pneumonia syndrome (IPS), transfusion-related lung injury (TRALI), diffuse alveolar hemorrhage (DAH), and peri-engraftment respiratory distress syndrome (PERDS), with both DAH and PERDS existing as a subset of IPS. Non-alloimmune conditions may include the direct cytotoxic effects of HCT conditioning regimens, cardiogenic dysfunction, and iatrogenic causes of pulmonary edema.

Chemotherapy-associated pneumonitis

Initially described in the 1970s, chemotherapy-associated pulmonary toxicity has been reported in association with multiple chemotherapeutic agents, including bis(chloroethyl)nitrosourea (BCNU), busulfan, and melphalan. BCNU-related lung injury typically presents within the first 3 months following HCT with a non-productive cough and increasing dyspnea in the context of rapidly progressing, bilateral, interstitial infiltrates radiographically [11]. Pulmonary function testing reveals restrictive lung disease with diminished forced vital capacity (FVC) and total lung capacity (TLC) measurements. Independent risk factors for BCNU pneumonitis include an absolute BCNU dose over 1000 mg (even when less than 450 mg/m^2), younger donor age (<54 years), and prior mediastinal irradiation [12]. Treatment with pulsed doses of corticosteroids early in the clinical course significantly decreases the morbidity and mortality; if untreated, or recognized late in its course, severe pulmonary fibrosis may develop.

Delayed pulmonary toxicity syndrome (DPTS) was frequently reported in autologous patients receiving chemotherapy with regimens that contain BCNU, cyclophosphamide, and cisplatin – typically in the setting of metastatic breast cancer. The incidence of DPTS was as high as 29–64% with a median time to onset of 45 days. Treatment with corticosteroids (1 mg/kg/day) leads to resolution in up to 92% of cases, and high-dose inhaled corticosteroid prophylaxis may also be beneficial [13]. Finally, busulfan, in combination with cyclophosphamide or melphalan, has also been implicated in post-transplant lung dysfunction. While pharmacokinetic targeting has minimized busulfan-related toxicity, an increased incidence of pulmonary complications has been observed when local lung radiotherapy is delivered prior to busulfan administration [14].

Transfusion-related acute lung injury (TRALI)

TRALI is the leading cause of mortality following infusions of plasma-containing blood products, and occurs following 1:1000 to 1:5000 transfusions [15,16]. All plasma-containing blood products, including whole blood, packed red blood cells, fresh frozen plasma, platelets, cryoprecipitate, granulocytes, immunoglobulin infusions, and stem-cell products have been linked to the development of TRALI, with albumin the sole exception. The diagnosis is suspected with acute onset of dyspnea and respiratory distress typically occurring 1–6 hours after transfusion. Chest radiographs reveal diffuse pulmonary infiltrates reflecting edema from increased pulmonary vascular permeability. Pathologically, neutrophilic infiltrates and over-expression of neutrophil-related cytokines and chemokines have been linked to TRALI. With associated mortality rates approximating 5–10%, TRALI accounts for nearly half of all transfusion-related deaths. Treatment is generally supportive. Discontinuation of the blood products and initiation of respiratory support result in recovery within 3–4 days in the majority of patients. TRALI is a capillary leak syndrome and not an issue with fluid overload, hence the use of steroids and forced diuresis should be instituted with caution to prevent hypovolemia. In over 70% of cases, antibodies directed against human leukocyte antigen (HLA) class I or II epitopes on recipient hematopoietic cells have been identified as the primary cause of the TRALI event, but in rare cases the antibody may be present in the recipient's plasma, and directed against transfused donor leukocytes [17].

Idiopathic pneumonia syndrome (IPS): definition, clinical course, and spectrum of disease

IPS is defined as widespread alveolar injury following HCT that occurs in the absence of an active lower respiratory tract infection or cardiogenic causes [4,18]. As shown in Table 95.2, diagnostic criteria of IPS include signs and symptoms of pneumonia, non-lobar radiographic infiltrates, abnormal pulmonary function, and the absence of infectious organisms as determined by BAL or lung biopsy [2,4,18]. A variety of histopathologic findings have been associated with IPS, and interstitial pneumonitis (a term historically used interchangeably with IPS) is the most frequently reported pattern [19]. The incidence of IPS in the first 120 days after allogeneic HCT following high-dose conditioning ranges between 3 and 15%, depending on the donor type (related versus unrelated) and degree of antigenic mismatch between donor and recipient [2–4,18]. The median time of onset for IPS was initially reported to be 6–7 weeks (range 14–90 days) after HCT [18], and mortality rates ranged from 50 to 80% overall, with more than 95% mortality for patients requiring mechanical ventilation [4]. A retrospective study in Seattle showed a lower incidence and earlier onset of IPS than previously reported, but the typical clinical course involving the rapid onset of respiratory failure leading to death remained unchanged [3]. Long-term survival in pediatric patients is also poor, with transplant-related mortality (TRM) significantly higher than in non-affected patients (5-year TRM: 52% vs. 13%, $p = 0.001$) [20]. Recent studies suggest that the history of prior viral respiratory tract infections, use of reduced-intensity conditioning (RIC) regimens, and umbilical cord blood as a stem-cell source may impact the frequency, time of onset, and overall mortality of IPS [4].

The clinical spectrum of IPS encompasses several forms of pulmonary toxicity (Figure 95.2, Table 95.3). In one small subset of patients, acute pulmonary hemorrhage or hemorrhagic alveolitis occurs. DAH generally develops in the immediate post-HCT period and is characterized by progressive shortness of breath, cough, and

Table 95.2 Definition of idiopathic pneumonia syndrome

I. Evidence of widespread alveolar injury:
  a. Multilobar infiltrates on routine chest radiographs or CT
  b. Symptoms and signs of pneumonia (cough, dyspnea, tachypnea, rales)
  c. Evidence of abnormal pulmonary physiology
    1. Increased alveolar to arterial oxygen difference
    2. New or increased restrictive pulmonary function test abnormality
II. Absence of active lower respiratory tract infection based upon:
  a. Bronchoalveolar lavage negative for significant bacterial pathogens including acid-fast bacilli, *Nocardia* and *Legionella* species
  b. Bronchoalveolar lavage negative for pathogenic non-bacterial microorganisms:
    1. Routine culture for viruses and fungi
    2. Shell vial culture for CMV and respiratory syncytial virus (RSV)
    3. Cytology for CMV inclusions, fungi and *Pneumocystis jiroveci (carinii)*
    4. Direct fluorescence staining with antibodies against CMV, RSV, HSV, VZV, influenza virus, parainfluenza virus, adenovirus, and other organisms
  c. Other organisms/tests also to consider:
    1. Polymerase chain reaction (PCR) for human metapneumovirus (HMP), rhinovirus, coronavirus, and HHV-6
    2. PCR for *Chlamydia*, *Mycoplasma*, and *Aspergillus* species
    3. Serum galactomannan ELISA for *Aspergillus* species
  d. Transbronchial biopsy if condition of the patient permits
III. Absence of cardiac dysfunction, acute renal failure, or iatrogenic fluid overload as etiology for pulmonary symptomatology

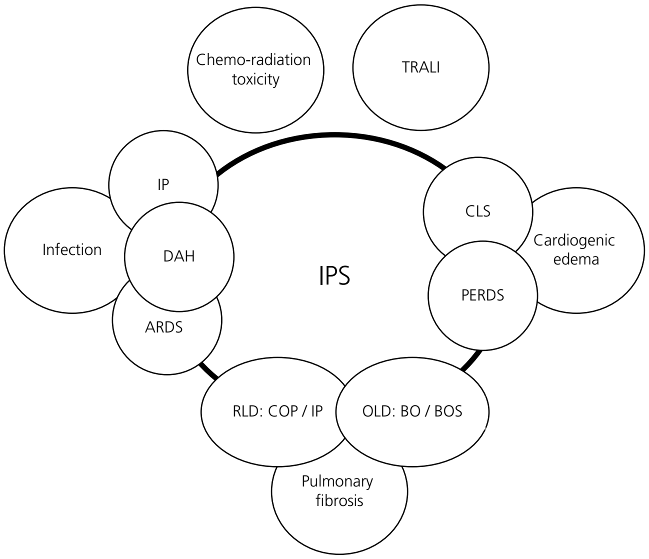

Figure 95.2 Clinical spectrum of IPS. The clinical spectrum of IPS includes a variety of descriptive forms of lung injury that may share clinical features with toxicity incurred by chemo-radiotherapy, cardiogenic edema, pulmonary fibrosis, infection, and transfusion-associated lung injury (TRALI). ARDS, acute respiratory distress syndrome; BO, bronchiolitis obliterans; BOS, bronchiolitis obliterans syndrome; COP, cryptogenic organizing pneumonia; CLS, capillary leak syndrome; DAH, diffuse alveolar hemorrhage; IP, interstitial pneumonitis; OLD, obstructive lung disease; PERDS, peri-engraftment respiratory distress syndrome; RLD, restrictive lung disease.

Table 95.3 The spectrum of non-cardiogenic, pulmonary toxicity defined by IPS

Interstitial pneumonitis (IP):
- Clinical symptoms: fever, cough, dyspnea, hypoxemia
- Onset: within first 100 days post-transplant
- Etiology: infectious (i.e. CMV, PCP) or non-infectious factors (chemotoxicity: BCNU, bleomycin, busulfan, methotrexate)
- Radiographic findings: bilateral interstitial infiltrates

Diffuse alveolar hemorrhage (DAH):
- Clinical symptoms: progressive dyspnea, cough, rare hemoptysis
- Key finding: progressively bloodier aliquots of lavage fluid
- Onset: early, within first 100 days post-transplant.
- Radiographic findings: diffuse infiltrates, central appearance initially noted
- Histology: diffuse alveolar damage with alveolar hemorrhage

Peri-engraftment respiratory distress syndrome (PERDS):
- Clinical symptoms: fever, dyspnea, hypoxemia
- Onset: very early, within 5–7 days of engraftment, classically after autologous SCT
- Radiographic findings: bilateral interstitial infiltrates

Non-cardiogenic capillary leak syndrome (CLS):
- Clinical symptoms: dyspnea, cough, weight gain, edema
- Onset: early, within first 30 days post-transplant
- Radiographic findings: bilateral peri-hilar infiltrates, pulmonary edema, pleural effusions

Cryptogenic organizing pneumonia (COP):
- Clinical symptoms: fever, dry cough, dyspnea
- Onset: 2–12 months post-transplant
- Radiographic findings: patchy airspace disease, ground glass appearance, nodular opacities
- Histology: peri-bronchiolar infiltration and fibrosis and the presence of intraluminal granulation tissue

Bronchiolitis obliterans (BO):
- Clinical symptoms: cough, dyspnea, wheezing, lack of fever
- Pulmonary function testing: obstructive findings (diminished FEV_1 or FEV_1/FVC)
- Onset: 3–24 months post transplant
- Radiographic findings: hyperinflation, otherwise routinely normal
- CT: bronchiectasis, centrilobular nodules, septal lines, ground-glass appearance
- Histology: lymphocytic bronchitis, bronchiolar inflammation with luminal obliteration

Table 95.4 Risk factors for IPS

Graft-versus-host disease prophylaxis (methotrexate)
Acute graft-versus-host disease
Increasing recipient age
Total body irradiation (≥1200 cGy)
Myeloablative conditioning
Decreased pre-transplant performance status
Longer duration from diagnosis to transplant
Transplantation for malignancy other than leukemia
HLA disparity (donor: recipient)

hypoxemia, with or without fever [21]. The definition of DAH requires the demonstration of progressively bloodier aliquots of BAL fluid, but frank hemoptysis is rare. Mortality is high despite aggressive treatment with high-dose steroids, and death usually occurs within weeks of diagnosis [21]. Some patients with DAH can have microorganisms isolated from blood, BAL fluid, or tracheal aspirate within 1 week of alveolar hemorrhage. Majhail et al. compared patients with DAH and infection-associated alveolar hemorrhage who presented with similar clinical and radiographic findings in the setting of progressively bloodier BAL fluid following allogeneic HCT [22]. Infectious and non-infectious alveolar hemorrhage causes were found to be related but distinct entities with extremely poor outcomes following conventional therapy, including steroids [22]. PERDS also falls within the definition of IPS. PERDS is characterized by fever, dyspnea, and hypoxemia that, by definition, occur within 5–7 days of neutrophil engraftment [23]. PERDS in the autologous setting responds promptly to corticosteroids and is associated with a favorable prognosis [23], whereas response to therapy and overall outcomes are poor after allogeneic HCT [3,4,24,25]. PERDS can be distinguished from chemotherapy-induced lung injury by both time of onset and the implication of engraftment-associated cytokine release and capillary leak as underlying cause for pulmonary inflammation.

Risk factors of IPS

As shown in Table 95.4, risk factors for IPS include conditioning with high-dose total body irradiation (TBI), acute GVHD, older recipient age, high-risk disease status, initial diagnosis of malignancy other than leukemia, and, in some reports, the use of methotrexate for GVHD prophylaxis [4,20,26]. In a meta-analysis, it was found that lung irradiation dose, cyclophosphamide dose, and the addition of busulfan were significantly associated with the development of interstitial pneumonitis, and that lung shielding reduced the incidence of HCT-related IPS [27]. The cumulative incidence of IPS within 120 days of HCT was found to be significantly lower following a non-myeloablative conditioning regimen using a single dose of TBI than observed following conventional conditioning despite greater patient age and a similar incidence of acute GVHD [28]. Once established, however, pulmonary toxicity remains severe and culminated in respiratory failure in the majority of patients. These findings suggest that the intensity of HCT conditioning contributes to the development of IPS, and are consistent with data generated from preclinical models showing that the lung is sensitive to the combined effects of radiation and alloreactive T cells. The impact that more robust but reduced-intensity regimens and other factors including stem cell source will have on IPS remains to be fully elucidated.

Table 95.5 Categorization of the clinical spectrum of lung injury following HCT

Clinical spectrum of disease as categorized by presumed site of primary tissue injury		
Pulmonary parenchyma	**Vascular endothelium**	**Airway epithelium**
Acute interstitial pneumonitis (AIP)[a]	Peri-engraftment respiratory distress syndrome (PERDS)*	Cryptogenic organizing pneumonia (COP)/bronchiolitis obliterans organizing pneumonia (BOOP)[a]
Adult respiratory distress syndrome (ARDS)[a]	Non-cardiogenic capillary leak syndrome (CLS)[a]	Bronchiolitis obliterans (BO)
BCNU pneumonitis	Diffuse alveolar hemorrhage (DAH)[a]	
Radiation pneumonitis	Pulmonary venocclusive disease (PVOD)	
Delayed pulmonary toxicity syndrome (DPTS)	Transfusion-related acute lung injury (TRALI)	
Post-transplant lymphoproliferative disease (PTLD)	Pulmonary cytolytic thrombi (PCT)	
Eosinophilic pneumonia (EP)	Pulmonary arterial hypertension (PAH)	
Pulmonary alveolar proteinosis (PAP)	Pulmonary thrombo-embolus (PTE)	

[a] Conditions routinely included under the classification of idiopathic pneumonia syndrome (IPS).

Table 95.6 Animal models of IPS

HCT donors	**HCT recipients**	**Mismatch**	**Conditioning**
B10.Br	Cba	Multiple minor Ags	TBI: 1100 cGy
C57bl/6	B10.Br	Complete mismatch	TBI: 750 Cgy ± Cy
C57BL/6	B6.C-H2^{bm1}/By	MHC class I	TBI: 675 cGy
C57BL/6	B6.C-H2^{bm12}/Kheg	MHC class II	TBI: 675 cGy
B6C3F1 hybrid	B6C3F1 hybrid	None (syngeneic)	Cy, cisplatin, BCNU
C57bl/6	B6D2F1 Hybrid	Haplo-identical	TBI: 1100–1300 cGy
C57bl/6	B6.C-H2^{bm1}/By	MHC class I	TBI: 1100 cGy
Lp/J	C57bl/6	Multiple minor Ags	TBI: 1300 cGy

Ags, antigens; cGy, centigray; Cy, cytoxan; TBI, total body irradiation.

Etiology of IPS

Potential etiologies for IPS include direct toxic effects of HCT conditioning regimens, occult pulmonary infections, and soluble and cellular inflammatory mediators implicated in the development of other forms of pulmonary injury and acute GVHD. Acute GVHD often precedes IPS, suggesting a possible causal relationship between the two disorders [4,29]. Moreover, the consistent association between lung injury and target organ GVHD in experimental models also supports such an etiology [30–33]. However, the lung has not been traditionally recognized as a classic GVHD target organ, and the specific contribution of alloreactive donor T-lymphocytes to IPS remains a topic of considerable debate. Epithelial apoptosis, a finding attributed to T-cell-mediated injury and considered pathognomonic for acute GVHD, is not consistently observed in allogeneic HCT recipients with IPS [19,34]. By contrast, a histologic spectrum of "pulmonary GVHD" ranging from early diffuse alveolar injury to cicatricial BO, a late and irreversible form of lung injury after HCT, has been described [19]. The heterogeneity of pulmonary histopathology is complicated further by the nonspecific changes that occur after mechanical ventilation, the limited frequency with which lung biopsies are completed on HCT recipients, and the quality and quantity of specimens obtained. In an attempt to address this issue, investigators recently categorized the disease entities falling under the umbrella of IPS by the primary anatomic site of cellular damage: the interstitial tissue, vascular endothelium, or airway epithelium (Table 95.5). Although some cases may remain unclassifiable, this approach may focus future lines of investigation on specific pathways of tissue injury and thereby foster the development of therapeutic interventions that can be tailored to distinct subtypes of disease [4].

Animal models of human disease

To define better the pathogenesis of IPS, several rodent models have been developed and used extensively to investigate immunologic mechanisms of injury that contribute to damage to the vascular endothelium and to the alveolar and bronchiolar epithelium (Table 95.6) (reviewed in [4]). These preclinical models have consistently shown that the development of significant, non-infectious lung injury correlates with the presence but not the severity of systemic GVHD [31]. A model using a complete major histocompatibility complex (MHC) mismatched donor–recipient strain combination best reproduces acute, early-onset IPS. In this system, pulmonary toxicity is caused by the influx of host monocytes and donor T cells into the lungs of lethally irradiated mice within the first 2 weeks of HCT [33]. Intensifying the pre-HCT conditioning with cyclophosphamide accelerates the development of IPS, consistent with human data. From a classification standpoint (see Table 95.5), lung injury is characterized by injured type II alveolar epithelial cells, endothelial cells, and increased frequencies of cytotoxic T-lymphocytes and immune cells expressing B7 family costimulatory ligands in the alveolar and interstitial spaces [33]. Lung dysfunction reflects this interstitial and alveolar damage and presents as reduced specific compliance, decreased total lung capacity, and increased wet and dry lung weights. BAL fluid of mice with IPS contains elevated levels of soluble mediators including cytokines, nitrites, lactate dehydrogenase, and total protein, indicative of lung inflammation and pulmonary endothelial damage and leak [35].

Other more clinically relevant murine systems exist wherein donor and host are haploidentical or differ only at multiple minor histocompatibility complex (HC) antigens or isolated MHC class I or class II loci. These systems specifically model IPS that develops during the first 2–3 months after HCT and is characterized by donor leukocyte infiltration into the lung [4]. Regardless of strain combination, two primary, reproducible abnormalities are apparent: a dense mononuclear cell infiltrate around both pulmonary vessels and bronchioles and an acute pneumonitis involving the interstitium and alveolar spaces representing each of the three anatomic categories outlined in Table 95.5 [31,36,37]. When measured, alterations in pulmonary function are associated with lung histopathology, demonstrating that the observed lung inflammation is physiologically relevant [31,33,38]. Despite the presence of endothelial damage, DAH is not regularly seen in preclinical IPS models. The only exception is when mice with severe GVHD are challenged with lipopolysaccharide (LPS). In this scenario, hemorrhage is associated with large increases in BAL fluid levels of neutrophils, tumor necrosis factor-alpha (TNF-α), and LPS [31,36].

The pathogenesis of IPS

The pathophysiology of IPS is complex. Data generated using experimental models described above support a shift away from the current paradigm of acute lung injury after HCT as an idiopathic clinical syndrome and towards a process in which the lung is susceptible to two distinct but interrelated pathways of immune-mediated injury: a T-cell axis and an inflammatory cytokine axis. Collectively, these include aspects of the adaptive and the innate immune responses, synergistic interactions between cells from the lymphoid and myeloid lineages, and the release of soluble inflammatory molecules (Figure 95.3). These distinct but related pathways of inflammation culminate in the recruitment of immune cells to the lung leading to tissue damage and dysfunction.

Soluble inflammatory mediators

Evidence for cytokine activation during the development of IPS has been demonstrated in clinical studies, which have shown increased pulmonary vascular permeability and increases in BAL fluid and serum levels of several cytokines (TNF-α, sTNFR receptors I and II, IL-6) and inflammatory chemokines (IL-8, MCP-1, MIG) that regulate leukocyte recruitment to sites of inflammation [1,25]. A causal role for TNF-α in the development of experimental IPS has been established using strategies that either neutralize its effects [36] or use TNF-α-deficient mice as HCT donors [39]. Neutralization of TNF-α at the time of LPS challenge effectively prevents enhanced pulmonary inflammation – confirming the linkage between LPS and TNF-α in this setting – and reduces the severity of lung injury when implemented during the natural course of disease [36]. Studies using genetically altered mice have shown that IPS is dependent upon donor-derived, rather than host-derived, TNF-α. Although TNF-α from both donor accessory cells (macrophage/monocytes) and T cells contributes significantly to lung injury, the T-cell component predominates [39].

The actions of TNF-α are mediated by two receptors: a 55–60-kDa type I receptor (TNFRI; p55/60; CD120a) and a 75–80-kDa type II receptor (TNFRII; p75/80; CD120b). TNFRI is constitutively expressed, whereas the expression of TNFRII is strongly regulated by various cytokines and other inflammatory stimuli. In one report, the absence of TNFRI was associated with decreased pulmonary edema and improved lung compliance on day 7 of allo-HCT [40]. However, cellular infiltration into the lung and BAL fluid levels of proinflammatory cytokines was actually higher in mice deficient in

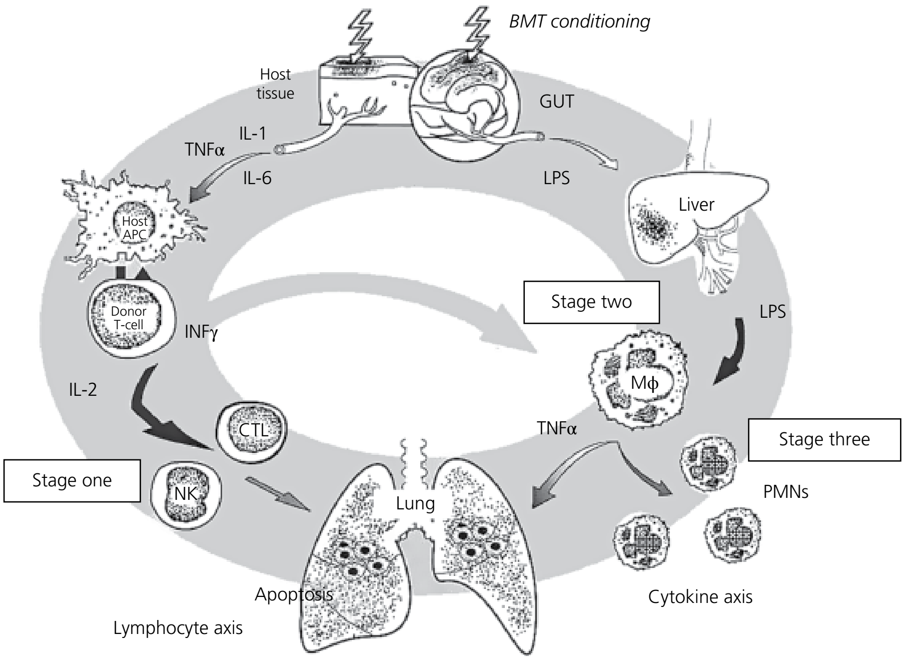

Figure 95.3 Pathophysiology of IPS. Data generated using murine HCT models have been incorporated into a working hypothesis of IPS physiology. This schema postulates that the lung is susceptible to two distinct but inter-related pathways of immune mediated injury that occur along a T-lymphocyte activation axis and a "gut–liver–lung" axis of inflammation. Chemo-radiotherapy of HCT conditioning causes cytokine release that enhances the ability of host antigen-presenting cells to present alloantigens to mature donor T cells and upregulates chemokine expression in the lung. Once engaged, donor T cells become activated and secrete IFN-γ and IL-2. IFN-γ primes donor macrophages (Mφ) and monocytes, whereas IL-2 facilitates T-cell activation and the generation of CXCR3 expressing Th1 lymphocyte effectors that migrate to the lung early after HCT (stage one) in response to inflammatory chemokine gradients and contribute to pulmonary toxicity via Fas/FasL-mediated cell killing. The inflammatory axis focuses on the relationship between the cellular activating effects of LPS and the downstream production of TNF-α as it occurs along a gut–liver–lung axis of inflammation. LPS enters the systemic circulation through gaps in the intestinal mucosa. The ability of systemic endotoxin to reach the alveolar space is related to the consequences of GVHD in other target organs, particularly the liver, which is pivotally located immediately downstream (via the splanchnic circulation) of the intestinal reservoir of Gram-negative bacteria and their toxic byproducts. CCR2 expressing donor macrophages primed by IFN-γ are recruited to the lung (stage two) where they are triggered by LPS to secrete inflammatory cytokines like TNF-α, resulting in enhanced chemokine expression, the recruitment of neutrophils to the lung, and increased tissue damage (stage three).

TNFRI compared with controls. These findings are consistent with a more recent study showing that compared with controls, TNFRII-deficient, allogeneic HCT recipients develop significantly less severe IPS, which is associated with reductions in pulmonary ICAM-1 expression and in leukocyte infiltration into the lungs [41].

The release of inflammatory cytokines, including TNF-α, has been linked to the cellular activating effects of endogenous microbial products such as LPS. Experimental results from established mouse models [42,43] are consistent with clinical observations showing that (1) monocytes upregulate CD14 expression following recruitment to an inflamed lung and show enhanced sensitivity to LPS stimulation [44], and (2) evidence of cytokine activation and components of the LPS activating system (including lipopolysaccharide binding protein and soluble CD14) in the BAL fluid and plasma of patients with IPS [1,25]. Recent plasma proteomic studies revealed striking similarities between the inflammation engendered during IPS in humans and mice and underscored a significant contribution of the acute phase response (TNF-α/IL-6) signaling pathway during disease progression. Moreover, the results identified a set of robust markers predictive for disease progression and response to therapy and suggested that patients whose innate immune response are "hot-wired" to respond to the release of microbial products with high level secretion of TNF-α may be more likely to be protected via strategies that neutralized the inflammatory effects of this protein [45]. Accordingly, strategies that neutralize TNF-α do not completely abrogate lung injury [36,39,46] in the experimental and clinical settings, suggesting that other inflammatory and cellular mechanisms also contribute to the development of IPS. Delivery of chemo-radiotherapy during HCT conditioning, concurrent presence of iron overload, and lung-infiltrating inflammatory cells all contribute to the release of molecules that contribute to the generation of both oxidative and nitrosative stress. Because of its oxygen-rich environment, the lung is particularly sensitive to the effects of these conditions, and experimental and clinical data suggest that amelioration of oxidative stress may be an effective strategy to reduce the severity of pulmonary toxicity during IPS (reviewed in [4]).

Pulmonary surfactant

Pulmonary surfactant is produced by alveolar type II cells and is composed of a complex mixture of lipids and at least four surfactant proteins (SPs). By reducing surface tension, surfactant decreases the work of breathing, allows alveoli to remain open at end expiration, and keeps alveoli dry. SP-A and SP-D also have a major role in host defense and in regulating immune responses in the lung [47]. Reduced production or dysfunction of SPs contributes to the clinical picture of hypoxemia, progressive dyspnea, and pulmonary edema that is associated with IPS. Mice lacking SP-A or SP-D exhibit exaggerated allogeneic T-cell-dependent inflammation that is associated with enhanced markers of lung injury, and intratracheal instillation of human SP-A attenuates the manifestations of IPS in this setting [48]. In humans, low pre-HCT serum levels of SP-D may predispose individuals to enhanced inflammatory responses following exposure to conditioning regimens and allogeneic immune responses and have been shown to (1) represent a risk factor for the development of IPS and bronchiolitis obliterans [49], (2) associate with certain polymorphisms in the human SP-D gene [50], and (3) be a valuable biomarker in acute lung injury that can predict outcome [51]. Despite its overwhelming therapeutic success in premature infants, results of surfactant replacement trials during acute lung injury after HCT in children and adults have been variable [52]. A phase III study is under way in pediatric HCT recipients to determine the efficacy of novel surfactant preparations that closely resemble natural surfactant.

Donor immune cells

The role of alloreactive donor T cells in the pathogenesis of IPS remains a topic of considerable debate. The importance of lymphocytes to lung injury after experimental HCT has also been shown by several groups [30–33,39]. Donor T cells are critical to the early proinflammatory events associated with lung injury that develops within the first week of HCT across MHC antigens, whereas in minor H antigen mismatch systems, donor lymphocytes continue to contribute to physiologically significant lung injury at later time points [32,33]. Donor cytotoxic T-lymphocytes (CTLs) can contribute to lung injury via three primary cytolytic mechanisms: the perforin–granzyme, Fas/Fas ligand (FasL), or TNF-α pathways. Each pathway has been shown to contribute to lung injury in non-HCT models. Furthermore, cytolytic T cells expressing granzyme B are present in the lungs of mice after allogeneic HCT, and they colocalize with macrophages expressing the costimulatory molecules B7.1/CD80 and B7.2/CD86 [33]. Alloantigen-specific killing by donor T cells using both the perforin and Fas/FasL pathways has also been identified in the lung after HCT. Pulmonary CTL activity is present as early as week 2 after transplant [53]. Whereas both FasL- and TNF-α-mediated cytolytic pathways are utilized during the evolution of IPS caused by Th1 ($CD4^+$) T cells, Tc1 ($CD8^+$) T cells use TNF-α exclusively.

Some studies have challenged the role of Th1/Tc1 effectors in the development of IPS and paradoxically showed that pulmonary inflammation is accelerated when interferon-gamma (IFN-γ) signaling is blocked [54]. These results were later independently confirmed and extended to show that IFN-γ negatively regulates the expansion of $Th17^+CD4^+$ T cells in the lungs during IPS [55]. In vitro differentiated Th17 cells also mediated severe pulmonary pathology in a mouse GVHD model [56] and the role of Th17 cells in tissue inflammation is currently an area of intense study [57]. These findings notwithstanding, robust lung inflammation has been reproducibly observed in several models when receptor–ligand interactions are completely intact, a scenario that is likely more applicable to the human disease state.

Experimental data suggest that synergistic interactions between cells from the lymphoid and myeloid lineage are critical to the development of lung inflammation after allogeneic HCT. Donor macrophages are recruited to the lung within the first several weeks after experimental HCT [58], are a significant, albeit not the primary, source of TNF-α [39], and their responsiveness to LPS stimulation correlates with the severity of IPS [42,43]. Experimental results obtained using CD14-deficient donors are consistent with reports showing that when recruited to an inflamed lung, monocytes upregulate CD14 expression and show enhanced sensitivity to LPS stimulation [44], and with the clinical observations made in the BAL fluid of patients with IPS [1,25]. Polymorphonuclear cells are a major component of the mixed inflammatory infiltrates, and increases in TNF-α seen in the lungs of animals with IPS [31,36], and their appearance in the bloodstream, are often temporarily associated with the onset of lung inflammation in humans [25]. Neutrophil products such as elastase, myeloperoxidase, metalloproteinases, and oxidants are abundant in the BAL fluid of patients with adult respiratory distress syndrome (ARDS) and during lung allograft rejection and are believed to contribute to the endothelial and epithelial damage that occurs in each setting [59,60].

Mechanisms of leukocyte recruitment to the lung during IPS

White blood cell (WBC) migration to sites of inflammation has been studied extensively and involves interactions between leukocytes and endothelial cells that are mediated by adhesion molecules, chemokines, and their receptors [61]. Mouse models have shown that the mRNA expression of intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin are increased in the lungs of mice after allogeneic HCT [62], and the severity of IPS is dramatically reduced when ICAM-1-deficient mice are used as HCT recipients of either MHC-matched or -mismatched allogeneic donor cells [37,63]. Investigators have also begun to explore the role of chemokine receptor–ligand interactions in IPS, which can be conceptualized in three distinct stages (reviewed in [4,64]). This hypothesis stipulates that the migration of each leukocyte subset controls the recruitment of the next wave of effectors. In this paradigm, the recruitment of Th1/Tc1 effectors [65–68] initiates the cascade, and plays an active role in regulating the inflammation engendered during the evolution of IPS [39,58].

Targets of inflammation and injury during IPS

Although yet to be definitively established, it is conceivable that pulmonary endothelial and epithelial cells can serve as targets for direct alloimmune-mediated damage. Endothelial cell (EC) damage can be induced by transfer of allogeneic lymphocytes to immunodeficient mice [69] and has been implicated as a direct contributor to the development of several complications, including GVHD, sinusoidal obstruction syndrome, and thrombotic microangiopathy [70]. Evidence for EC injury and leak, as demonstrated by pulmonary edema, enhanced total protein levels in BAL fluid, and increased wet to dry lung weight ratios, are also observed after clinical and experimental IPS [25,33]. In preclinical models, EC apoptosis coincides with the onset of pulmonary pathology, is associated with elevations in BAL fluid TNF-α levels, and is accompanied by evidence for EC activation as measured by enhanced mRNA expression of adhesion molecules [62]. Neutralization of TNF-α early after allogeneic HCT significantly reduces EC apoptosis and lung histopathology observed in mice [62]. TNF-α may therefore function as both an effector and a facilitator of lung injury by contributing directly to EC injury and death, and by regulating the chemokine milieu in the lung during the early stages of IPS.

By contrast, epithelial apoptosis, generally ascribed to T-cell-mediated injury and considered pathognomonic for acute GVHD in other target tissues, has not been consistently observed in allogeneic HCT recipients with lung injury. The unique aspects of epithelial anatomy in the lung may help explain this discrepancy. Since there is no stratification or layering of pulmonary epithelial cells as in the skin or intestine, identification of epithelial cell apoptosis by histologic criteria can be more challenging. Experimental studies have, however, provided evidence for epithelial injury during IPS. Panoskaltsis-Mortari et al. demonstrated that IPS was associated with injured alveolar type II cells and increased frequencies of cytotoxic T-lymphocytes in a model of early-onset IPS [33]. The same group later showed that keratinocyte growth factor, a mediator of epithelial cell proliferation and a growth factor for type II pneumocytes, diminished IPS injury by damping the immune response to chemo-radiotherapy and by accelerating repair of the damaged tissue, specifically alveolar type II epithelial cells [71]. Finally, a new murine model of bronchiolitis obliterans following allogeneic HCT revealed that bronchiolar epithelial cells express MHC class II and are surrounded by cells expressing granzyme B prior to the occlusion of the airways [72].

Table 95.7 Treatment options for IPS

Supportive therapy:
- Supplemental oxygen, mechanical ventilation
- Empiric broad-spectrum antimicrobial agents pending culture results
- Management of iatrogenic fluid overload
- Continuous veno-venous hemofiltration (CVVH)

Immunosuppressive therapy:
- Corticosteroids (2 mg/kg/day)
- Investigational: cytokine inhibitors, including anti-TNF agents

The diagnostic and therapeutic approach to patients with IPS

As discussed earlier in the section Diagnostic considerations and role of bronchoalveolar lavage, and outlined in Figure 95.1, the approach to HCT patients with acute pulmonary dysfunction requires the timely coordination of care across multiple medical subspecialties and a number of diagnostic tests. Once established, current standard treatment regimens for IPS (Table 95.7) include supportive care measures in conjunction with broad-spectrum antimicrobial agents and intravenous corticosteroids [4]. Unfortunately, responses to standard therapy (including high-dose steroids) are limited, and the mortality of patients diagnosed with IPS remains unacceptably high. The early institution of continuous veno-venous hemofiltration may help to improve survival in some pediatric patients [73], but prospective studies addressing the use of this treatment are lacking in the literature. As described above, translational research studies have suggested that neutralization of TNF-α may be a useful option for IPS. In an early phase I/II study, etanercept, a soluble dimeric TNF-α-binding protein, showed promise when given subcutaneously at a dose of 0.4 mg/kg twice weekly (maximum of eight doses) in combination with systemic steroids and empiric antimicrobial therapy to patients with IPS [25]. Ten of 15 patients were able to withdraw completely from supplemental oxygen support and survival at days 28 and 56 (from the first etanercept dose) was significantly improved. In addition, in a retrospective study of 22 patients treated with corticosteroids alone versus 18 patients treated with corticosteroids plus etanercept, overall survival was significantly higher in the etanercept-treated group (88.2% vs. 36.4%, $p = 0.003$) [74].These encouraging results led to the development of two multi-institutional studies that were recently completed. Although etanercept in combination with systemic corticosteroids was associated with high response rates in a phase II study in children with IPS, results were inconclusive in a phase III placebo-controlled trial in adults that closed prematurely secondary to slow accrual [75,76].

Consistent with animal studies, not all patients with IPS respond to etanercept therapy. A recent plasma proteomic study conducted on samples obtained from patients with IPS provided a set of robust markers predictive for disease progression and response to therapy [45]. Indeed, patients prone to respond to alloimmune activation with high-level secretion of TNF-α may be more likely to be protected via strategies that neutralize the inflammatory effects of this protein. In addition, an effort to categorize patients with IPS based on the presumed anatomic site of primary injury and exploiting mechanistic insights gained in the laboratory may avail the use of other promising, non-cross-reactive therapeutic or preventative agents [4]. For example, it is conceivable that approaches to maintain EC integrity may be effective at preventing or treating IPS. The administration of molecules that function as survival factors for ECs has been successful in preventing endothelial damage and mortality from septic shock and radiation injury.

Specifically, keratinocyte growth factors have been shown to be efficacious in reducing epithelial damage and the severity of experimental IPS [71] in addition to protecting pulmonary endothelium from oxygen-induced injury. Similarly, ongoing studies examining the role of surfactant replacement therapy might prove useful in overcoming the effects of epithelial injury and dysfunction on ventilation and oxygenation. Since IPS develops and progresses to respiratory failure despite significant systemic immune suppression, it is possible that novel strategies directed towards inhibiting pathways of leukocyte recruitment to the lung may serve as future adjuncts to standard therapy intended to prevent or treat this serious complication. Such strategies have been successful in early-phase studies for GVHD prevention [77].

Non-infectious, late-onset lung injury: obstructive and restrictive lung disease

Definition, risk factors, and clinical course

Two forms of chronic pulmonary dysfunction are commonly observed in patients surviving more than 100 days after allogeneic HCT: obstructive lung disease (OLD) and restrictive lung disease (RLD). The incidence of both patterns of lung toxicity ranges from 20 to 50%, depending upon donor source and the time interval after HCT [5,6]. In each scenario, collagen deposition and the development of fibrosis either in the interstitial (RLD) or peri-bronchiolar (OLD) space are believed to contribute to the patterns of lung dysfunction displayed on pulmonary function testing. While both forms of pulmonary dysfunction exist as late-onset, non-infectious lung complications following allogeneic HCT, RLD and OLD can be distinguished by a number of clinical parameters (Table 95.8).

Restrictive lung disease

RLD is defined by reductions in forced vital capacity (FVC), total lung capacity (TLC), and diffusion capacity of the lung for carbon monoxide (DLCO) as measured by standard pulmonary function tests (PFTs). In restrictive disease, the ratio of the forced expiratory volume in 1 second (FEV_1) to FVC (FEV_1/FVC) is maintained near 100% [6,78]. RLD is common after HCT; significant decreases in FVC or TLC are reported at day 100 in nearly half of allogeneic HCT recipients. Although at times transient in nature, a decline in TLC or FVC at 100 days and 1 year after HCT compared with pre-transplant values (even if the absolute values for each measurement remained within the normal range) has been associated with an increase in non-relapse mortality [78]. TBI-containing conditioning regimens and the presence of acute GVHD have been associated with RLD, but a relationship with chronic GVHD has yet to be fully characterized.

Table 95.8 Clinical factors present in OLD versus RLD

Presentation	Obstructive lung disease	Restrictive lung disease
Clinical syndrome	BO	COP
Onset	Late (3–12 months)	Early (within 3 months)
Symptoms	Dyspnea, non-productive cough	Dyspnea, non-productive cough
Physical examination	Wheezing	Rales
PFTs	Obstructive physiology	Restrictive physiology
FEV_1/FVC	Decreased	Normal
TLC	Normal	Decreased
DLco	Decreased	Decreased
CT scan findings	Air trapping Bronchial wall thickening Centrilobular nodule	Patchy consolidation: COP "Ground-glass" opacities
Chronic GVHD	Strong association	Variable, association with COP

BOOP, bronchiolitis obliterans organizing pneumonia; BOS, bronchiolitis obliterans syndrome; COP, cryptogenic organizing pneumonia; CT, computed tomography; DLCO, diffusion capacity of the lung for carbon monoxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GVHD, graft-versus-host disease; OLD, obstructive lung disease; PFTs, pulmonary function tests; RLD, restricted lung disease; TLC, total lung capacity.

Histologic features of RLD after HCT are rarely described in the clinical literature. Although reported in less than 2% of HCT recipients, bronchiolitis obliterans organizing pneumonia (BOOP), also known as cryptogeneic organizing pneumonia (COP), is associated with restrictive (rather than obstructive) changes on PFTs [5,78]. Clinical features resemble idiopathic COP and include dry cough, shortness of breath, and fever, while radiographic findings show diffuse, peripheral, fluffy infiltrates consistent with airspace consolidation [78,79]. In the context of allogeneic HCT, the development of COP is strongly associated with prior acute and chronic GVHD [80]. When possible, the diagnosis of COP should be confirmed on lung biopsy specimens by several signature features: patchy fibrosis, granulation tissue within alveolar spaces, alveolar ducts and respiratory bronchioles, and the absence of infectious organisms. While often the case, the term COP or BOOP should not be used interchangeably with BO to describe a patient with chronic lung dysfunction after HCT. The two disorders differ with respect to histopathology, pulmonary function characteristics, and, most importantly, response to therapy; COP after HCT is quite responsive to corticosteroids, and in other settings may resolve spontaneously, whereas BO is not [5,78].

Obstructive lung disease/bronchiolitis obliterans (BO)

OLD was first recognized as a complication of allogeneic HCT in the mid-1980s and is now a well-documented cause of morbidity and mortality. OLD is characterized by enhanced resistance to airflow on expiration and results from narrowing and injury to smaller airways and bronchioles. The diagnosis of OLD is based primarily on spirometric measurements as demonstrated by decreases in FEV_1 and in FEV_1/FVC [6,78]. Obstructive defects as defined by an FEV1/FVC of <70% have been observed in approximately 15–25% of allogeneic HCT recipients by day 100 and can persist for years [6,78,81]. Lung biopsies from patients with OLD have shown several histopathologic patterns, but BO, which depicts small airway inflammation with fibrinous obliteration of the bronchiolar lumen, remains the most common [6,78] (Figure 95.4a) This variation in histopathology is complicated by the methods used to procure lung tissue; trans-bronchial biopsies rarely include an adequate sampling of distal bronchial structures and therefore such specimens can reveal cellular infiltrates involving larger airways and the interstitium without definitive bronchiolar inflammation. Biopsy-confirmed BO has been used historically to describe "chronic GVHD of the lung" [6]. However, significant airflow obstruction may exist without BO, and in most cases, OLD is diagnosed by PFT findings without histopathologic confirmation. In this context, two clinical phrases are described in the literature. "Air flow obstruction" is defined as a more than a 5% per year decline in percent predicted FEV_1 with the lowest post-transplant FEV_1/FVC ratio less than 0.8 [82]. "Bronchiolitis obliterans syndrome" (BOS) denotes the deterioration of graft function that accompanies chronic lung allograft rejection [83].

The lack of consistent terminology and the variability in diagnostic criteria have contributed to wide variations in the reported incidence of OLD after HCT. Afessa et al. found that OLD was reported

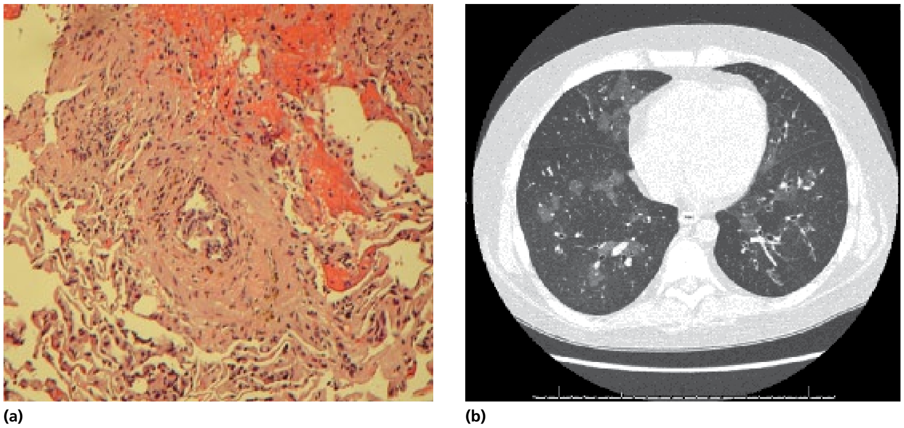

Figure 95.4 Histology and CT of OLD/BO. The cicatricial form of obstructive lung disease/bronchiolitis obliterans includes concentric, periluminal fibrous bands and obliteration of small airways (100×) (a). Expiratory image of chest CT scan shows classic areas of air trapping consistent with inflammation of terminal airways found in obstructive lung disease (b).

in 8.3% of over 2000 allogeneic HCT patients in nine studies and was identified in 6–20% of long-term survivors with chronic GVHD [5]. By contrast, using the definition described above for airflow obstruction, Chien et al. found that new-onset OLD following HCT with high-dose conditioning was more frequent (26% overall and 32% among patients with chronic GVHD) than previous estimates [82]. The onset of OLD ranges from 3 to 18 months after HCT, and is more insidious than IPS [6,78]. Respiratory symptoms include cough, dyspnea, and wheezing, but many patients remain asymptomatic despite showing signs of moderate to severe airway obstruction on PFTs. Chest X-rays are often normal except for signs of hyperinflation, but patchy, diffuse infiltrates can also be present. Likewise, chest CT findings can range from "normal" early in the course of disease to extensive peri-bronchial inflammation and bronchiectasis with significant air trapping at later time points [84] (Figure 95.4b). Non-contrast, high-resolution, chest CT scans are currently the study of choice to evaluate patients with suspected OLD.

Risk factors for OLD include lower pre-transplant FEV_1/FVC, concomitant infections, chronic aspiration, acute and chronic GVHD, HCT using mismatched donors, and high-dose (versus reduced-intensity) conditioning regimens [6,82,85]. Chien et al. examined the impact of age and conditioning intensity and found that the risk for experiencing a yearly decline in FEV_1 in excess of 20% was significantly lower for recipients of RIC than for patients receiving high-dose therapy who were more than 50 years of age [85]. Although donor/host CMV status does not correlate with the development of OLD, previous RSV and adenoviral infections are possible risk factors in the pediatric population [86]. The development of OLD is strongly associated with chronic GVHD, particularly in patients with low serum immunoglobulin G (IgG) levels and with chronic hepatic GVHD [6].

In 2005, the National Institutes of Health (NIH) defined consensus criteria for BO/OLD (Table 95.9) [87]. Using these NIH guidelines, a large retrospective study identified BO/OLD in 5.5% of allogeneic HCT recipients and in 14% of patients who developed chronic GVHD [88]. Pre-transplant FEV_1 and FEV_1/FVC ratios were significantly lower in patients who met the NIH consensus criteria for BOS compared with patients who never met the criteria. Mortality rates were doubled in patients who met the NIH consensus definition compared with those who did not [88]. Once established, the clinical course of OLD can vary from mild to severe with necrotizing bronchiolitis, a rapid decline in FEV_1, and mortality rates of 25–50%. Reports suggest that early detection of disease may be important to improving outcomes. To this end, a decrease of maximum mid-expiratory flow rates (FEV_{25-75}) may represent an earlier indicator of OLD than changes in FEV_1. In addition, rapid rates of airflow decline between 100 days and 1 year after HCT have also been shown to correlate with higher mortality [82]. Finally, pre-transplantation risk scores based solely or in part on pre-HCT lung function may be useful in predicting early respiratory failure and death in patients undergoing allogeneic HCT [89,90]. The clinical scope of OLD/BO along with challenges related to diagnosis, characterization, and approach to treatment have been discussed in detail in several recent comprehensive reviews [81,91–93].

Table 95.9 NIH consensus criteria for BOS

1. FEV_1 <75% predicted, plus FEV_1/FVC ratio <0.7
2. Evidence of air trapping, bronchiectasis or small airway thickening on high-resolution CT (inspiratory and expiratory views). If radiographic findings not evident, then a residual volume (RV) >120% predicted or histologic confirmation of constrictive bronchiolitis
3. Absence of acute respiratory infection. Documentation may include microbiology cultures (bronchoalveolar lavage, sinus aspiration, sputum cultures, or upper respiratory tract viral screen) or radiographic features (CT or chest radiograph) suggestive of infection
4. If BOS is diagnosed by pulmonary function and radiologic testing (without tissue confirmation), then at least one other distinct manifestation of chronic GVHD in a separate organ system must be present

Pathogenesis of chronic lung injury

The pathophysiology of chronic lung injury after HCT is poorly defined, but likely involves an initial insult to lung parenchyma followed by an ongoing dysregulated inflammatory process involving the interplay between recruited immune cells of donor origin and the resident host cells of the pulmonary vascular endothelium and interstitium. A triphasic model of chronic, non-infectious lung injury after HCT has been proposed that involves T-cell activation, leukocyte recruitment, the deposition of collagen, and the development of fibrosis [6,94]. In this model, an allogeneic response to lung epithelial cells initiates a dysregulated reparative response resulting in scarring of either the terminal airways, leading to OLD, or the interstitial space, leading to RLD (Figure 95.5). Precisely what determines the anatomic specificity (peribronchiolar versus interstitial) of chronic lung injury remains

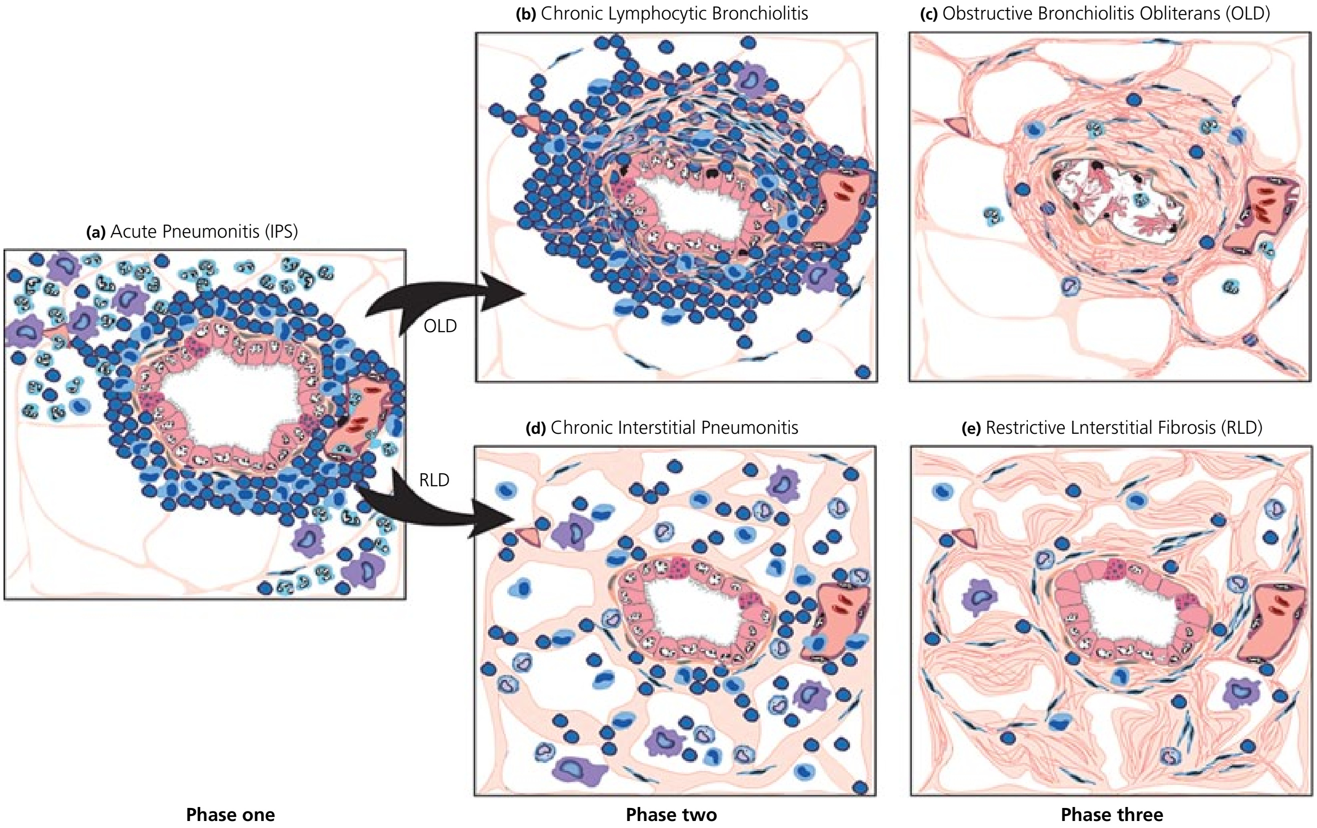
(b) Chronic Lymphocytic Bronchiolitis
(c) Obstructive Bronchiolitis Obliterans (OLD)
(a) Acute Pneumonitis (IPS)
OLD
RLD
(d) Chronic Interstitial Pneumonitis
(e) Restrictive Lnterstitial Fibrosis (RLD)
Phase one
Phase two
Phase three

Figure 95.5 A triphasic model of OLD and RLD following allogeneic HCT. Immune-mediated injury to the lung after allogeneic SCT can be conceptualized in three phases. In phase one, acute lung injury develops as a consequence of an allogeneic immune response and results in the sequential influx of lymphocytes, macrophages and neutrophils into an inflamed pulmonary parenchyma (a). Persistence of an inflammatory signal in the setting of dysregulated repair mechanisms promotes the transition from acute to chronic injury in phase two (b and d). As chronic inflammation proceeds to phase three, lung fibroblasts increase dramatically in number and contribute to the enhanced deposition of collagen and granulation tissue in and around bronchial structures, ultimately resulting in complete obliteration of small airways and fixed OLD (c). If, by contrast, the principal target of early damage is the alveolar epithelium, fibroblast proliferation and intraseptal collagen deposition ultimately result in interstitial fibrosis and RLD (e). (For a color version of this figure, please see the color plate section.)

unclear, and the development of one pattern of chronic lung injury (OLD or RLD) does not necessarily exclude the other [95]. Furthermore, an early, robust inflammatory phase may not be a prerequisite for subsequent fibrosis, a scenario that could explain why some patients with chronic lung dysfunction do not have a clear antecedent history of acute lung inflammation. In this context, pulmonary fibroblasts may represent an important link in the development of both RLD and OLD after allogeneic HCT, and evidence now exists that circulating fibrocytes can be used as a surrogate marker for the severity of BO [96].

The contribution of innate immune dysregulation to the proposed triphasic model is underscored by recent experimental and clinical studies. Compelling evidence for a role of TNF-α in the transition from acute to chronic lung injury is provided by work using transgenic rodents with targeted over-expression of TNF-α in the lungs; early lung histopathology includes a lymphocytic infiltrate similar to that seen in experimental IPS models, whereas the histologic changes associated with more prolonged exposure to TNF-α closely resemble those seen at later time points after HCT. Moreover, in a recent prospective study of TNF-α inhibition for the management of chronic lung injury post-HCT, significant increases in plasma levels of TNFR1 and TNFR2, both surrogate markers for TNF-α, were noted compared with non-HCT controls and with transplant patients who did not develop either BO/OLD or RLD [97]. In addition, two clinical studies demonstrated that genetic variations in both bactericidal/permeability-increasing protein- and nucleotide-binding oligomerization domain containing 2/caspase recruitment domain family member 15 (NOD2/CARD15) influence the risk of airflow obstruction and BO after allogeneic HCT [98,99].

Finally, the recent development of new animal models has underscored the interplay between local innate immunity and non-Th1 cell subsets and the contribution of donor B cell alloantibody production in the development of chronic pulmonary GVHD [72,100,101]. The latter observation was validated mechanistically as infusion of donor cells incapable of making IgG alloantibodies and blockade of germinal center formation abrogated lung fibrosis [140]. Importantly, these experimental findings are supported by the clinical observation that patients with newly diagnosed BO have significantly higher percentages of $CD19^+CD21^{low}$ B cells and levels of B-cell-activating factor (BAFF) in the peripheral blood compared with allogeneic HCT recipients without chronic GVHD [102]. Collectively, genetic approaches along with the scientific exploitation of relevant animal models for BO [72] may ultimately prove useful for deciphering mechanisms of disease that are more relevant to OLD that occurs in the context of HCT.

Treatment of chronic lung injury after HCT

The accepted association between BO/OLD and chronic GVHD has led to a consensus that this form of late pulmonary toxicity is immunologically mediated. Therefore, once the diagnosis of non-infectious OLD is established, standard therapy has included a combination of enhanced immune suppression with supportive care (including bronchodilator and supplemental oxygen therapy) and broad-spectrum antimicrobial prophylaxis. Since airflow obstruction tends to be “fixed” rather than reversible, response to bronchodilator therapy is usually marginal. Similarly, responses to immune suppressive therapy (including various combinations of steroids, calcineurin inhibitors, and azathioprine) are limited, and are typically associated with preservation of remaining lung function rather than in significant improvement. Furthermore, no combination of agents has been shown to be particularly effective [93]. Given the inherent increased risks of opportunistic infection, the duration of immune suppressive therapy should be carefully considered if a clinical response is not seen within the first several months of treatment or when pulmonary dysfunction is longstanding. Patients with more severe disease at the start of treatment have a poorer prognosis and higher mortality rates, reinforcing the importance of early diagnosis. The poor response to standard therapy and the unacceptable morbidity and mortality experienced by afflicted patients has resulted in the consideration of lung transplantation in some HCT recipients with severe OLD [103] and underscore the need for a better understanding of the pathogenesis of disease [104].

Corticosteroids - systemic and inhaled

In 2009, a Consensus Conference on the diagnosis and treatment of chronic GVHD of the lung was held in Regensburg, Germany, resulting in a summary of accepted treatment options [91]. The conference recommended a combination of systemic and inhaled corticosteroids as front-line therapy for patients with BO/OLD. Although systemic corticosteroids form the backbone of therapy for both acute and chronic GVHD, there have been no randomized clinical trials to assess its efficacy in patients with BO/OLD. A small retrospective study noted stabilization of lung function in seven of nine patients treated with pulsed corticosteroids at 10 mg/kg/day [105]. No other prospective trials on systemic corticosteroids alone have been published. The potential additive benefits of inhaled steroids have been examined in a number of clinical trials, with variable results [106,107].

Azithromycin and FAM therapy

Several small case series have attempted to exploit the anti-inflammatory effects of the antibiotic azithromycin to treat BO/OLD in both allogeneic HCT and lung allograft recipients. Each study suggested a beneficial effect of this drug on pulmonary function when administered for 12 weeks or longer [108,109]. Treatment with fluticasone, azithromycin, and montelukast (FAM therapy) has been used at a number of medical centers and is now widely employed for BO/OLD management. A retrospective review of patients treated with FAM therapy noted a reduction in the use of systemic corticosteroids compared with matched historical controls treated with high-dose corticosteroids [110]. Prospective phase II and III clinical trials are currently in progress to examine the role of combination therapy with FAM in the management of BO/OLD [111].

Inhibition of TNF-α and other approaches

The potential role for TNF-α in the pathogenesis of both OLD and RLD suggests that agents such as etanercept, which neutralize this protein, may have promise, and studies have demonstrated a potential benefit of this drug in some HCT patients with chronic lung injury [97,112]. A phase II clinical trial using etanercept for the treatment of chronic lung injury after HCT was recently published [97]. Etanercept was administered for 4 or 12 weeks to 31 patients with either BO/OLD (n = 22) or RLD (n = 9). BAL procedures were performed pre- and post-therapy in all patients. The addition of etanercept therapy to conventional immune suppression was well tolerated, with no observable infusional toxicities and a limited frequency of opportunistic infections. Objective responses in pulmonary function were observed in one-third of patients with non-infectious OLD or RLD and the 5-year overall survival was 90% (95% confidence interval 73–100%) for those patients who responded to therapy. Study subjects showed increases in plasma levels of sTNFRI, sTNFRII, IL-8 and TGF-β at the time of study entry and attempts to identify biomarkers of OLD represent an ongoing line of investigation. The role of extracorporeal photopheresis (ECP) for management of BO/OLD has yet to be defined. A recent report of 10 patients undergoing ECP for BO/OLD noted stabilization in six of nine patients treated with extended courses of ECP therapy [113]. Finally, the administration of inhaled cyclosporine showed promise for lung allograft recipients [114], but studies in HCT recipients have not been reported.

Optimizing the management of patients with chronic pulmonary dysfunction

The comprehensive approach to patients with late lung toxicity includes completion of a thorough history and physical examination, assessment of pulmonary function and radiographic findings, consideration of bronchoscopy/BAL and procurement of lung tissue, and consultation with a variety of medical specialists (Figure 95.6). However, practice guidelines regarding (1) the frequency of medical surveillance (including completion of PFTs) to identify early onset of disease, (2) the optimal diagnostic approach (including the role of radiographic imaging, BAL/tissue procurement) once pulmonary dysfunction is identified, (3) clinical criteria for when to start and stop systemic or inhaled therapy, (4) the route, dose, class, and duration of immune suppressive and supportive care agents, and (5) consideration of lung allograft procedures in severely affected individuals have not been established. The outstanding diagnostic and therapeutic dilemmas that exist with respect to the management of BO/OLD were thoughtfully reviewed by Chien and Martin [93].

Although some centers recommend quarterly PFTs following day 100 post-HCT for routine screening, the frequency of and methods for such measurements vary widely across institutions. Once new pulmonary toxicity is suspected, chest CT scans are frequently considered. While air trapping on expiratory views supports a diagnosis of OLD, a normal radiographic appearance of the lungs is not uncommon, nor does it rule out the presence of developing lung disease. Using an overlay of inspiratory and expiratory views from routine high-resolution CT scans of the chest, investigators at the University of Michigan have adapted the parametric response map, a voxel-wise image analysis technique to assess the evolution and extent of lung disease in non-HCT patients with chronic obstructive lung disease. This novel attenuation mapping technique can detect evidence for small airway and parenchymal disease before changes are observed on conventional radiographic or PFTs [115]. Studies are ongoing to test and validate this approach in HCT recipients with and without suspected pulmonary dysfunction. The role of bronchoscopy and BAL in managing patients with OLD or RLD

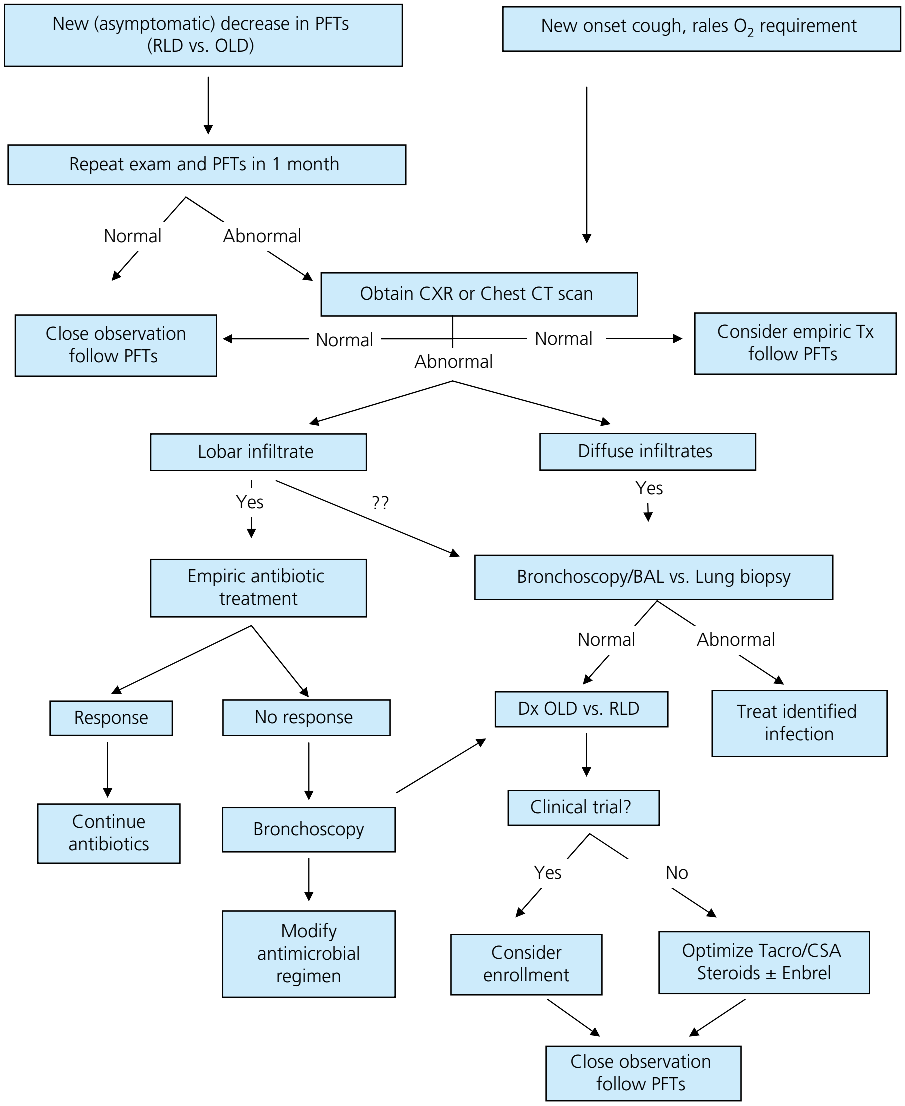
New (asymptomatic) decrease in PFTs (RLD vs. OLD)
New onset cough, rales O2 requirement
Repeat exam and PFTs in 1 month
Normal
Abnormal
Obtain CXR or Chest CT scan
Close observation follow PFTs
Normal
Normal
Consider empiric Tx follow PFTs
Abnormal
Lobar infiltrate
Diffuse infiltrates
Yes
??
Yes
Empiric antibiotic treatment
Bronchoscopy/BAL vs. Lung biopsy
Normal
Abnormal
Response
No response
Dx OLD vs. RLD
Treat identified infection
Continue antibiotics
Bronchoscopy
Clinical trial?
Yes
No
Modify antimicrobial regimen
Consider enrollment
Optimize Tacro/CSA Steroids ± Enbrel
Close observation follow PFTs

Figure 95.6 Approach to HCT patients with late-onset respiratory dysfunction. The comprehensive approach to patients with late lung toxicity includes completion of a thorough history and physical examination, assessment of pulmonary function, and radiographic assessment. Results of the initial work-up will guide subsequent decision-making towards initiation of empiric antimicrobial therapy versus consultation with medical and surgical specialists and consideration of bronchoscopy/BAL and procurement of lung tissue. When pulmonary dysfunction is determined to be non-infectious in origin and immune suppressive therapy is considered, enrollment on open clinical trials is desirable whenever possible. BAL, bronchoalveolar lavage; CXR, chest X-ray; OLD, obstructive lung disease; PFTs, pulmonary function tests; RLD, restrictive lung disease; Tx, therapy.

also deserves further consideration. One of the more intriguing findings of the recent TNF-α inhibition trial was the preponderance of patients with suspected BO/OLD and RLD who had subclinical but active pulmonary infections [97]. All 57 patients enrolled in this prospective study underwent a pretherapy BAL and 20 (35%) were ruled ineligible for subsequent study therapy because pathogenic organisms were identified in the BAL fluid. Of note, none of the 20 patients exhibited clinical signs of acute or chronic infection. Hence, the high incidence of occult infections in this setting supports the consideration for bronchoscopy as part of the standard approach to this patient population.

Finally, what composite of correlative clinical data should trigger the initiation of systemic or inhaled therapy? Are FEV_1 values and FEV1/FVC ratios the best measures of OLD or are measurements of FEV_1/SVC or FEV_{25-75} more sensitive indicators of small airway obstruction? Should therapy be initiated based upon a specific percentage decline in FEV1 or FEV_1/FVC ratio over a specified time period, regardless of the absolute FEV_1 or FEV_1/FVC ratio? Thus, would patients with a >10–15% decline in FEV_1 over a defined period, but whose FEV_1 remains >75% of predicted, benefit from early interventional therapy? What therapies should be considered for patients with significant non-infectious RLD? Is this a manifestation of an alloimmune attack on the recipient lungs? If one considers the proposed triphasic model for the progression of chronic fibrotic pulmonary dysfunction (Figure 95.5), treating affected patients at the onset of bronchiolar (or interstitial) inflammation and prior to significant collagen deposition would have merit. Such an approach underscores the potential benefits of early detection and the procurement of lung tissue via trans-bronchial or surgical approaches.

Conclusion

Lung injury remains a significant problem following allogeneic HCT in both the immediate post-transplant period and in the months to years that follow. Although lung injury occasionally occurs following autologous HCT, the allogeneic response significantly exacerbates toxicity in both the acute and chronic settings. Historically, much of this injury was assumed to be due to occult and often unidentifiable infections, but a large preponderance of experimental data now demonstrates that non-infectious disorders such as IPS and BO have major immunologic components. Despite these findings, establishing the lung as a true target of GVHD remains a hotly debated topic. Like the gut and skin, the lung is a critical immunologic interface between the sterile body sanctuary and the outside environment. As such, the lung is a rich source of histocompatibility antigens and professional antigen-presenting cells and the site of complex immunologic networks. Inflammatory mediators such as TNF-α along with donor-derived effector cells, which contribute to GVHD, are associated with lung injury in the experimental and clinical settings. However, the absence of consistent evidence for epithelial apoptosis remains a major obstacle to considering the lung a target organ of acute GVHD. In the chronic setting, the lung is widely accepted as a target of GVHD. The clear similarities between the histopathologic features of OLD observed after allogeneic HCT and during lung allograft rejection strongly support this concept. The case for immunologic mechanisms contributing to RLD is evolving, but conventional wisdom has yet to embrace this form of lung dysfunction as a manifestation of chronic GVHD. However, in the absence of infection, it remains unclear what (if any) other process could be responsible for the influx of (donor) immune cells into the lung parenchyma and interstitial space. It is hoped that as animal models yield further insights, our understanding of these disease processes will improve and ultimately lead to new therapeutic strategies to diagnose, treat, and prevent pulmonary toxicity in our patients.

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CHAPTER 96

Kidney and Bladder Complications of Hematopoietic Cell Transplantation

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Introduction

Renal dysfunction is a common complication of hematopoietic cell transplantation (HCT) and a major cause of morbidity and mortality. There are many definitions used to describe both the acute kidney injury (AKI) and chronic kidney disease (CKD) that occur after HCT (Table 96.1). The heterogeneous nature of the definitions, types of HCT, and diverse patient populations often make it difficult to draw comparisons across studies. The bladder is also commonly involved after HCT, and processes that affect the bladder can also lead to renal injury.

This chapter focuses on the epidemiology, pathogenesis, and treatment of renal and urologic problems. The time course of renal injury varies from days after transplant to months later. The difference between acute and chronic is arbitrarily defined by timing, as CKD usually refers to manifestations of renal disease that occur after day 100, and "acute" covers the early injury up to day 100. Similarly, bladder injury, most commonly hemorrhagic cystitis (HC), is divided into early onset and late onset, and the distinction between the two is also one of timing, lending itself to different etiologic factors. In this chapter, the early-onset or acute injuries will be discussed first, followed by the later or chronic injuries. The disease processes can overlap: AKI that persists is later termed CKD. Although this chapter discusses the injury that occurs after HCT, baseline or pre-transplant renal function can impact outcome [1]. Therefore, baseline assessments not only of serum creatinine, but also urinalyses and more formal estimations of glomerular filtration rate (GFR), are warranted. In addition, the hematopoietic cell transplantation-specific comorbidity index (HCT-CI) score (intermediate and high risk) is associated with an increased risk of AKI after HCT [2]. Accurate assessment of baseline renal function can help guide later medication dosing.

Acute kidney injury

Epidemiology

In the majority of studies of AKI in the HCT population, AKI is defined as a doubling of baseline serum creatinine within the first 60–100 days after HCT. The incidence of AKI varies from a low of 6.5% in autologous transplants to as high as 79% in patients admitted to the intensive care unit (ICU) after transplant. The incidence also varies based on the type of HCT and the conditioning regimen. AKI is more common in allogeneic transplant recipients than autologous transplant recipients, and high-dose regimens compared with reduced-intensity conditioning (RIC). The less severe, or grade 2, AKI is defined as less than a doubling of baseline serum creatinine with a >25% decrease in GFR. Grade 2 is a doubling of baseline serum creatinine without the need for dialysis, and grade 3 is dialysis-requiring AKF. Two new definitions proposed by nephrologists for AKI are based on the so-called RIFLE (risk, injury, failure, loss, and end-stage) criteria and the AKIN (acute kidney injury network) definition (Table 96.1). The stages are based on changes in GFR and serum creatinine and/or decreases in urine output. Risk is an increase in serum creatinine of 1.5× baseline or a decrease in GFR of >25%. Injury is a doubling of baseline serum creatinine or a 50% decrease in GFR. Failure is either a tripling of baseline serum creatinine, a serum creatinine of >4 mg/dL or a 75% decrease in GFR. Loss and end-stage refer to duration of renal failure of 1 and 3 months, respectively [3]. These criteria were established because the degree of renal failure affects outcome in adult patients admitted to the ICU. They are also applicable to the HCT population and should be used in future studies of AKI after HCT. Regardless of the definition chosen, increasing severity of AKI is associated with a stepwise increase in the hazard ratio of mortality [4–6].

Risk factors for AKI by type of conditioning regimen and transplant

High-dose conditioning regimens and allogeneic HCT

The incidence of AKI in these patients is as high as 69%. AKI in this group of patients occurs early after transplant, often before day 28. Risk factors for AKI in this group of patients include lung toxicity, hepatic toxicity, sinusoidal obstruction syndrome (SOS), amphotericin use, and sepsis [7–14] (Table 96.2). In a smaller study in Turkey, cyclosporine (CSP) use was associated with an increased risk of AKI after high-dose regimens, whereas sepsis played a major role in autologous recipients. It was also found that a low serum albumin at baseline increased the risk of later renal dysfunction [15]. Those patients who develop AKI after high-dose conditioning require dialysis more frequently and have a higher mortality than patients without AKI. In the patients who require dialysis, the mortality approaches 83%. In a meta-analysis of patients with AKI after HCT, AKI was an independent predictor of mortality [16].

RIC regimens and allogeneic HCT

In a retrospective cohort study comparing 140 high-dose versus 129 RIC transplant recipients, it was found that despite the older age of

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the RIC patients and the greater percentage of patients with a GFR of <89 mL/min/1.73 m^2 at baseline, only 47% of patients developed AKI compared with 73% in the high-dose treatment group within the first 3 months post-transplant. AKI occurred slightly later in the RIC group, at a median of 26–60 days post-transplant. Fewer patients required dialysis in the RIC group (3% versus 12%) and mortality was significantly lower. The need for artificial ventilation increased the risk of AKI 10-fold in the RIC group of patients. Other risk factors included graft-versus-host disease (GVHD) and the source of the donor cells, with AKI being more common in patients who received marrow compared with peripheral blood, diabetes, SOS, sepsis, methotrexate use, and cytomegalovirus (CMV) reactivation [6,13,17–21].

Table 96.1 Definition of terms: A comparison of post-SCT AKI[a] definition and classification according to serum Cr levels in the RIFLE, AKIN, and grading criteria

RIFLE	
Risk	Increase in serum Cr ≥1.5 × baseline or decrease in GFR ≥25%
Injury	Increase in serum Cr ≥2.0 × baseline or decrease in GFR ≥50%
Failure	Increase in serum Cr ≥3.0 × baseline or decrease in GFR ≥75% or an absolute serum Cr ≥4.0mg/dL (354 μmol/L) with an acute rise of at least 0.5 mg/dL (44 μmol/L)
Loss	Persistent AKI >4 weeks
ESKD	ESKD >3 months
AKIN	
Stage 1	Increase in serum Cr ≥0.3 mg/dL (26.5 μmol/L) or increase to 150–199% (1.5–1.9-fold) from baseline
Stage 2	Increase in serum Cr to 200–299% (>2.0–2.9 fold) from baseline
Stage 3	Increase in serum Cr to ≥300% (≥3-fold) from baseline or serum Cr ≥4.0 mg/dL (354 μmol/L) with an acute rise of at least 0.5 mg/dL (44 μmol/L).
Grading	
Grade 0	Decrease in GFR <25% of baseline
Grade 1	Increase in serum Cr <2-fold from baseline with a decrease in GFR >25% but <50% of baseline
Grade 2	Increase in serum Cr ≥2-fold from baseline but not requiring dialysis
Grade 3	Increase in serum Cr ≥2-fold from baseline and need for dialysis

GFR, glomerular filtration rate; RIFLE, risk, injury, failure, loss of kidney function, and end-stage kidney disease; AKIN, acute kidney injury network; Cr, creatinine; AKI, acute kidney injury; ESKD, end-stage kidney disease.

[a] Post-SCT AKI is defined within the first 100 days after SCT.

Source: Ando et al. 2010 [179]. Reproduced with permission of Nature Publishing Group.

High-dose conditioning regimen and autologous HCT

There are two case series of AKI in autologous transplant recipients. Compared with the allogeneic setting, the incidence is lower – approximately 21% – in patients after autologous HCT. In a study of patients with amyloid light-chain (AL) amyloidosis, risk factors for AKI identified at baseline included decreased creatinine clearance, proteinuria, and cardiac involvement. In addition, transplant-related risk factors included melphalan use and bacteremia. A second study of 232 women who underwent HCT for breast cancer found a similar incidence of AKI but identified liver and lung toxicity in addition to sepsis as risk factors for acute injury, similar to risk factors for AKI after high-dose conditioning followed by allogeneic transplantation. It has been suggested that the lower incidence after autologous HCT is related to the avoidance of calcineurin inhibitors (CNIs).

Pathogenesis of AKI after HCT

Although many risk factors have been associated with the development of AKI after HCT, only the most common ones are discussed in detail below (Table 96.3). Thrombotic microangiopathies (TMAs), including thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS), can occur early after HCT, but changes in serum creatinine may not be present at the time of diagnosis. TMAs are discussed in the section on CKD. Tumor lysis syndrome is a rare cause of AKI in patients after HCT, seen more commonly in patients with residual disease or an underlying lymphoproliferative disorder at the time of HCT. The renal injury is related to tubular obstruction and dysfunction secondary to hyperuricemia, hyperphosphatemia, and deposition of uric acid and phosphate within the kidney. Management involves the use of uric acid inhibitors, aggressive hydration, and, when needed, dialysis. Tumor lysis syndrome is relatively uncommon in the HCT population as most patients are in remission at the time of transplant.

Sepsis

Sepsis leads to a combination of insults, both hemodynamic and inflammatory, that coalesce to cause renal failure. The pathophysiology of AKI in this setting is complex and not well understood. There is an initial inflammatory response leading to cytokine-induced

Table 96.2 Summary of the literature on acute kidney failure (AKF) after hematopoietic cell transplantation (HCT)

No. of patients	Type of transplant	Incidence: autologous/ allogeneic (%)	Definition of AKF	Postulated risk factors	Onset time (days) (median)	Ref.
143	100% allogeneic	48.9	Grade 1 and 2	Increased bilirubin levels, SOS, aGVHD 3–4	33	[5]
232	100% autologous	21	Grades 2 and 3	Liver and lung toxicity, sepsis	28	[10]
170 pediatric	55.3% allogeneic myeloablative, 44.7% allogeneic non-myeloablative	45.7/17.1	Grade 2	Myeloablative conditioning	30	[178]
57	26% autologous, 73% allogeneic	60/79	Grades 2 and 3	Liver failure, low serum albumin, APACHE II score	27	[12]
269	100% allogeneic	60	Grades 2 and 3	Myeloablative conditioning, female gender, high-risk malignancy, comorbidity	22	[13]
147	100% allogeneic	36	Grade 2	SOS, amphotericin use	33	[14]
88	100% allogeneic	69	Grades 2 and 3	SOS, sepsis, lung and liver toxicity	16	[9]
47	53% autologous, 47% allogeneic	32/68	Grades 2 and 3	SOS, sepsis, cyclosporine	44	[15]
253	100% allogeneic, non-myeloablative	40	Grades 2 and 3	Ventilator use, GVHD, product source (marrow versus peripheral blood)	60	[19]
62	100% allogeneic, non-myeloablative	29	Grades 1–3	HLA match, 1 or >aGVHD, SOS, sepsis	38	[21]
96	37.5% RIC, 62.5% myeloablative	29.2	Grades 1–3	aGVHD	33	[7]

GVHD, graft-versus-host disease; SOS, sinusoidal obstruction syndrome.

Table 96.3 Potential risk factors and mechanisms for AKI in the HCT population

Intravascular volume depletion
- Vomiting and diarrhea associated with acute gut GVHD

Systemic vasodilation
- Sepsis

Renal vasoconstriction
- Sinusoidal obstruction syndrome
- Calcineurin inhibitors

Endothelial injury
- Acute GVHD
- Calcineurin inhibitors
- Total body irradiation
- Thrombotic microangiopathy

Tubular injury
- Medications: amphotericin, vancomycin
- Conditioning chemotherapy: vancomycin

Source: adapted from Sharfuddin and Molitoris 2011 [23].

systemic arteriolar vasodilatation and endothelial injury [22]. The resultant capillary leak that occurs leads to renal hypoperfusion. In addition, early after sepsis there is a constriction of the renal vessels, further decreasing renal perfusion. Injury to the tubules themselves causes a local release of cytokines and chemokines that cause local inflammation and further intrarenal injury [23]. In addition to the effects of sepsis, the medications used to treat the infections are often nephrotoxic. Several reports have identified amphotericin, in the conventional or liposomal formulation, as a risk factor for AKI.

Hepatic SOS

There is a well-known association between sinusoidal liver injury and renal insufficiency. It has been postulated that portal hypertension resulting from hepatic sinusoidal injury leads to both decreased renal perfusion and tubular injury, the former probably being the more important in the genesis of AKI. Clinically, SOS is associated with marked sodium avidity, low urinary sodium resulting in volume overload, edema, and weight gain, as seen in hepatorenal syndromes [24]. Weight gain likely serves as a marker of impending renal failure rather than being a result of the renal injury; in addition, the association between weight gain and the development of AKI described in the literature is conflicting [14,25]. Although some have advocated keeping weight gain per se to a minimum (<10% fluid overload) to improve outcome in this patient population, it is unclear whether the weight gain is causal or the result of other insults to these patients. Hence prevention of SOS may be the more important strategy.

Nephrotoxic medications

Although there are many potential nephrotoxic medications that are given to patients after HCT, the one that is most commonly associated with development of AKI is amphotericin. The finding by many of an increased risk of AKI among patients receiving amphotericin is not surprising because the nephrotoxicity of conventional amphotericin is well known, as is its association with renal failure in the HCT population [26–28]. However, in a study by Hingorani *et al.* [14], liposomal preparations of amphotericin were also associated with an increased risk of AKI. In the same study, neither the administration of vancomycin nor gentamicin in the 2 weeks prior to development of AKI increased the risk of AKI. In cord blood transplant recipients, vancomycin was associated with an increased risk of AKI post-transplant [29].

Use of amphotericin for fever of unclear etiology carries significantly increased risks of AKI in the transplant population. Given the marked increase in risk of AKI in patients who have received either conventional or liposomal amphotericin, we recommend that patients be treated with amphotericin only if clear indications exist, such as documented fungal infection or prolonged use of prophylactic triazoles. Fortunately, newer antifungal drugs such as fluconazole, itraconazole, voriconazole, and caspofungin are now available for indications formerly covered by amphotericin. Depending on the end-points chosen, each of these medications has been shown to be equally efficacious to amphotericin but with better safety profiles [30].

Management of AKI

In the majority of cases, the management of AKI is supportive. The choice of dialysis modality often depends on hemodynamic stability of the patient, degree of volume overload, and blood pressure, which makes it difficult to interpret outcomes as sicker patients are more likely to be placed on continuous renal replacement therapy. In two studies of pediatric HCT patients, those patients placed on convective therapies [continuous veno-venous hemofiltration (CVVH) or continuous veno-venous hemodiafiltration (CVVHDF)] rather than diffusive therapy had a better survival rate, especially when initiation of therapy was early, and in one study at the time of intubation and mechanical ventilation regardless of renal function and urine output [31,32]. What is apparent is that early intervention, regardless of modality choice, can improve outcome.

Chronic kidney disease

Epidemiology

The cumulative incidence of CKD varies from 13–60% in adult studies [33–35] to as high as 62% in children [36]. CKD usually becomes apparent 6–12 months after HCT, although it has been described as early as 2 months and as late as 10 years post-transplant. As in AKI, numerous definitions are used to define CKD in these patients. Most definitions are based on serum creatinine or an estimated or calculated measurement of GFR based on the Modification in Diet and Renal Disease or the Schwartz formula in children. The National Kidney Foundation has divided GFR into stages 1–5 based on an estimated GFR (eGFR) to help guide clinical treatment plans. The range of GFR is from ≥90 mL/min/1.73 m^2 with some renal damage for stage 1 to <15 mL/min/1.73 m^2 for stage 5 and end-stage renal disease (Table 96.4).

Many terms have also been suggested to describe the chronic forms of renal dysfunction occurring after HCT, including "bone marrow transplantation nephropathy" [33], "radiation nephritis" [37], and "conditioning-associated HUS" [38]. None of these terms defines the degree of renal dysfunction, anemia, or hypertension. The renal pathologies of bone marrow transplantation nephropathy, conditioning-associated HUS, and radiation nephritis are indistinguishable and may reflect a common injury. Total body irradiation

Table 96.4 National Kidney Foundation Kidney Disease Outcomes Quality Initiative: chronic kidney disease by stage

Stage	Description	eGFR (mL/min/1.73 m^2)
1	Kidney damage with normal or GFR	≥90
2	Kidney damage with mild ↓ GFR	60–89
3	Moderate ↓ GFR	30–59
4	Severe ↓ GFR	15–29
5	Kidney failure	<15 or dialysis

eGFR, estimated glomerular filtration rate.

(TBI) may play a role in the endothelial injury that leads to the development of a TMA and its subsequent renal injury.

In addition to a TMA, there are other manifestations of CKD in this patient population, including nephrotic syndrome and GVHD-related CKD. The latter form of CKD after HCT is often defined as abnormalities in serum creatinine or estimated and calculated GFR at 6–12 months after transplant. The lack of a consistent definition of post-HCT CKD has made it difficult to draw comparisons across studies, to identify risk factors for its development, and to devise prevention and treatment strategies.

Clinical syndromes of CKD after HCT

Acute kidney injury

AKI has been shown to be a risk factor for CKD in various adult and pediatric studies [14,35], suggesting that some patients do not recover fully from their AKI and progress to CKD.

TMAs: TTP and HUS

Definitions and clinical diagnosis

TMA syndromes represent a spectrum of clinical diseases characterized by systemic or intrarenal platelet aggregation, thrombocytopenia, and microvascular fragmentation of erythrocytes. Platelet aggregation can result in ischemia and organ injury. When renal injury is predominant, a diagnosis of HUS is usually rendered, while the presence of extensive extrarenal manifestations leads to a diagnosis of thrombotic TTP (reviewed in [39]).

TMA syndromes are not uncommon in the setting of HCT. In a series of both retrospective and prospective studies, the incidence of microangiopathic disease after HCT ranged between 2 and 21% [40–42]. The incidence seems to be lower in patients receiving high-dose conditioning regimens than in patients receiving RIC regimens [43,44]. TTP tends to occur early after transplant, whereas HUS seems to occur much later after transplant. When the presentation is fulminant, TMA syndromes are often associated with severe AKF and death. More commonly, however, they follow an indolent course, resulting in the eventual development of CKD.

For the diagnosis of TMA after HCT, the following clinical characteristics must be met: microangiopathic hemolytic anemia demonstrated by red blood cell fragmentation with two or more schistocytes per high-power field, elevated lactate dehydrogenase levels, renal dysfunction (defined as a >50% increase in baseline serum creatinine or a 50% decrease in creatinine clearance from baseline), and/or neurologic involvement without other identifiable causes, and a negative direct and indirect Coombs test [45]. The new grading system proposed is based on the common toxicity criteria for HCT TMA, and severity is based primarily on serum creatinine level, need for dialysis, and/or encephalopathy [45]. However, a comparison between the Clinical Trials Network definition of TMA and the International Working Group suggested that requirement of concurrent renal and neurologic dysfunction in the Clinical Trials Network definition and the requirement of ≥4% schistotocytes of the International Working Group definition could lead to exclusion of patients with probable TMA who are at high risk for a poor outcome [46]. Those patients with more severe forms of TMA have a higher risk of mortality [47].

Risk factors for TMA after HCT

Although no clear relationships have been found to date for the development of TMA after HCT, a number of risk factors have been examined. In earlier studies, where HUS was the primary diagnosis, risk factors identified were TBI [33,36,48] and CNI use [33,38,47,49,50]. However, in later studies of TMA, acute GVHD grades II–IV, older age, and transplant from an unrelated donor were the primary risk factors identified [40,42]. Other investigators have also identified SOS, grafts from matched unrelated donors or haploidentical donors, and lymphoid malignancy as significant predictors of TMA after HCT in addition to the above risk factors [11,51–53]. No decrease in the incidence of TMA has been found in patients undergoing RIC regimens, and it is thought that fludarabine may directly contribute to the endothelial injury in these patients [44,53]. In many of these cases, the renal injury is not present at the time of diagnosis of the TMA but occurs later in the course of the disease. TMA can also occur in the HCT population as a result of viral infections [54].

Total body irradiation

The entity known as “radiation nephritis” was described as early as the 1920s. The clinical course is variable and manifests as nephritis, hypertension, and proteinuria and/or anemia [55]. The time frame for each of these clinical presentations is also variable, with acute nephritis presenting 6–12 months after irradiation, and the subacute, chronic, and late forms occurring 2–5 years later. Although radiation can damage the vascular endothelium, tubular epithelium, and glomerulus, the primary site of injury has not been identified [55]. The doses of TBI traditionally associated with radiation nephritis are usually >2000 cGy [56]. To test the theory that radiation causes endothelial injury, Rubin and Casarett performed renal scans at several time points after irradiating rats unilaterally with 1000–6000 R [55,57]. A baseline renal scan outlined both kidneys and remained essentially unchanged for 3 months post-exposure. Between 3 and 6 months, subtle changes were noted, and at 9 months, the irradiated left kidney showed clearly diminished uptake of radiolabel. The changes on renal scan correlated with microangiopathic pathology showing poor vascularization and disruption of glomerular capillaries.

Given the variable time to presentation and degree of renal injury, a secondary insult(s) to the kidney may potentiate the radiation injury. For example, it is known that irradiated vessels are more sensitive to the effects of hypertension. It is therefore possible that hypertension in these patients may cause further vascular injury or impair the kidney’s ability to repair such injury. As with other dividing cells, radiation inhibits endothelial cell proliferation in culture [58]. The normal turnover time of endothelial cells is approximately 2 months [59], which may explain the delayed development of renal injury in some patients after HCT.

Based on the dose of radiation received, Miralbell *et al.* determined the risk of having an abnormal creatinine level at 18 months after HCT [60]. There appeared to be a dose-dependent relationship between radiation and renal injury. At a dose of 12 Gy, the relative risk was 2.9, and at a dose of 13.5 Gy, the relative risk was 8.4 compared with the group of patients who received 10 Gy. In a prospective study using renal shielding, a trend towards a decreased incidence of CKD was observed in the shielded versus the unshielded groups [48]. In this study, all patients received TBI in addition to cytarabine and cyclophosphamide as part of their conditioning regimen. Twenty of the 157 adults (13%) surviving beyond 100 days developed renal insufficiency; 18% were from the unshielded group and 10% were from the single-shielded group. No patients who received double shielding (diminishing the total radiation dose to the kidneys to 9.8 Gy) developed renal insufficiency throughout the follow-up period of 2.5 years. A retrospective analysis of 100 patients who underwent T-cell-depleted transplantations found that 11/70 patients who received TBI as part of their conditioning regimen developed TMA, whereas none of the patients who did not receive TBI developed a TMA. None of the patients received a CNI [61].

Immunosuppressive agents

Immunosuppressive agents have also been associated with the development of CKD. Agents such as CSP and tacrolimus can initiate HUS-like syndromes after HCT [33,41,47,49,50,53]. On histologic examination of kidneys from three HCT patients on CSP who died with renal failure, thrombus formation was observed in capillary tufts and arterioles with subendothelial and mesangial widening and sclerosis. Conversely, none of 55 patients not on CSP had renal capillary or arteriolar thromboses [62]. CSP is also a potent vasoconstrictor of the afferent arteriole of the glomerulus. In a small study of seven renal transplant recipients on CSP, decreases in GFR and renal plasma flow were found and correlated with CSP dose. The decreases seen here were transient and reversed as drug levels returned to normal [63]. The nephrotoxic effects of CSP correlate with serum levels of the drug and duration of therapy. CSP is also known to cause arteriolar injury, glomerular sclerosis, and interstitial fibrosis, in addition to diffuse expansion of the mesangial matrix [64]. Studies of pediatric cardiac transplant patients also found an association between CSP use within the first 2 months after transplant and decreases in GFR years after transplant [65]. High CSP levels (>500 μg/L) in the first 6 months after cardiac transplant were associated with the development of end-stage renal disease at any time after cardiac transplant [66]. However, in a study of approximately 69,000 patients receiving a non-renal transplant including heart, lung, liver, intestine, and combined heart–lung, CSP use at initial hospitalization was associated with only a slightly increased risk of developing CKD after transplant [hazard ratio (HR) = 1.24, 95% confidence interval (CI) 1.17–1.30] [67]. In both adult and pediatric studies, the combination of sirolimus and a CNI, whether used for prophylaxis of GVHD or treatment, has been associated with an increased risk of TMA compared with CNIs alone [68–70]. The numbers in these studies were small; however, 11–23% of patients developed TMA and it seemed to be associated with high levels of tacrolimus, sirolimus, or both. This risk may be higher in heavily pretreated patients, such as those undergoing their second HCT [70]. Close monitoring of levels and clinical and laboratory signs of TMA are warranted in patients on combination therapy. In some cases, discontinuation of sirolimus, tacrolimus, or both was needed to improve signs and symptoms of TMA.

Histopathology of TMA after HCT

Microscopic examination of kidney biopsy specimens from patients with TMA-associated CKD demonstrates mesangiolysis and loss of endothelial cells with expansion of the subendothelium and occlusion of capillary loops (Figure 96.1). On electron micrographs, there is extensive widening of the space between the glomerular basement membrane and the subendothelium with amorphous deposits that are not immune complexes. Recent renal pathology studies of renal biopsy specimens and autopsy specimens have demonstrated staining of C4d, a complement split product, in glomerular and peritubular capillaries, suggesting an antibody-mediated process and complement activation as the cause of the endothelial injury in the cases of TMA. Patients also had evidence of tubulitis and peritubular capillaritis in their kidneys. The median time to biopsy or autopsy was 7 months post-transplant and all patients had either acute or chronic GVHD. Five of the seven patients did not have clinical evidence of TMA at the time of biopsy or death but did have elevations in serum creatinine and proteinuria/hematuria [71].

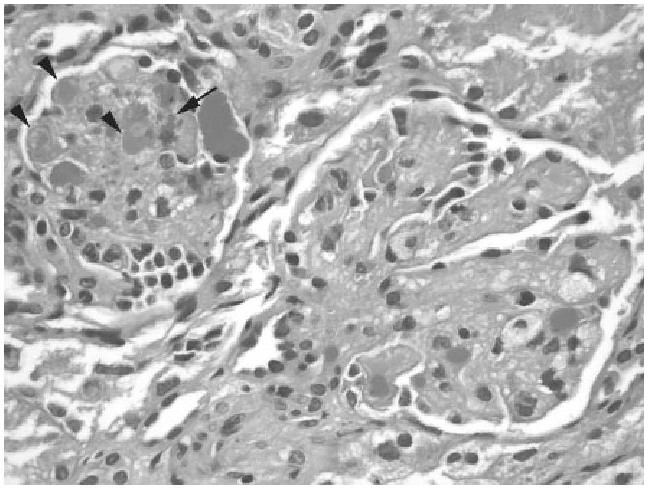

Figure 96.1 Thrombotic microangiopathy. The glomerular mesangium is fibrillary and blends with unapparent capillary lumina that are obscured by markedly swollen endothelial cells that impart a "bloodless" appearance. Fibrin thrombi fill some of the capillaries in the left glomerulus (arrowheads), which has erythrocyte fragments in the mesangium (arrow). (Hematoxylin and eosin; magnification: ×200).

Pathophysiology of TMA after HCT

Regardless of the offending agent, endothelial damage is thought to represent the inciting factor in the development of TMA syndromes (Figure 96.2). Endothelial injury activates the coagulation system, leading to thrombin formation and fibrin deposition. The ability to repair such injury depends on the balance between fibrinolytic and coagulant activity [72]. Although histologically similar, TMA appears to be distinct from TTP, in that TA-TMA patients do not have markedly low levels of ADAMTS13 [73,74]. Current theories on the pathogenesis of renal endothelial injury include hypotheses based on abnormalities of the coagulation cascade and the complement system [75,76].

More recent studies have identified both acute GVHD grade II–IV and chronic GVHD as risk factors for the development of TMA. Endothelial injury has been described in patients with chronic GVHD, and it is thought that endothelial cells are direct targets of cytotoxic donor T-lymphocytes in these patients [77]. The risk of TMA injury in the kidney may be secondary to circulating inflammatory cytokines related to GVHD elsewhere in the body or may reflect direct injury to endothelial cells of the kidney by GVHD. Plasma markers of endothelial injury and coagulation activation are elevated in patients with acute GVHD after HCT, suggesting an association between endothelial injury, acute GVHD, and the subsequent development of TMA [78,79]. Loss of function of vascular endothelial growth factor (VEGF) or inhibition of VEGF leads to the development of proteinuria and glomerular endothelial injury consistent with TA-TMA [78], and lower levels of VEGF have been associated with more severe forms of acute GVHD [79]. Perhaps GVHD leads to endothelial injury in the kidney through a reduction in serum VEGF levels and therefore loss of the protective effects of VEGF on the glomerular filtration barrier. The increased risk of TA-TMA in recipient–donor gender mismatch pairs further supports the theory that acute GVHD may play a role in the pathogenesis of TA-TMA after HCT [80].

An association between inflammatory cytokines, interleukin-6, interleukin-8, and tumor necrosis factor-alpha (TNF-α) and the

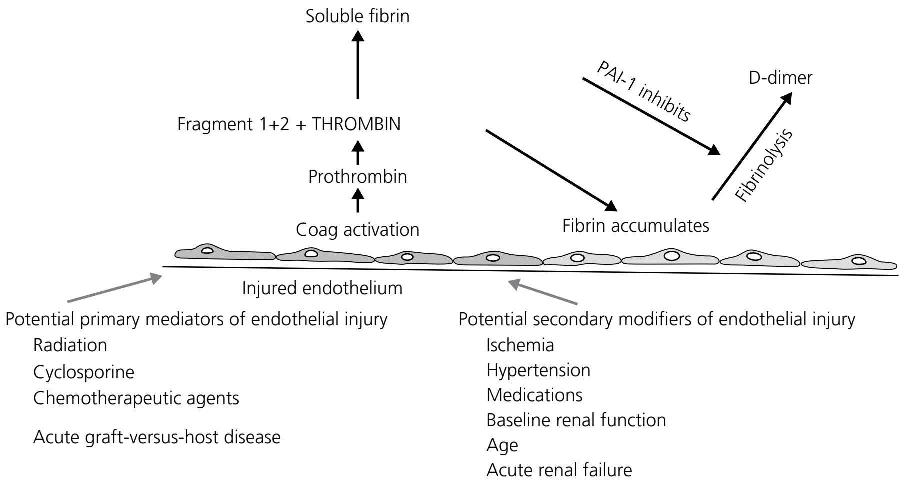

Figure 96.2 Potential risk factors and pathophysiology of thrombotic microangiopathy after hematopoietic cell transplantation. PAI-1, plasminogen activator-1.

balance between ultralarge von Willebrand factor release from endothelial cells and the rate of breakdown by ADAMTS13 resulting in thrombosis has been described [81]. Other investigators have found systemic release of TNF-α correlated with levels of PAI-1 and tissue plasminogen activator release, supporting the idea of cytokine-induced endothelial damage [82].

Natural history of TMA after HCT

Several studies have been carried out to elucidate the clinical course of TMA-related CKD following HCT. Again, most have involved small numbers of patients and have had a relatively short follow-up (1–5 years) [33,36,83–85]. What is apparent from the studies reported is that often an acute deterioration of renal function is followed by a period of stabilization, but rarely is full renal function restored. Patients with CKD after HCT can progress to end-stage renal disease and have decreased survival compared with other patients with end-stage renal disease [86].

In the study by Cohen *et al.* of 149 patients who survived 100 days or more after HCT during 1985–1990, 19 (13%) developed HUS within 6–12 months after transplantation [33]. Four patients progressed rapidly to end-stage renal disease with a clinical picture resembling HUS; three of the four died. The one surviving patient had a markedly diminished GFR of 17 mL/min (normal 80–100 mL/min). Seven patients suffered a more gradual but continual decline, and the remaining eight patients had stabilization of their renal function after an initial period of decline. Four of the seven patients eventually progressed to dialysis. Elevated lactate dehydrogenase levels and lower platelet levels seemed to predict a poor prognosis.

Nephrotic syndrome

Chronic GVHD may manifest itself in the kidney as nephrotic syndrome with or without renal insufficiency (reviewed in [87]). Patients usually present with proteinuria, edema, and hypoalbuminemia. The majority of these case reports demonstrated membranous nephropathy with subepithelial deposits on biopsy; it is postulated that these deposits are antigen–antibody complexes representing GVHD in the kidney (Figure 96.3).

However, cases of minimal-change disease (MCD), which is thought to be a T-cell-mediated process, have been described [87] and one case in a cord blood transplant patient without GVHD was thought to be associated with recipient T-cell activation leading to both graft failure and MCD [88]. Comparisons between case reports of membranous nephropathy and MCD after HCT have found that membranous nephropathy occurs in 61% of cases compared with 22% of cases with MCD [89]. The majority of reported patients with membranous nephropathy were male, slightly older, and had a history of acute and chronic GVHD. Both MCD and membranous nephropathy occur later after transplant at 8 and 14 months, respectively, and tend to occur within 1–5 months of the development of GVHD and/or the tapering of immunosuppression for chronic GVHD. Membranous nephropathy is more difficult to treat, with only 27% of patients reported achieving remission compared with 90% of patients with MCD. Others have reported cases of diffuse proliferative glomerulonephritis, antinuclear cytoplasmic antibody-related glomerulonephritis, focal segmental glomerulosclerosis, and immunoglobulin A nephropathy [90–94] occurring after HCT. The development of each of these diseases seems to be associated with chronic GVHD and/or tapering of immunosuppression. Treatment with high-dose prednisone and/or reinstitution of CNIs usually results in resolution of nephrotic syndrome. Some physicians have used rituximab successfully in patients with nephrotic syndrome after HCT, typically in cases of membranous nephropathy [95].

GVHD-related CKD

Many survivors with CKD after HCT will not present with nephrotic syndrome, meet the definition of a TMA, or have a documented viral infection, and therefore are labeled idiopathic CKD. The incidence of idiopathic CKD varies from 13% in patients receiving high-dose conditioning to 16–66% in patients who have been treated with RIC. Risk factors identified in the high-dose group include older age, TBI, CSP use, AKI, and acute and chronic GVHD. In the RIC transplant group, long-term CNI use and prior autologous transplant, and also AKI and GVHD, were associated with an increased risk of CKD.

However, in other studies, TBI was not associated with an increased risk of CKD [96]. Miralbell *et al.* found that those patients receiving a lower dose of TBI because of renal shielding actually had greater decreases in GFR at 4 months compared with patients who received higher doses of TBI. The effects were no longer apparent

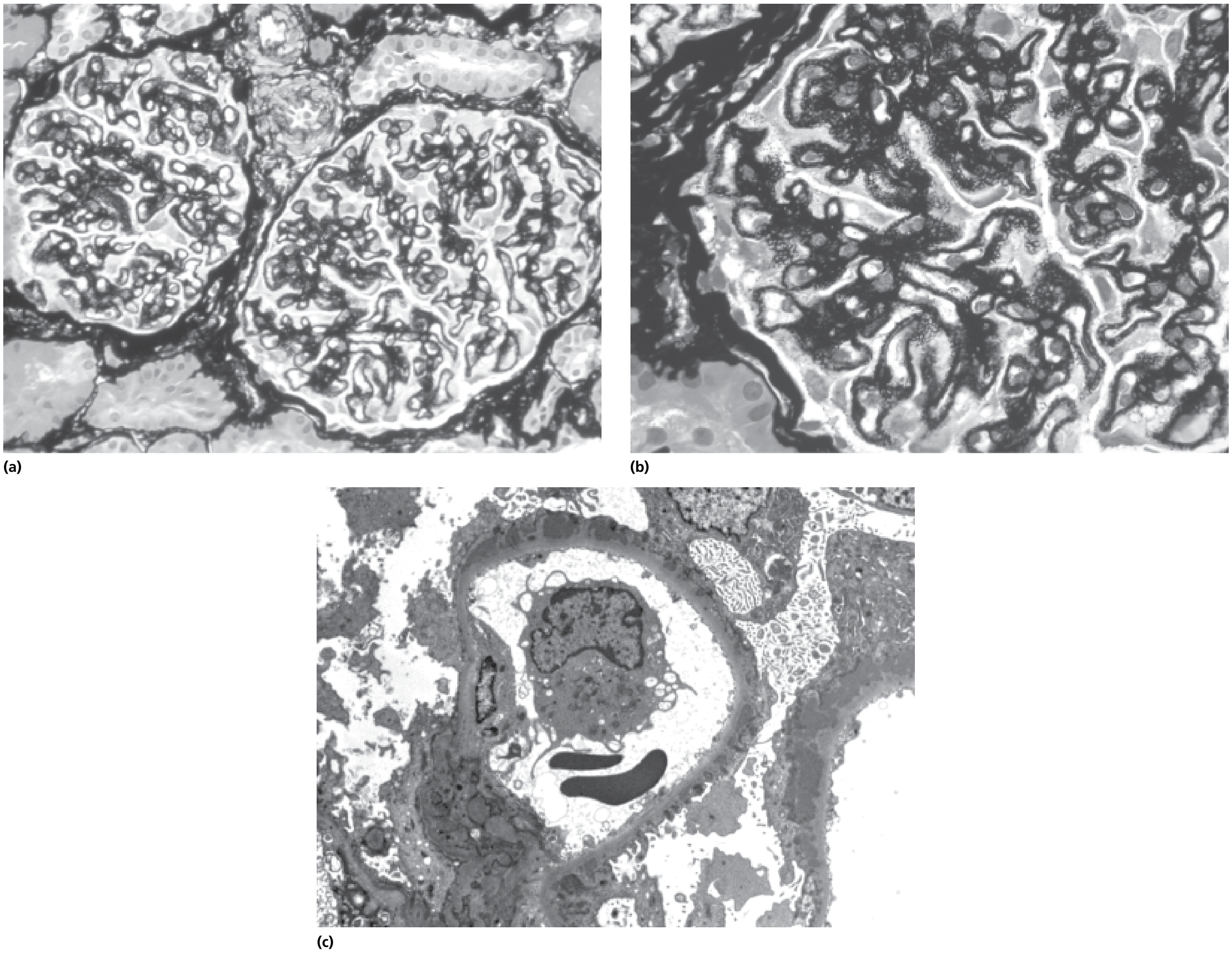

Figure 96.3 Membranous nephropathy. (a) The normocellular glomeruli in this nephrotic bone marrow transplantation patient have uniformly thickened glomerular basement membranes. (Methenamine–silver–hematoxylin; magnification: ×200). (b) Eosinophilic subepithelial deposits are visible between membrane spikes. (Methenamine–silver–hematoxylin; magnification: ×400). (c) Variably sized immune complex deposits stud the subepithelial glomerular basement membrane. (Uranyl acetate and lead citrate; magnification: ×6000).

after 12 months following HCT [97]. In a cohort study of 142 children undergoing HCT over a period of 5 years in The Netherlands, Kist-van Holthe *et al.* [98] found no correlation between radiation dose used and renal insufficiency at 1 year. In a large, retrospective study of 1635 HCT patients, TBI, irrespective of dose (range 200–1600 cGy), was not associated with the development of CKD after adjusting for demographic data and baseline characteristics, including original diagnosis, donor type, transplant type, and transplant year [34]. In the same study, TBI (HR = 1.0, 95% CI 0.7–1.4) was not associated with an increased risk of CKD among patients who received an allogeneic graft (n = 1228).

The finding that GVHD is a risk factor for the development of CKD in patients after HCT who may not present with nephrotic syndrome or evidence of TMA [34,35,97] suggests that either the kidney is a direct target organ of GVHD via T-cell-mediated renal damage or the chronic systemic inflammatory state of GVHD leads to CKD (Figure 96.4). A third explanation is that chronic exposure to CNIs, such as CSP, leads to CKD. These are not mutually exclusive hypotheses as T-cell-mediated injury in GVHD is intertwined with cytokine effects [99]and the effects of CSP may be potentiated

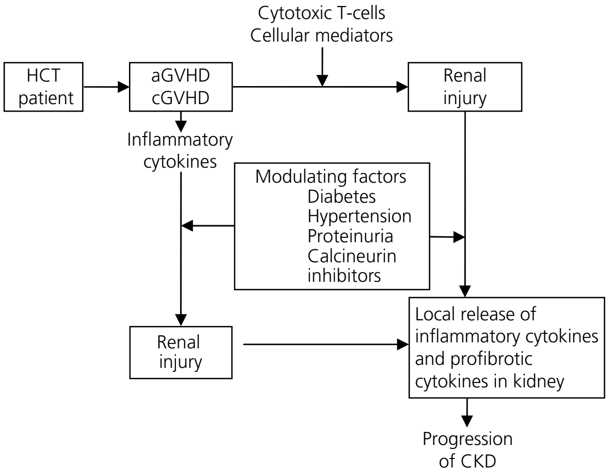
Cytotoxic T-cells
Cellular mediators
HCT patient
aGVHD cGVHD
Renal injury
Inflammatory cytokines
Modulating factors
Diabetes
Hypertension
Proteinuria
Calcineurin inhibitors
Renal injury
Local release of inflammatory cytokines and profibrotic cytokines in kidney
Progression of CKD

Figure 96.4 Proposed conceptual representation of pathogenesis of graft-versus-host disease (GVHD)-related chronic kidney disease (CKD) in recipients of hematopoietic cell transplantation (HCT). aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.

in the presence of a chronic inflammatory state. In an autopsy study of renal pathology in six autologous and 20 allogeneic HCT patients, renal tubulitis identical with that seen in renal allograft rejection was present in 67% of patients, whereas a later study found that only 12% of patients had tubulitis associated with more severe forms of GVHD [100,101]. In a case report of minimal-change nephrotic syndrome after HCT, large numbers of $CD8^+$ donor T cells were found infiltrating the interstitium and periglomerular areas of the kidney [102]. In a mouse model of GVHD in which the kidneys were examined, venulitis, endothelialitis, and tubulitis were present as early as 2 weeks post-transplant. This lymphocytic inflammatory process then progressed, and by 6 months more severe endothelialitis and venulitis were present along with an increased number of peritubular infiltrates [103]. The authors suggested that these findings represent a continuous process of tissue injury that is different from the early injury that is seen in other target organs of GVHD [103]. This observation may explain why CKD often occurs beyond the first 3 months after transplant.

The inflammatory and cytokine cascade that accompanies GVHD may also affect the kidney without invoking the T-cell-mediated injury that is present in GVHD involving the skin, gut, and liver [104,105]. In support of this hypothesis, several studies have demonstrated that elevations in plasma cytokine levels correlated with the development of post-transplant complications and organ dysfunction in the HCT population [106,107]. In animal models of GVHD, tissue destruction of acute GVHD does not require alloantigen expression on the target epithelium for cellular cytotoxicity but can be mediated by inflammatory cytokines [108]. In a case report of minimal-change nephrotic syndrome post-HCT, increased production by donor T cells of TNF-α and interferon-gamma was related to the development of nephrotic syndrome; the lack of cellular infiltrates on biopsy suggested that the glomerular injury was secondary to cytokine production stimulated by alloantigens at extrarenal sites [107].

In addition to the T-cell activation and cytokine release in acute GVHD, chronic GVHD also involves the activation of B cells and the production of cytokines, including transforming growth factor-beta (TGF-β_1) [109]. TGF-β_1 is important for collagen synthesis and matrix deposition in the kidney and other organs [110,111] and may also be important in the development of CSP-associated nephropathy in non-renal transplant patients [112]. The effects of CSP on the kidney can be potentiated by increases in the production of TGF-β [113]. All CKD is not secondary to TBI or CSP use but rather a combination of factors including GVHD and the chronic inflammatory state that accompanies it.

Albuminuria and CKD

Albuminuria, defined as a urine albumin-to-urine creatinine ratio (ACR) of 30–299 mg/g creatinine, is thought to be a marker of endothelial dysfunction and inflammation, reflecting a systemic endothelial injury that affects multiple organs including the kidney. Newer work postulates that albuminuria results from tubular dysfunction in the trafficking and degradation of albumin [114,115]. In a prospective study of 142 patients with normal baseline serum creatinine undergoing their first HCT, ACRs were measured as an early marker of renal injury. Urine samples were collected weekly from baseline to day 100 after transplant. Total (intact) monomeric albumin was determined in untreated urine samples by liquid chromatography. Albuminuria was defined as an ACR (mg/g creatinine) over 30 and overt proteinuria as an ACR of 300 or more.

The prevalence of albuminuria at baseline, day 100, and 1 year was 37, 64, and 50%, respectively. Overt proteinuria occurred in 4% of patients at baseline, 15% at day 100, and 4% at 1 year. Characteristics associated with albuminuria include age, gender, donor type, hypertension, and acute liver injury with portal hypertension, but not exposure to TBI. Early albuminuria increased the risk of acute GVHD and sepsis, but not AKI. Albuminuria at day 100 was associated with CKD at 1 year (odds ratio = 4.0, 95% CI 1.1–14.6). Non-relapse mortality risk was elevated (HR = 6.8, 95% CI 1.5–50.6) among patients with overt proteinuria at day 100, and overall survival was decreased [116]. Other studies have suggested that proteinuria may be an early marker of TMA [117]. In studies with longer follow-up, the prevalence of proteinuria was 22.8% in patients at a median of 6 years post-HCT [4].

Given the increased risk of mortality in patients with proteinuria at day 100 post-HCT, a renal biopsy may benefit these patients as we can better understand the pathophysiology of proteinuria and the role of GVHD and chronic inflammation in the development and progression of CKD in these patients. Perhaps proteinuria is a marker of GVHD and/or its effects in the kidney and in those patients with evidence of inflammation on biopsy, continuation of immunosuppression at day 100 may be warranted even in the absence of GVHD elsewhere in the body. There is evidence in patients with diabetes and albuminuria that treatment with an angiotensin-converting enzyme inhibitor (ACEI) or an angiotensin receptor blocker (ARB) slows the progression of CKD [118,119]. Extrapolating from the studies in the diabetic population, we speculate that ACEIs and ARBs would be useful in patients with albuminuria after HCT.

Hypertension and CKD

In pediatric and adult HCT recipients, Majhail et al. reported a hypertension prevalence of 70% during the first 2 years with a median time to onset of 1 month post-transplant [120]. Almost 90% of these patients were receiving drug therapy. Cyclosporine use was an independent risk factor for hypertension. Other studies have reported a hypertension prevalence of 15–72%, depending on the length of follow-up [8,35,121–123]. In a retrospective analysis of children and adults followed for a median of 16 years, the prevalence of hypertension was 17% [124]. Antihypertensive therapy was prescribed for 65% of patients with hypertension. Independent predictors of hypertension include AKI, TBI, autologous transplant (mostly neuroblastoma), obesity, and diabetes. Hypertension has been associated with a fourfold increased risk of CKD [4].and with the development of TA-TMA [8,61,117].

Effective treatment of hypertension is important for decreasing cardiovascular risk and slow the progression of CKD [8,125]. Blood pressure screening should be performed at all follow-up visits, and at least once per year [120,123,126]. ACEI or ARB therapy should be considered first-line chronic therapy, given their antiproteinuria and renoprotective properties [127,128]. However, caution must be exercised in those with hyperkalemia, AKI, and females of reproductive age. Additional therapeutic options should include sodium restriction and lifestyle medication [124].

Treatment of TMA and GVHD-related CKD

Although the described pathology of the kidney in selected patients with CKD after HCT has demonstrated microangiopathic changes or changes associated with nephrotic syndrome, the pathophysiology of idiopathic chronic renal injury after HCT remains obscure. Histopathology is often not obtainable because patients with CKD after HCT are often not considered candidates for biopsy because of the perceived risks of the procedure in this patient population.

Therapeutic options for the treatment of TMA after HCT are limited. Case series of plasma exchange have been described, with mixed results [53,129,130]. One study suggests that plasma exchange may be beneficial in patients with TMA in the absence of active acute GVHD only [131]. Potential offending agents such as CSP and tacrolimus have been discontinued with some improvement clinically, but again the response depends on the severity of disease. Also, in some cases, where acute GVHD is contributing to disease, resolution of TMA has not occurred until GVHD was treated [53]. Given the finding of GVHD as a risk factor for the development of TMA, some authors have hypothesized that an immune etiology may be a contributing factor and attempted to treat TMA with rituximab. In a case report, four of five patients who developed TMA after HCT were successfully treated with rituximab [132]. In a report of 12 patients with TTP early after transplant, 50% of them responded to treatment with defibrotide [133]. In a series of 13 patients with TMA, substitution of daclizumab, an anti-CD25 antibody, for the CNI and sirolimus for treatment of GVHD resulted in complete remission in five and partial remission in two patients; however, only four of the 13 patients survived [134].

Based on treatments used in animal models, primarily of radiation-induced HUS, there are other potential interventions for patients with HUS after HCT. ACEIs have been used in rodent models of HCT-related renal injury, with intriguing results. The use of captopril or enalapril at the time of TBI in these animals resulted in less azotemia, lower blood pressures, and long-term preservation of renal function [56]. In addition, when it did occur, the onset of renal dysfunction was delayed and there was a decrease in proteinuria. However, these mouse models were of radiation-induced injury, and since it is likely that TBI does not play a role in GVHD-related CKD, it may be that the potential beneficial effects of ACEIs and ARBs in these patients are their potential to reduce blood pressure and also inflammation and inflammatory markers in patients. A randomized controlled trial of captopril in 50 patients demonstrated a trend towards a decrease in the development of CKD at 1 year post-HCT and an improvement in patient survival. Given the small numbers of patients, the differences did not achieve statistical significance [135]. There have been case series of patients undergoing renal transplantation successfully after HCT, and it is a viable option for patients with end-stage renal disease after HCT [136–138]. In patients who received their kidney from the same donor as their hematopoietic cells, little or no immunosuppression is required.

Bladder complications after HCT

Hemorrhagic cystitis

Epidemiology

Hemorrhagic cystitis (HC) is a common complication after HCT and a significant cause of morbidity. Clinical syndromes vary from microscopic hematuria to macroscopic hematuria, dysuria, and flank pain with or without renal insufficiency. The grading system for HC based on the National Cancer Institute Common Toxicity Criteria defines grade 1 as the presence of microscopic hematuria, grade 2 as the presence of gross hematuria without clots managed with hydration and diuresis, grade 3 as persistent gross hematuria or clots that require cauterization or transfusions, and grade 4 as gross hematuria that requires surgical intervention. In some studies, HC has been divided into early onset (within 48 hours of the conditioning regimen) and late onset (occurring any time after 48 hours). Early onset is often attributed to the toxic effects of conditioning regimen, including the use of cyclophosphamide, whereas late onset is thought to be related primarily to viral infections [139,140].

Early-onset HC

Early-onset HC occurs during conditioning therapy with cyclophosphamide with or without TBI. Acrolein, a breakdown product of cyclophosphamide, is directly toxic to the bladder mucosa and is one of the primary causes of early-onset HC. The risk of HC from cyclophosphamide is dose dependent [141]. In a retrospective analysis of 81 patients who developed HC at one institution, Tsuboi *et al.* found that sodium 2-mercaptoethanesulfonate (mesna), often used as prophylaxis against HC, increased the risk of developing early-onset HC fivefold, and bladder irrigation increased the risk ninefold; additionally, the use of busulfan in the conditioning regimen was associated with an increased risk of early-onset HC [140]. The authors hypothesized that the combination of mesna and busulfan had a synergistic effect; however, mesna alone also led to increased risk of early-onset HC. The authors suggested that mesna itself may be toxic to the bladder mucosa. These findings contradict others in the literature (described in the section on treatment) regarding the use of mesna.

Late-onset HC

Both BK polyoma virus and adenovirus are known to cause HC after HCT; however, it is not clear whether viremia and viruria leads to renal insufficiency or HC in all patients [142,143]. Pre-transplant anti-BK virus immunoglobulin G titers of 1:20 or more increased the risk of BK viruria in patients, but donor BK viral serology was not associated with a risk of reactivation of BK infection in patients who were seronegative at the time of transplant [144]. In addition, peak BK viral load (≥3 log increase from baseline) in the urine has been shown to increase the risk of HC in patients after HCT [144]. In a study of 130 HCT patients, 51% had antibodies to adenovirus prior to transplant. In this study, risk factors for clinical adenovirus infection were positive antibody status of the donor and grade IV acute GVHD [145]. In a report of 21 patients, adenovirus was implicated in the development of acute renal failure (ARF) after HCT in 90% of the patients [146]. Additional case reports of ARF associated with adenovirus have been described [147]. Other risk factors that have been identified for late-onset HC include older age, pre-transplant conditioning with busulfan, allogeneic HCT, and acute GVHD grade II–IV [140,144,148,149]. Risk factors in children include male gender and unrelated donor graft recipients [150].

Treatment of HC post-HCT

First-line management of HC often involves supportive care, increased hydration and diuresis, and occasionally continuous bladder irrigation. In a randomized prospective study by Bedi *et al.*, no difference was found between the use of mesna versus forced diuresis during the conditioning regimen to prevent the development of early-onset HC secondary to the uroepithelial toxicity of conditioning [151]. One hundred patients were randomized to receive mesna or hyperhydration to prevent HC during conditioning therapy. Patients in the mesna arm received 1.5 mL/m^2/day of fluids compared with the 3 mL/m^2/day of fluids given to the patients in the hyperhydration arm. Again, no difference was found in the incidence of early-onset HC [152]. Ballen *et al.* found that hyperhydration with 5% dextrose normal saline with potassium at 250 mL/hour was more cost-effective and a well-tolerated alternative to the

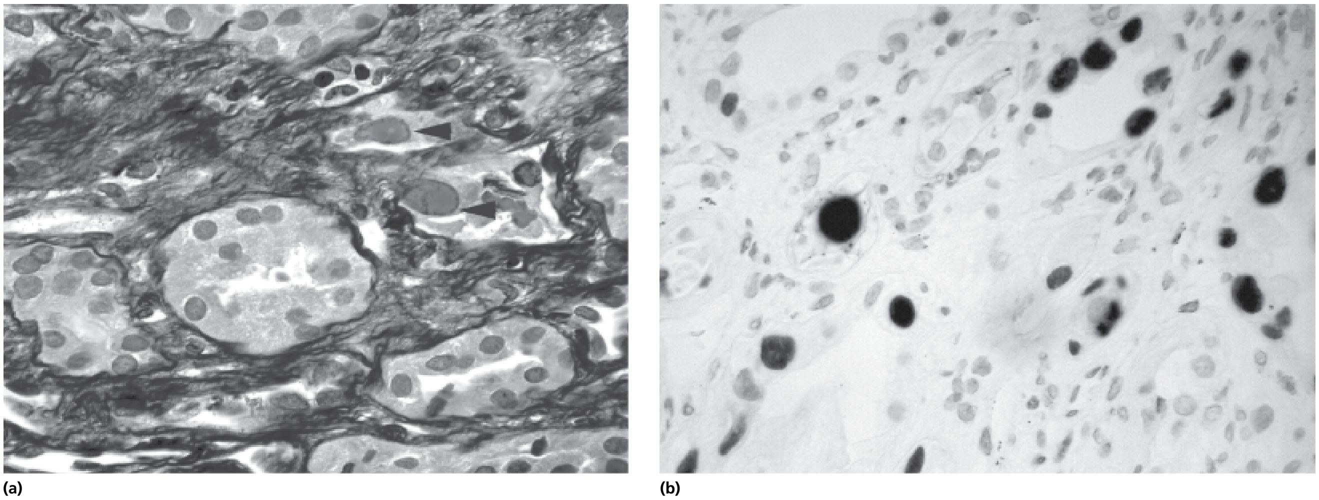

Figure 96.5 BK nephropathy. (a) The nuclei of tubular epithelial cells infected with BK virus have basophilic inclusions and peripheral chromatin rimming (arrowheads). (Methenamine–silver–hematoxylin; magnification: ×400). (b) Infected nuclei stain positively with antibodies directed against the large T antigen of SV40. (Diaminobenzidine; magnification: ×400).

use of mesna to prevent early-onset HC attributed to cyclophosphamide used for conditioning. The nursing time involved to monitor these patients carefully and avoid volume overload was not factored into the cost [153].

A third randomized study evaluated hyperhydration with continuous bladder irrigation or continuous mesna infusion. The incidence of early-onset HC was similar in the two groups but, as expected, the continuous bladder irrigation group had more hematuria. There was a significantly higher number of urinary tract infections in the bladder irrigation group, and these patients reported more discomfort, restriction of movement, and bladder spasms than the mesna group [154]. Based on these studies, it is clear that hyperhydration and maintenance of adequate diuresis are important. Most centers use mesna when cyclophosphamide-based conditioning is used, but it may not offer additional benefit if hydration and diuresis are adequate. Continuous bladder irrigation should not be used to prevent HC unless patients have markedly decreased or no urine output. It may have a role in dissolution of clots in more severe forms of HC.

There are numerous case reports of improvements in symptoms and stabilization of renal function in patients with HC secondary to BK or adenovirus HC treated with cidofovir [155–160]. Ciprofloxacin has also been shown to reduce BK viral replication, decrease the risk of BK reactivation, and quantitatively decrease BK viruria [161], and the authors suggested that it may be beneficial to prevent BK-associated HC [162]. Leflunomide has been used to treat both BK nephropathy in renal transplant patients and CMV infections in HCT patients with promising results and may have a role in the treatment of BK nephropathy in the HCT population (reviewed in [163]). There are isolated case reports of the successful use of intravesicular sodium hyaluronate [164], prostaglandin E2 [165], and cidofovir [157] to treat virally induced HC in patients after HCT. Previous intravesicular therapies have been tried (including formalin, alum, and silver nitrate), with varying results and many complications secondary to therapy (reviewed in [166]). Intravenous ribavarin for adenovirus-associated HC and factor VIIa have also been used successfully to treat HC in patients after HCT [167,168]. Other therapies that have been reported to manage the bleeding associated with severe HC include selective embolization of vesical arteries [169] and cystectomy in a patient who developed renal failure and pulmonary edema secondary to ongoing HC after HCT [170]. There is one case report of the use of hyperbaric oxygen therapy to treat intractable HC successfully [171]. In cases of HC where obstruction of urine flow occurs, percutaneous nephrostomy tubes or ureteral stent placements may be necessary to relieve the obstruction and allow drainage of the urine.

There have been case reports of patients with biopsy-proven BK nephropathy (Figure 96.5) after transplant resulting in CKD and in some cases end-stage renal disease requiring dialysis [172]. Thus, in patients with BK viruria after transplant, it is important to measure serum BK levels by the polymerase chain reaction as these have been associated with the development of CKD and renal failure [172]. Serum BK levels are more reflective of renal involvement and, in renal transplant patients, are associated with BK nephropathy in the graft. After HCT, a blood BK level of >10,000 copies/mL has been associated with the development of HC [173]. Similarly, Haines et al. reported that children with a peak plasma BK viral load of >10,000 copies/mL during the first year after HCT, regardless of urine viral load, had worse renal and urological disease compared with those with viremia of <10,000 copies/mL [174]. BK viremia was an independent predictor of the development of renal injury in patients post-HCT [175]. In cases of BK viremia or nephropathy, first-line therapy is often reduction of immunosuppression when possible prior to institution of cidofovir and other antiviral agents. A renal biopsy should be performed in patients with BK viremia and elevated serum creatinine levels prior to initiation of cidofovir to confirm the diagnosis of BK nephropathy. In the absence of biopsy-proven BK nephropathy, cidofovir may not be indicated.

Complications and imaging findings with HC

Various complications have been described in patients who develop HC after HCT; these include hydronephrosis, obstructive uropathy [141,150], ureteral obstruction secondary to clot formation and/or ureteral stenosis [176], necrotizing tubulointerstitial nephritis [147], and ARF [126,132,133,136,141,146,147,150]. Bladder perforation has also been described [150], as has intractable hemorrhage requiring cystectomy.

Imaging findings of HC will vary based on severity of disease and imaging modality. On renal and bladder ultrasonography, focal or diffuse bladder wall thickening can be seen [177]. Clots may be visible within the bladder, and if the clot is causing an obstruction, unilateral or bilateral hydronephrosis may also be visualized. Sloughed debris secondary to obstruction, clot, inflammation or infection can also cause obstruction and is visualized on ultrasound. In the presence of hydronephrosis, additional imaging studies such as a voiding cystourethrogram and a lasix renogram may be necessary to differentiate obstruction versus reflux. Additionally, the upper tracts may be involved, and thickening of the suburothelial lining of the renal pelvis has been described. Perinephric fluid collections secondary to hemorrhage or rupture of a fornix can also be found. With the addition of Doppler studies to the ultrasound, decreased flow to areas of the bladder and also hypervascularity and areas of active bleeding can be identified. Direct visualization with cystoscopy and cauterization of areas of active bleeding may be necessary. Computed tomography and magnetic resonance imaging can enhance the images and may offer more definition of the bladder wall than ultrasound. However, first-line imaging in cases of HC should be carried out with ultrasound and Doppler studies.

Conclusion

There are clearly distinct clinical entities involving the kidney that occur after HCT: ARF, TMA, nephrotic syndrome, and GVHD-related CKD. HC is the most common urologic complication reported in patients after HCT.

There are patients who will develop CKD that is not related to TBI or the conditioning regimen, but rather to complications and/or therapy that occur after HCT, specifically acute and chronic GVHD and prolonged CNI use. The burden of management will fall not only on the nephrologists, but also on the transplant physician to ensure close monitoring of renal function, blood pressure, and urinalyses post-transplant. It may be that our energies have been misdirected in trying to reduce exposure to TBI and that we should rather try to decrease the inflammatory and cytokine effects of GVHD and reduce exposure to CNIs to prevent CKD in this population of patients.

Although physicians are well attuned to HC after HCT, increasing awareness regarding the potential for both BK and adenovirus to lead to nephropathy, both acute and chronic, is needed. Careful monitoring of patients after the acute hemorrhage is controlled may be needed to prevent ongoing injury. Imaging with renal ultrasound is an important aspect of managing the acute complications such as obstruction but may also be helpful in monitoring ongoing injury and late complications.

Renal injury after HCT is a relatively common occurrence. As the indications for and number of transplants performed worldwide increase, so will the burden of kidney disease. Identifying those patients at risk for the development of both AKI and CKD will be important for potential intervention and prevention of kidney injury and progression to end-stage renal disease in this patient population.

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CHAPTER 97

Endocrine Complications Following Hematopoietic Cell Transplantation

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Introduction

The advent of hematopoietic cell transplantation (HCT) has resulted in a significant extension of life for many children and adults affected with hematologic, immunologic, genetic, and metabolic diseases; however, the conditioning regimens for HCT, and also the process of HCT itself, may lead to some new complications for some patients. As continued improvements in post-HCT survival rates now often span decades, the manifestation of long-term endocrine complications in terms of growth, skeletal development, metabolic function, and gonadal and sexual function has become increasingly recognized, and the goal of maintaining an extended, superior quality of life post-HCT has likewise attained even greater importance. This chapter reviews the endocrine abnormalities that may result from HCT, and outlines suggested approaches to the investigation and management of these disorders.

Hypothalamic–pituitary dysfunction

Radiation exposure can result in hypothalamic–pituitary dysfunction in the following three clinical situations: (1) radiotherapy of brain or extracranial tumors (doses ≥30 Gy), (2) cranial radiotherapy for acute lymphoblastic leukemia (ALL) (doses 18–24 Gy), and (3) total body irradiation (TBI) prior to HCT for hematologic malignancy or solid tumors (doses 10–14 Gy). Since the functions of the hypothalamus and the pituitary are intimately related, it is not always easy to discriminate between damage at these two levels. In general, however, the hypothalamus is more radiosensitive than the pituitary, and more often the site of damage [1]. Hyperprolactinemia, for example, often occurs following external radiotherapy to the hypothalamic–pituitary area, suggesting a reduction in dopamine secretion by the hypothalamus. The degree of pituitary dysfunction is typically related to the dose of irradiation received; hypopituitarism has been observed with cumulative total doses of ≥20 Gy to the brain [2]. The threshold radiation dose may be lower in patients treated simultaneously with high doses of methotrexate or intrathecal chemotherapy; however, Kauppila et al. [2] noted that hypopituitarism was not seen after TBI doses of 12 Gy. In fact, it is generally thought that, in most cases, the hypothalamic–pituitary axis remains intact after HCT, and that hormonal disturbances usually occur as a result of injury at the target endocrine organ [2]. Nevertheless, it is possible that lower doses of cranial irradiation prior to HCT in patients with hematologic malignancies may cause hypopituitarism and/or long-term hypothalamic–pituitary hormone insufficiency. In contrast, end-organ hormone disturbances are common post-HCT, even with chemotherapy-only regimens [2]. Further discussions of the pathophysiology of specific hormone disorders, and methods for their investigation and treatment, are presented in the respective sections below.

Growth disorders

Pituitary growth hormone (GH) is secreted in a pulsatile manner that is superimposed on a schedule of diurnal variation. GH secretion is stimulated by growth hormone releasing-hormone (GHRH) and suppressed by hypothalamic somatostatin. GH stimulates the production of insulin-like growth factor (IGF)-1 proteins by the liver, which, in turn, exerts negative feedback control on the secretion of hypothalamic GHRH and pituitary GH itself (Figure 97.1). The immediate neonatal period generally represents the highest level of GH secretion. GH levels then decrease throughout childhood, but rise during puberty as a result of increased pulse amplitude (but not frequency). GH secretion falls once again with increasing age.

The anabolic effects of GH on the linear growth of bones are mainly mediated by IGFs and their binding proteins (IGFBPs), which extend the serum half-life of the IGFs by aiding in their transport to target cells and modulating their interactions with membrane surface receptors. Given that IGF-1 concentrations are more dependent on GH than IGF-2, the measurement of IGF-1 levels is more likely to provide useful clinical correlations in the evaluation of GH deficiency.

GH also has direct metabolic effects, such as lipolysis, increased amino acid transport into tissues, and increased liver protein synthesis. Further, GH is a diabetogenic hormone, and its excesses can produce insulin resistance via altered post-receptor insulin action, resulting in decreased carbohydrate utilization and impaired glucose uptake into cells. GH additionally increases hepatic glucose output, which further worsens hyperglycemia.

GH deficiency in the adult may be associated with reduced lean body mass and musculature, increased body fat, reduced bone mineral density (BMD), reduced exercise performance, and increased plasma cholesterol, comprising an overall increased mortality risk from cardiovascular disease. Further, adults with GH deficiency may suffer from depression, anxiety, reduced energy, and social isolation. Despite these observations, there are

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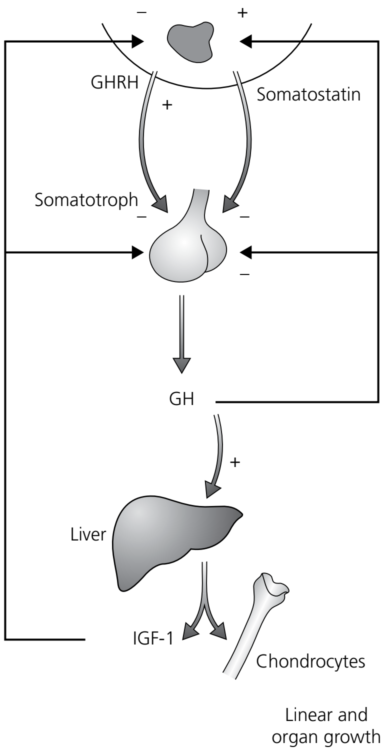

Figure 97.1 Neuroendocrine control of growth hormone and IGF-1 secretion. GH, growth hormone; GHRH, growth hormone-releasing hormone; IGF-1, insulin-like growth factor-1. Source: adapted from Melmed et al. 2008 [3].

Table 97.1 Risk factors for growth dysfunction following HCT

TBI, particularly single-dose irradiation
Cranial irradiation
High-dose chemotherapy
Younger age at transplantation

no clinical syndromes of adult GH deficiency that have been widely recognized.

It is believed that the type of conditioning regimen prior to HCT has a significant influence in the development of potential growth dysfunction (Table 97.1). A large body of data suggests that radiation (particularly cranial and TBI) is mostly responsible for diminished growth in children who have undergone HCT. Potential pathogenic mechanisms include the direct impact of combination chemotherapy and radiation on the skeleton (i.e. skeletal dysplasia, particularly of the spine), radiation-induced hypothalamic–pituitary dysfunction, and hypothyroidism. Radiation may also prompt the development of precocious puberty, which can result in premature epiphyseal fusion and subsequent reduced final height. Children who have received preparation regimens consisting of cranial irradiation followed by high-dose chemotherapy and single-dose TBI are most likely to demonstrate growth defects 2–5 years after HCT, with pubertal growth most affected. With no previous cranial irradiation and fractionated TBI, however, growth was not compromised 3 years after HCT [1].

Because GH deficiency is strongly related to irradiation, growth rates may be adversely affected by HCT; in fact, GH deficiency is found in 32% of children after HCT with TBI [4]. Inadvertent exposure of the liver and other tissues during TBI via secondary radiation may additionally contribute to the defective production of essential growth factors such as IGF-1 [2]. Nevertheless, reports of final short stature after HCT range from 4% [5] to 60% [6], and other sources report final stature that generally falls within the normal adult range [1]. This variability in final height outcome may be attributable to the different patient ages at HCT, as the risk of impaired growth is highest in the youngest children transplanted prior to the onset of puberty; conversely, some reports have noted improved growth after HCT with younger patient age [7].

In the absence of cranial irradiation, there is actually a poor correlation between GH production and concentrations of IGF-1 or IGFBP-3 and growth in children after HCT, suggesting the influence of other factors on growth dysfunction in addition to primary GH deficiency. Endogenous or exogenous glucocorticoid excess, for example, may blunt GH secretion. Chemotherapy alone, particularly BU–CY (busulfan–cyclophosphamide), may cause growth deficiencies due to toxicities at the epiphyseal growth plate, even after corticosteroid administration has ceased. Additional considerations include nutritional or vitamin deficiencies, genetics, the nature of the underlying disease (e.g. red cell aplasia, Fanconi anemia, thalassemia), and the presence of general illness or graft-versus-host disease (GVHD). The manifestation of other endocrine hormonal dysfunction may further contribute to impaired growth dynamics. As another example, bone age advancement can be severely delayed in hypothyroidism – usually more so than with GH deficiency – and epiphyseal dysgenesis may be seen when calcification of the epiphyses progresses. The administration of gonadal sex steroids for the treatment of hypogonadism may cause premature growth cessation by accelerating the fusion of long bones and the closure of the growth plates, but the absence of these factors during the prepubertal period appears to have no major significance. As with sex steroids, glucocorticoid deficiency seems to have little effect if the individual is otherwise healthy (i.e. if hypotension and hypoglycemia are absent).

Evaluation of growth disorders post-HCT

Children should be closely monitored and serially measured for appropriate growth (height velocity) after HCT in the context of normal standards, such as the growth charts provided by the National Center for Health Statistics (NCHS), part of the Centers for Disease Control (CDC). Height velocity standards remarkably encompass the range of normal growth patterns between 2 years of age and puberty with great predictability, hence any deviation of the patient's plotted growth from the normal curve should be immediately suspect for an aberrant growth process. Growth potential may be estimated by the measurement of "bone age," or the level of ossification of the tubular bones, via radiographs of the left hand and wrist. Final adult height may be predicted with greater accuracy with increasing bone age.

In the assessment of growth hormone levels, the possibility for hypothalamic or pituitary dysfunction must always be considered, particularly with known radiation exposure and the presence of other endocrine hormone abnormalities [thyroid-stimulating hormone (TSH), adrenocorticotropoid hormone (ACTH), gonadotropin, etc.) and potential worsening of the underlying disease process (e.g. local extension of Hodgkin disease of the nasopharynx to the hypothalamus).

The identification of actual GH deficiency, however, is not often easily established, owing to low levels of basal GH secretion in both normal and truly GH-deficient patients. Further, the sequential

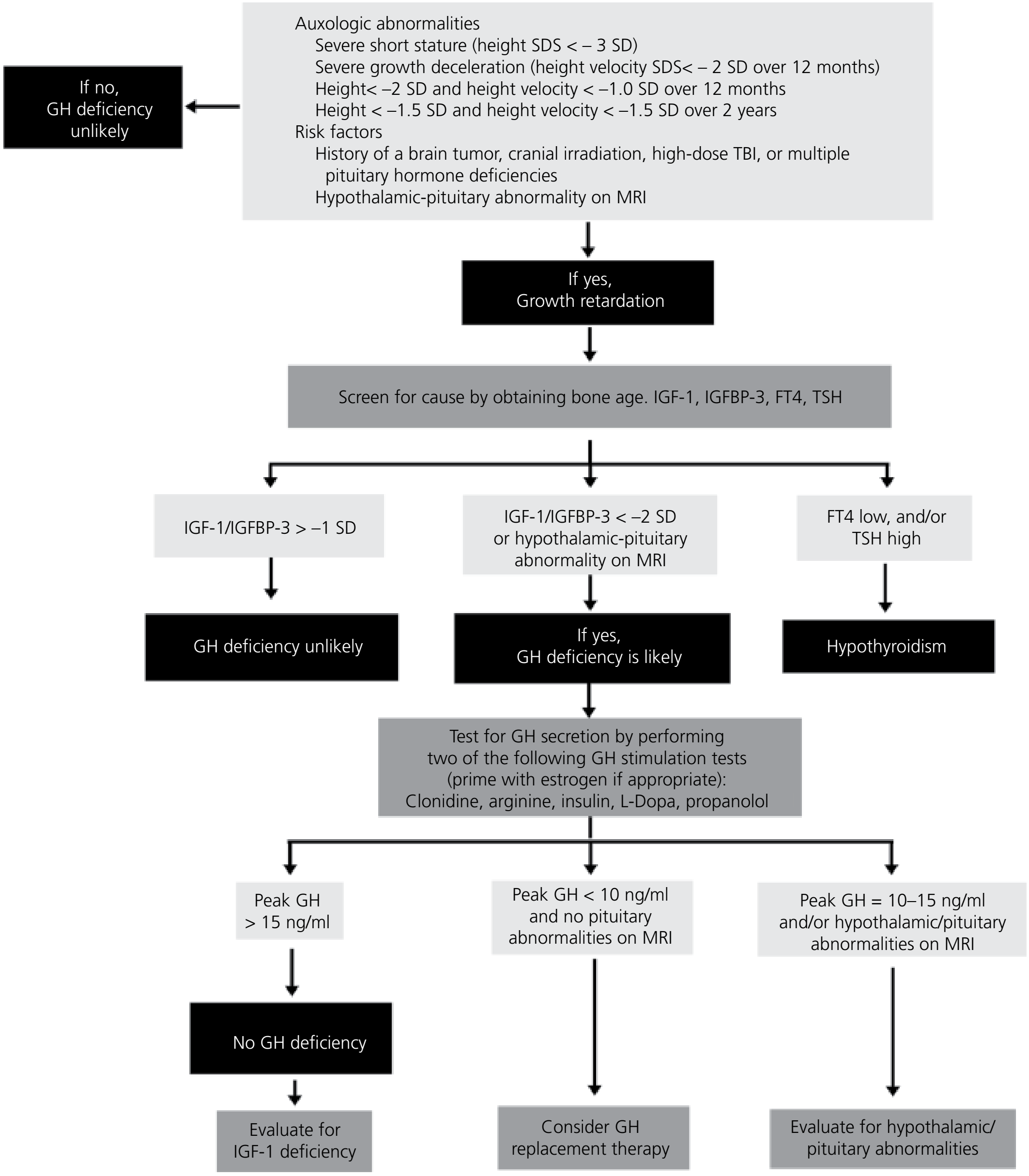
Auxologic abnormalities
Severe short stature (height SDS < – 3 SD)
Severe growth deceleration (height velocity SDS< – 2 SD over 12 months)
Height< –2 SD and height velocity < –1.0 SD over 12 months
Height < –1.5 SD and height velocity < –1.5 SD over 2 years
Risk factors
History of a brain tumor, cranial irradiation, high-dose TBI, or multiple pituitary hormone deficiencies
Hypothalamic-pituitary abnormality on MRI
If no, GH deficiency unlikely
If yes, Growth retardation
Screen for cause by obtaining bone age. IGF-1, IGFBP-3, FT4, TSH
IGF-1/IGFBP-3 > –1 SD
IGF-1/IGFBP-3 < –2 SD or hypothalamic-pituitary abnormality on MRI
FT4 low, and/or TSH high
GH deficiency unlikely
If yes, GH deficiency is likely
Hypothyroidism
Test for GH secretion by performing two of the following GH stimulation tests (prime with estrogen if appropriate): Clonidine, arginine, insulin, L-Dopa, propanolol
Peak GH > 15 ng/ml
Peak GH < 10 ng/ml and no pituitary abnormalities on MRI
Peak GH = 10–15 ng/ml and/or hypothalamic/pituitary abnormalities on MRI
No GH deficiency
Evaluate for IGF-1 deficiency
Consider GH replacement therapy
Evaluate for hypothalamic/ pituitary abnormalities

Figure 97.2 Algorithm for the evaluation of growth dysfunction following HCT. FT4, free thyroxine; GH, growth hormone; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; SDS, standard deviation score; TSH, thyroid-stimulating hormone. Source: adapted from Reiter and Rosenfeld 2008 [8].

measurement of spontaneous GH secretion is cumbersome and expensive. The development and availability of sensitive radioimmunoassays for IGF-related proteins have greatly simplified the diagnostic process (Figure 97.2). Thus, the laboratory workup for GH deficiency should primarily involve the measurement of IGF-1 and IGFBP-3 levels, and also the results of stimulated GH secretion. GH secretion can be stimulated with either physiologic (fasting, sleep, exercise) or pharmacologic stimuli (levodopa, clonidine, glucagon, propranolol, arginine, insulin). GH deficiency may generally be suspected in cases of subnormal growth velocity combined with GH levels <10 ng/mL after each of two pharmacologic stimulations carried out on two separate occasions. Testing should be performed after an overnight fast in order to prevent carbohydrate or fat ingestion from suppressing a GH response. Nevertheless, pulsatile secretion of GH may cause varying results.

Management of growth disorders post-HCT

It has been suggested that GH should be administered to children with decreased clinical growth rates, and/or decreased IGF-1 and IGFBP-3 levels, and/or low GH values on stimulation tests, while giving due consideration to the potential necessity of replacing other deficient hormones necessary to achieve optimal growth. Although no randomized trial has yet been reported that clearly delineates the benefits of GH treatment in children, some available long-term outcome data are promising with regard to positive growth response to treatment. In a French study [9], GH therapy of children who received TBI and/or cranial irradiation for leukemia resulted in a median gain of height that was equal to 0.59 × Z score (standard deviation from their aged-match peers). The benefit was greater in girls than boys.

Supplementation is commonly given with a starting dose of recombinant human growth hormone (rhGH) 0.18–0.35 mg/kg body weight per week, administered in seven daily doses. GH treatment after HCT typically results in an increase in growth velocity, albeit smaller than in children with idiopathic GH deficiency. Age at the initiation of treatment is inversely correlated with expected growth responses, possibly indicating the need for early treatment. First-year height velocity is the most predictive factor with regard to later growth that may occur in years 2–4; GH therapy in children typically is given for 2 years, but can be extended until the closure of long bone epiphyses if there are no contraindications. Suboptimal response to GH therapy may be attributed to poor compliance, improper administration, subclinical (undiagnosed) hypothyroidism (see later discussion), underlying illness, concurrent glucocorticoid therapy, prior irradiation of the spine, epiphyseal fusion, the presence of anti-GH antibodies (which develop in 10–20% of patients receiving rhGH), or an incorrect diagnosis of GH deficiency. It has not yet been possible to determine the link, if any, between GH therapy and the new development of leukemia; if present, the risk of leukemia subsequent to GH therapy appears to be limited to children already at high risk for that disease (e.g. prior malignancies, radiation exposure, the presence of syndromes associated with leukemia), but further studies are needed. In the National Cooperative Growth Study of more than 19,000 children treated with rhGH, no evidence of increased risk of recurrence of leukemia or central nervous system (CNS) tumor was observed [10].

The treatment of prepubescent HCT recipients with GH deficiency in addition to hypogonadism can be challenging; generally, administration of exogenous sex steroids is low in dosage or is postponed altogether until the desired patient height is reached in order to reduce the likelihood of premature closure of the growth plates.

Dysfunctional bone metabolism and osteoporosis

Certain hematologic malignancies (lymphomas) may also be associated with hypercalcemia and high levels of 1,25-dihydroxyvitamin D_3 (leading to increased calcium absorption). Increased bone resorption and impaired renal excretion contribute to hypercalcemia.

The loss of bone mass, or compromised bone strength, has been well documented as a sequel to HCT, preferentially within the first 6 months post-transplant. The mechanism of bone loss is complex and related to the effects of HCT on bone marrow stem cells. Typically, a 5–15% loss of bone mineral density (BMD) can be expected in the lumbar spine and femoral neck within 1 year after HCT [11]. Osteopenia is observed in as many as 50–60% of all HCT recipients, and osteoporosis develops in 20% [12]. Studies in adult survivors of childhood cancer demonstrate that BMD is reduced at multiple skeletal sites, supporting the premise that inadequate bone mineralization during childhood may have long-term adverse effects on BMD in adulthood [13]. The only study to have reported BMD in children after HCT using dual-energy X-ray absorptiometry (DEXA) technology showed that BMD was significantly lower than in adults [14]. Fracture rates in both male and female pediatric HCT recipients have been estimated at 172 per 10,000 person-years by one center (J. E. Sanders, unpublished data, cited in [15]); when compared with the peak childhood fracture rate of 133 per 10,000 person-years in a large, population-based cohort study of boys and girls [16], this suggests that reduced BMD in children as the result of HCT may be clinically significant.

In transplanted adults, several studies have confirmed that bone loss due to osteopenia or osteoporosis may occur even prior to HCT, due in part to the direct effects of underlying hematologic cancers on the integrity of bone, such as malignant changes in bone structure, particularly with acute leukemia or multiple myeloma. In one study, 39% of patients demonstrated evidence of osteopenia or osteoporosis prior to HCT in the lumbar spine, and 25% in the femoral neck; these numbers increased to 50% and 45% by 1 year after transplant, respectively [11].

In general, the pathogenesis of metabolic bone dysfunction is multifactorial and not completely understood, particularly with regard to the effects of high-dose treatment followed by HCT on bone mineral metabolism. The three major pathogenic mechanisms thought to be responsible for reduced BMD are (1) failure to achieve peak bone mass (due to altered calcium or vitamin D homeostasis, or deficiencies in growth or gonadal hormone secretion), (2) bone loss caused by increased bone resorption (disturbance in osteoclast function), and (3) inadequate replacement of lost bone due to decreased bone formation (disturbance in osteoblast function). HCT and post-transplant therapies may specifically affect bone homeostasis by inducing premature menopause and/or hypogonadism, directly poisoning bone cells, and altering calcium and magnesium metabolism, causing elevated parathyroid hormone (PTH). The leading cause of reduced BMD after HCT is prolonged glucocorticoid therapy for GVHD, likely due to the production of cytokines that trigger inappropriate osteoclastic activation, decreased bone formation, and decreased levels of 1,25-dihydroxyvitamin D_3. Glucocorticoids affect trabecular bone more than cortical bone, resulting in fractures of the vertebrae, ribs, and the ends of long bones. Bone loss is most rapid within 6 months of initiating glucocorticoid therapy, and may occur with doses as low as prednisone 5 mg daily. The use of chemotherapy and, to a lesser extent, radiation therapy as part of the pre-transplantation regimen exacerbates bone loss, due to the dose-dependent toxicity of high-dose chemotherapy to bone marrow osteoprogenitors, TBI-related radiation damage, and the suppressive effects of both of these modalities on the gonads. Cyclosporine, for example, potentiates hypomagnesemia and hypocalcemia. Physical inactivity due to long hospitalization or illness and nutritional deficits further compound these effects. Additional general risk factors include older age, thin body habitus, family history of osteoporosis or low-trauma fractures, smoking, and a diet low in calcium or vitamin D.

Interestingly, at the end of the first year post-transplantation, bone mass either begins to recover (spine and total body), or bone loss is attenuated (femoral sites); however, one study has shown that femoral bone loss is not regained. Improvement in bone mass is likely due to multiple factors, including a reduction in immunosuppressant dosage, resolution of immobilization or malnutrition, and convalescence from the transplant procedure itself. Long-term bone loss is mainly determined by the intensity of steroid use and untreated hypogonadism.

Osteonecrosis, or avascular necrosis, is not infrequent, and indeed is a significant sequela of HCT, affecting 10% of allo-bone marrow transplant (BMT) recipients in a single-center study [17]. Avascular necrosis seems to be related to a decreased number of bone marrow CFU-f colonies in vitro and may be facilitated by regeneration of mesenchymal stem cells (MSCs) and low osteoblast numbers. The mean time between HCT and development of osteonecrosis is 15 months.

Osteonecrosis involves bone infarction and the rapid and focal deterioration of bone quality that primarily affects the femoral head, although the ankles and shoulders may also be involved. Osteonecrosis is painful, and may lead to the ultimate collapse of the bone, often necessitating a hip replacement. Osteonecrosis can occur in all age groups, but is usually related to the use of corticosteroids, as glucocorticoids inhibit osteoblast function; relatively low doses, or even brief courses of high-dose therapy, have been implicated. The cumulative incidence of osteonecrosis after HCT is 5–20% at 5–15 years post-BMT, as osteonecrosis often occurs long after transplantation [18]. It is difficult to separate the effects of chronic inflammation on the microvasculature versus glucocorticoids in the development of osteonecrosis. In one large retrospective study among 4388 patients, the reported odds ratio of osteonecrosis was 3.75 for acute GVHD and 3.52 for chronic GVHD. Severity of GVHD was also associated with osteonecrosis.

Other risk factors for osteonecrosis include the presence of sickle cell disease and other hemoglobinopathies, polycythemia vera and other lymphoproliferative disorders, pregnancy, septic emboli, alcoholism, pancreatitis, Gaucher disease, lupus, rheumatoid arthritis, trauma (fracture/dislocation or direct tissue damage), radiation (direct injury to vascular supply leading to occlusion), and Cushing disease. Osteonecrosis of the jaw is also a rare complication of bisphosphonate therapy.

Evaluation of metabolic bone dysfunction post-HCT

Bone loss and fractures may present with pain and loss of function, negatively impacting quality of life. The strongest risk factors for metabolic bone dysfunction post-HCT are female gender and older age. Among the other factors, corticosteroid use is most significant; however, the dose–effect curve for steroid use in the development of osteoporosis is still not completely clear, and prospective data are sparse, hindering the ability to establish general recommendations for the prophylaxis and treatment of bone mineral loss post-HCT. The significance of the changes in BMD after HCT with regard to fracture risk has not been established. Long-term fracture risk is not only determined by BMD; vertebral fracture itself is a potent risk for future fracture, and also a history of falls and attributes of bone geometry. In children, growth charts, radiographic assessment of bone age, serum levels of IGF-1 and IGFBP-3, and GH provocation tests may be used to monitor for the occurrence of growth dysfunction, and possible metabolic bone disease (see the previous section for further discussion). In patients with demonstrated osteoporosis, it is reasonable to check serum calcium, magnesium, and 1,25-dihydroxyvitamin D levels. In adults, dual-energy X-ray absorptiometry (DXA) is currently the best and most well-referenced method to diagnose transplant-related bone loss. DXA may be performed as part of the pre-transplantation evaluation in order to assess the degree of bone loss before transplantation. After transplantation, bone densitometry can be repeated yearly for the first 2 years; if decreased BMD is seen, then densitometry is continued annually; otherwise, densitometry can be repeated every 3–5 years. DXA is also the most common method for measuring BMD in children, now that age-related normative reference data are currently available [19]. Bone turnover can also be measured by markers such as urinary hydroxyproline, urinary *N*-telopeptide cross-linked collagen, and serum osteocalcin. Once osteonecrosis is established, almost any imaging modality will reveal its presence, although magnetic resonance imaging (MRI) has the best sensitivity and specificity.

Management of metabolic bone dysfunction post-HCT

The prevention and treatment of osteoporosis focus on therapeutic modalities that act either to decrease the rate of bone resorption or to increase bone formation. The aggressiveness of disease intervention depends upon the degree of osteoporosis and the severity of bone loss, including T-scores and a history of prior fractures. Management should be tailored to each patient's specific response to therapy, level of tolerance to adverse effects, and the complicating presence of other concurrent illnesses.

For adult patients with osteopenia, calcium, vitamin D supplementation, and weight-bearing exercise are recommended. Patients should be counseled with regard to lifestyle management (exercise, smoking cessation, limiting alcohol intake) and instructed on body mechanics and posture in order to minimize the risk of falls. Treatment of osteopenic adults with chronic GVHD or osteoporotic patients should additionally include oral or intravenous bisphosphonates. Denosumab is a monoclonal antibody acting against receptor-activated nuclear kappa B ligand (RANKL) that has been shown to be highly effective at reducing bone loss in patients with osteoporosis, and decreases the rate of vertebral, non-vertebral, and hip fractures of both sexes. Denosumab is also effective in hypogonadal men and women, including those on glucocorticoid therapy, making it an appropriate choice for menopausal female patients [20].

In a large prospective study, pamidronate 90 mg per month for a period of 12 months was started prior to conditioning, and was found to be superior to placebo in reducing bone loss compared with calcitriol and calcium; however, femoral neck bone density after 1 year of therapy was still 2.8% below the baseline BMD [21].

Patients with Hodgkin disease and a history of mantle radiation therapy, however, already have a high risk (35%) for the development of breast cancer [22]; in these cases, selective estrogen response modulators (SERMs) such as raloxifene may be utilized, although venous thrombosis is a risk. Osteonecrosis of the jaw (ONJ) is a serious but rare complication of amino bisphosphonate therapy in adults (and also denosumab); however, the incidence of ONJ may approach 1% when bisphosphonates are administered intravenously and monthly to adults for the management of bone malignancy, and may occur more frequently in this setting after recent dentoalveolar trauma [23]. Oral bisphosphonates have been implicated less frequently as a cause of ONJ among patients without malignant bone disease, but have the potentially severe side effect of esophagitis. Intranasal or injectable calcitonin may be employed to alleviate pain related to bone fractures, but it has not proved useful in preventing or treating osteoporosis itself. Intermittent administration of injectable, low-dose, synthetic (recombinant human) PTH or a peptide analog as anabolic therapy has been shown to produce substantial increases in trabecular bone mass with little loss of (and even gains in) cortical femoral bone, and has reduced the risk of fractures. PTH may be reserved for patients in whom antiresorptive therapy is insufficient at preventing fractures, and may be particularly useful in steroid-induced osteoporosis. Patients must be monitored for the occurrence of hypercalcemia and hypercalciuria, however. Recombinant PTH cannot be used in patients who have received radiation therapy to the skeleton owing to the potential risk of osteosarcoma.

BMD should be evaluated in children at 3 months after allogeneic HCT, with an initial follow-up DEXA scan at 1 year [15]. Supplementation with calcium, vitamin D, and weight-bearing exercise is recommended, although these measures have not proved effective in preventing osteopenia, osteoporosis, or fractures when administered individually. As noted above, sex hormone replacement therapy has increased bone mass in both adult women and men, and is recommended for hypogonadal adolescents if age-appropriate [24,25]. Bisphosphonate therapy may also be pursued in pediatric patients, as some evidence seems to suggest that it may improve mean annualized BMD in children and adolescents after HCT by 33%, which is threefold higher than in children receiving calcium and vitamin D alone [15]; however, the effects of various pretreatment regimens were not sufficiently randomized in this study, and the group receiving bisphosphonates also had a higher proportion of patients receiving GH therapy and sex hormone replacement. The long-term effects of bisphosphonate and denosumab treatment in children remain to be established. Denosumab has been used in children for the treatment of osteogenesis imperfecta with acceptable safety [26].

Vitamin D deficiency

It is estimated that 1 billion people around the world are deficient in vitamin D [27]. Vitamin D deficiency has been linked to various inflammatory diseases; however, the mechanism by which vitamin D reduces inflammation is unclear.

Vitamin D deficiency and GVHD

Vitamin D deficiency has been demonstrated in a large number of subjects with autoimmune disease, and in GVHD. It remains unclear, however, whether vitamin D deficiency is the cause or the consequence of disease. In patients undergoing allogeneic stem-cell transplantation (allo-SCT), it was reported that 70% of patients were vitamin D deficient on day 0 and 87% were deficient on day 100 [28]. The high incidence of vitamin D deficiency in allo-SCT patients emphasizes the need for further study of the impact of vitamin D deficiency on clinical outcomes.

Vitamin D receptor (VDR) is upregulated during alloreactivity [29], suggesting that vitamin D consumption and possibly deficiency may play a role in the development of GVHD. It has been hypothesized that vitamin D may reduce GVHD in the allo-SCT setting through its tolerogenic effect on maturation towards Treg cell production [30], and also a direct inhibitory effect on T cells [31].

The idea of vitamin D-targeted therapy was used by a group of investigator in Spain to modify the course of GVHD [32]. In their retrospective study, the outcome of chronic graft-versus-host disease (cGVHD) in a series of 12 patients receiving vitamin D because of osteopenia or osteoporosis (confirmed by bone densitometry) after allo-HCT was assessed. These patients also had active cGVHD at the time when vitamin D treatment was initiated. Interestingly, there was a marked improvement in cGVHD for most of these patients, without appreciable secondary effects observed. This study was observational and retrospective, however, and therefore had significant limitations related to the methodology used.

Factors that contribute to low vitamin D_3 levels among patients undergoing bone marrow transplantation are lack of exposure to sun, anorexia and poor intake, and malabsorption and diarrhea due to chemotherapy or development of GVHD. Another possible explanation is the rapid metabolization of vitamin D.

The dose necessary to treat hypovitaminosis D during bone marrow transplantation needs further study. Observational experience suggests at least 2000 units PO of cholecalciferol daily among those in whom the level of 25-hydroxyvitamin D is between 20 and 30 ng/mL, provided that the level of PTH is elevated. In the absence of elevated PTH, the therapy may not be necessary. If the level of vitamin D_3 is between 10 and 20 ng/mL, the recommended empirical therapy consists of 5000 units of cholecalciferol daily; if the level is less than 10 ng/mL, a dosage of 10,000 units daily is needed. Vitamin D should be taken with meal for better absorption. Since the half-life of cholecalciferol is long, its level needs to be monitored regularly.

Thyroid dysfunction

Overall, thyroid dysfunction is a common disorder. Similarly, thyroid dysfunction of any type is the most common endocrine complication after allogeneic and autologous bone marrow transplantation (HCT), occurring in up to about half of patients. There is existing controversy over which aspects of HCT are most responsible for thyroid dysfunction: the process of HCT itself, the conditioning regimens used prior to HCT, or patient-related parameters (Table 97.2, Figure 97.3).

Radiation therapy involving the head and neck can lead to the occurrence of thyroid dysfunction, via direct damaging effects to the

Table 97.2 Risk factors for thyroid dysfunction following HCT

TBI
Single-dose irradiation
Pre-transplant cranial or neck irradiation
Occurrence of significant euthyroid sick syndrome (ETS)
Pediatric patient age at transplantation, particularly <10 years old
Presence of or predisposing factors for thyroid autoantibodies in donor and/or recipients of HCT

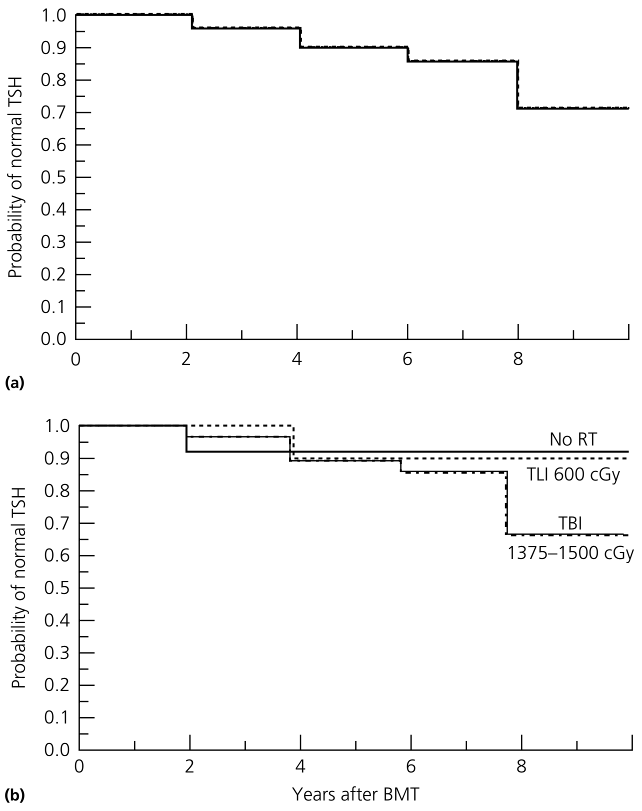

Figure 97.3 Actuarial risk of thyroid dysfunction in 150 patients after bone marrow transplantation and the effect of TBI. (a) Actuarial risk for the overall cohort. (B) Actuarial risk according to type and dose of radiation administered. RT, radiotherapy; TLI, total lymphoid radiation; TBI, total body irradiation; TSH, thyroid-stimulating hormone; BMT, bone marrow transplantation. Tick marks indicate follow-up. Source: Boulad et al.1995 [33]. Reproduced with permission of Nature Publishing Group.

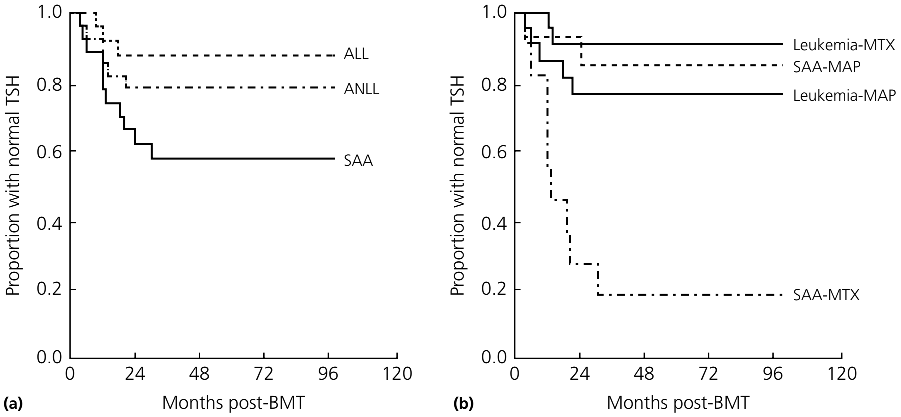

Figure 97.4 Life table estimates of the proportion of patients with normal TSH levels following BMT, (a) according to disease and (b) according to disease and GVHD prophylaxis. ALL, acute lymphoblastic leukemia; ANLL, acute non-lymphoblastic leukemia; BMT, bone marrow transplantation; GVHD, graft-versus-host disease; MAP, methotrexate, antithymocyte globulin, prednisone; MTX, methotrexate; SAA, severe aplastic anemia; TSH, thyroid stimulating hormone. Source: Katsanis et al. 1990 [35]. Reproduced with permission of Nature Publishing Group.

Table 97.3 Late consequences in children treated more than 10 years before with two different doses and types of fractionated total body irradiation (FTBI)

Late effects	**FTBI**			
	990 cGY (*N* = 25) *n* (%)	**1200 cGy (*N* = 17) *n* (%)**	**Total (*N* = 42) *n* (%)**	***p***
Hypothyroidism	4 (16)	1 (6)	5 (12)	0.63
Thyroid nodules	20 (80)	4^{a} (27)	24 (57)	0.002
Thyroid carcinoma	5 (20)	1 (6)	6 (14)	0.37

[a] Two patients excluded because they were never evaluated by ultrasound.
Source: adapted from Faraci et al. 2005 [36].

Table 97.4 Post-BMT hypothyroidism following cytoreductive therapy regimen without the use of irradiation

Study[a]	**No. of patients**	**Median follow-up (years)**	**Chemotherapy**	**No. of hypothyroidisms**
Sklar et al. (1982)	4	2	CY or CY + 6-mercaptopurine or CY + procarbazine	0
Urban et al. (1988)	10	3.1	CY or CY + BU or melphalan or BACT	0
Sanders (1991)	11	1	CY + BU	0
Galimberti et al. (1991)	58	3.2	CY + BU	0
Liesner et al. (1994)	26	4	CY + BU	0
Boulad et al. (1995)	7	6.2	CY or CY + BU	1
Tourbert et al. (1997)	77	2.6	CY + BU or BU + Alkeran or CY + ATG	11

ATG, antithymocyte globulin; BACT, BCNU, cytarabine, cyclophosphamide, and 6-thioguanine; BU, busulfan; CY, cyclophosphamide.
[a] See the cited source for original references.
Source: Tourbert et al. 1997 [38]. Reprinted with permission of John Wiley & Sons, Ltd.

thyroid tissue (primary dysfunction), and to the hypothalamus or pituitary gland. Further, the use of pre-transplant cranial or neck irradiation (e.g. for Hodgkin disease) is also implicated in the significant increase in rates of thyroid dysfunction post-HCT, including frank hypothyroidism, decreased thyroid reserve, hyperthyroidism, thyroiditis, the occurrence of thyroid nodules, and thyroid malignancy. Similarly, the use of total body irradiation (TBI) or total lymphoid irradiation (TLI) during the preparation regimen of HCT is associated with a greatly increased risk of some type of thyroid dysfunction occurring in the months to years after transplantation. The incidence of thyroid dysfunction is also higher in patients given single-dose irradiation (23–73%), rather than fractionated (10–28%) doses [33,34].

In one study, the prevalence of hypothyroidism appeared to be higher among children receiving HCT for severe aplastic anemia (SAA) than for leukemia, although this difference appeared to be the result of the type of GVHD prophylaxis used, rather than the disease itself (Figure 97.4). Patients with SAA who received methotrexate alone appeared to be at increased risk for developing thyroid dysfunction, but the reasons for this are unclear. It is possible that additional immunosuppression with methotrexate, antithymocyte globulin (ATG), and prednisone suppressed immunologic injury of the thyroid gland, whereas methotrexate alone following TLI may be comparatively inadequate to prevent thyroid injury. On the other hand, patients with acute leukemia, having received greater immunosuppression from TBI and previous long-term chemotherapy, may not require additional immunosuppression to avoid this type of radiation-induced "immune" thyroid injury [35].

Overall, the most common thyroid-related complications post-HCT are either functional in type [hypothyroidism due to destruction of thyroid tissue or autoimmune thyroid disease (AITD)], or present as mass lesions of the thyroid (benign nodular goiter, or malignant carcinoma) (Table 97.3). The prevalence of thyroid abnormalities is higher in allogeneic than in autologous HCT recipients [37], even in the absence of TBI [37,38]. In fact, Tourbert et al. [38] noted a remarkably high incidence of both frank hypothyroidism (10–15%) and euthyroid sick syndrome (ETS) (48% at 3 months after treatment) in a patient group who had received only a BU- or CY-based conditioning regimen without TBI, or had a history of previous radiotherapy (Table 97.4). ETS is actually the most commonly observed endocrine dysfunction in the 3-month period after HCT, and is typically characterized by low free triiodothyronine (T3), free thyroxine (T4), or both, with normal, elevated, or low TSH, along with an increased serum level of reverse T3, without pre-existing hypothalamic–pituitary and thyroid gland dysfunction (Table 97.5). A short-term fall in thyroid hormone levels after HCT generally reflects ETS, with increased conversion of T4 to reverse T3 (metabolically inactive), rather than intrinsic

Table 97.5 Thyroid function before and after BMT (3 and 14 months)[a]

		n	FT3 (pmol/L) [normal 3.6–9.4]	FT4 (pmol/L) [normal 8.5–22]	TSH (mIU/L) [normal 0.2–4]
Before BMT[b]	Normal	59	6.7 [3.7–10.9]	15.5 [9.7–21]	1.88 [0.3–3.9]
	PTI	10	6.6 [3.5–8.9]	13.8 [9.0–19.7]	5.9 [4.0–11.7]
	ETS	6	2.6 [1.2–3.1]	11.1 [7.4–13]	1.15 [0.1–2.0]
After BMT (3 months)[c]	Normal	23	5.7 [4.0–8.7]	12.7 [8.9–20.0]	2.00 [0.5–3.9]
	PTI	6	5.2 [1.2–7.5]	10.5 [4.2–13.9]	8.73 [4.4–17.0]
	ETS	29	2.5 [1.2–3.5]	8.8 [3.7–14.6]	1.23 [0.03–4.0]
After BMT (14 months)[d]	Normal	34	6.5 [4.1–10]	14.2 [10.0–19.2]	1.81 [0.55–3.3]
	PTI	5	5.8 [4.3–7.4]	13.0 [10.3–16.4]	6.12 [4.2–11.0]
	ETS	9	2.6 [1.2–3.5]	8.6 [4.2–14.1]	1.78 [0.1–3.2]

PTI, peripheral thyroid insufficiency, defined if TSH >4 mIU/L; ETS, euthyroid sick syndrome, defined if FT3 <3.6 pmol/L and/or FT4 <8.5 pmol/L and TSH < 4 mIU/L; FT4, free serum thyroxine; FT3, free serum triiodothyronine; TSH, ultrasensitive serum thyrotropin level; BMT, bone marrow transplantation.

[a] Biochemical thyroid hormone levels are given: mean (range). To convert SI units to gravimetric units, divide by following conversion factors:
Gravimetric conversion factor for TSH:1.0 = mU/L.
Gravimetric conversion factor for FT3: 0.0154 = pg/dL.
Gravimetric conversion factor for FT4: 12.87 = ng/dL.

[b] Total = 77 (not done = 2); death (0–2 months) = 8.

[c] Total = 69 (not done = 8); death (3–14 months) = 18.

[d] Total = 51 (not done = 3); death (14–24 months) = 6.

Source: Tourbert et al. 1997 [38]. Reprinted with permission of John Wiley & Sons, Ltd. Additional reference information for studies included in the table is provided in the original article.

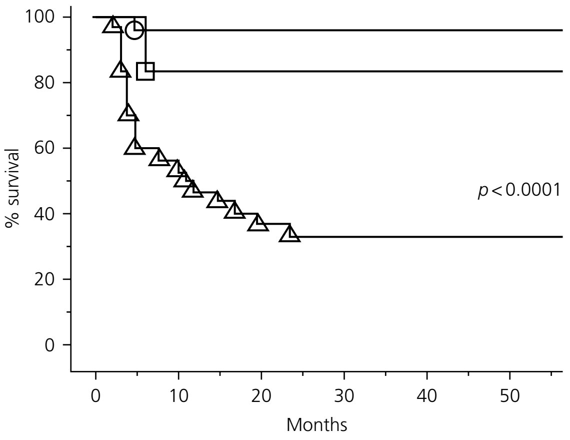

Figure 97.5 Actuarial survival curves at 3 months: normal thyroid profile compared with peripheral thyroid insufficiency and euthyroid sick syndrome. Source: Tourbert et al. 1997 [38]. Reproduced with permission of John Wiley & Sons Ltd.

change in the hypothalamic–pituitary–thyroid (HPA) axis. Thus, patients with ETS-related thyroid function abnormalities are not considered to have hypothyroidism. Notably, however, the occurrence of significant post-HCT ETS, as defined by a precipitous drop in serum total T4 levels, is predictive of lower survival rates compared with patients who do not develop ETS (34.5% vs. 96.2%, $p < 0.0001$) [38] (Figure 97.5). If total T4 levels fall below 4 μg/dL, the probability of death is about 50%; with serum T4 levels below 2 μg/dL, the probability of death reaches about 80%. Reasons for poor prognosis associated with ETS remain unclear, but it has been suggested that ETS may be a reflection of global hormonal dysregulation within the context of severely deranged homeostasis, and also the fact that the occurrence of ETS may be an independent prognosticator in itself.

Although controversy exists as to whether or not the age at transplantation is associated with an increased risk of thyroid dysfunction, several compelling studies cite an age of less than 10 years as being a significant risk factor [34], whereas other studies have found no association.

Functional derangement of the thyroid post-HCT

Hypothyroidism of any type is the most frequent type of clinical or laboratory thyroid dysfunction seen in long-term survivors of HCT [34]; however, the incidence and time of onset for hypothyroidism requiring therapy are highly variable, depending on the type of pretransplant conditioning therapy applied. The median time to the diagnosis of hypothyroidism after HCT is nearly 4 years, while the cumulative incidence of overt hypothyroidism in 308 patients treated with loco-regional radiotherapy for non-thyroid head-and-neck cancer was 20% at 5 years and 27% at 10 years [39]. However, when hypothyroidism is defined as an elevation in serum TSH, the cumulative incidence is even higher, with 48% at 5 years and 67% at 8 years [40]. Other reports more modestly estimate the occurrence of subclinical hypothyroidism after allogeneic HCT to range from 7 to 15%.

The mechanisms by which hypothyroidism may develop post-HCT are unclear. As with thyroid dysfunction in general, a history of radiation exposure, particularly TBI, seems to be a significant risk factor [34]. Single-dose ablative TBI is associated with a 50% incidence of overt hypothyroidism, whereas fractionated TBI is associated with about 15%, compared with pretreatment regimens with BU–CY treatment alone, which represent approximately 11% of hypothyroid cases. The incidence of primary, symptomatic hypothyroidism after HCT without the use of TBI during the pretransplant conditioning regimen has been reported to be around 10–15% [38] (see Table 97.4).

Because hypothyroidism can occur with such relative frequency in HCT recipients who have not received radiation prior to transplant, non-radiotherapy-related mechanisms for post-HCT hypothyroidism have been proposed. Several cytokines regulate thyroid function, and these may be involved in the pathogenesis of thyroid disorders, including ETS. Additionally, the effect of conditioning chemotherapy and the graft-versus-host effect of infused lymphocytes may trigger human leukocyte antigen (HLA)-related AITD. The de novo development of autoimmune thyroid disorders, such as Hashimoto thyroiditis or Graves disease, has been reported in the presence of cGVHD or other pre-existing autoimmune dysfunction [41], although antithyroid autoantibodies occur more rarely than other autoantibodies with cGVHD [42]. While a limited number of case reports note the transfer of AITD from donor to recipient without any autoimmune phenomena by the mechanism of adoptive autoimmunity (i.e. the adoptive transfer of even a small number of pathogenic lymphoid clones) following allogeneic HCT [43], this type of adoptive transfer is likely a minor cause of autoimmune hypothyroidism, as most cases of post-HCT autoimmunity more likely result from an immunologic dysregulation (or "immunologic chaos") [44]. Although it is also possible for the remission of pre-existing autoimmune disease to occur after transplantation by the reverse mechanism, this has not been reported with respect to autoimmune thyroiditis, except for one case of Graves disease [45]. It has been suggested that up to 3% of the post-HCT population may suffer from autoimmune thyroiditis after 5 years of follow-up; meaning that the 5-year actuarial rates for autoimmune thyroiditis were 2.9 and 4% for allogeneic and autologous transplantation, respectively, which are very similar to the 2–4% lifetime risk of de novo autoimmune thyroiditis, which favors a female-to-male ratio of 3:1 [41], but the true incidence of clinically significant autoimmune thyroiditis after HCT remains unclear.

Presentation of functional thyroid derangement post-HCT

The diagnosis of functional thyroid disease after HCT depends on obtaining a careful history, a thorough search for the physical signs of hypothyroidism or thyrotoxicosis, and detailed evaluation of laboratory results.

While the onset of hypothyroidism may be insidious in the adult general population, after HCT disease development may be rapid, with frank manifestations of hypothyroidism present by 6 weeks, and myxedema by 3 months. The signs and symptoms of mild hypothyroidism may be quite subtle, variable, and non-specific, such as dry/coarse skin, cold intolerance, periorbital swelling, and a delayed ankle reflex relaxation phase. Unusual symptoms at presentation may include the following: neurasthenia with muscle cramps, paresthesias, and weaknesses; refractory anemia; disturbances in reproductive function, including infertility, delayed puberty, or menorrhagia; idiopathic edema or pleuropericardial effusions; retarded growth; obstipation; chronic rhinitis or hoarseness due to edema of the nasal mucosa or vocal cords; and severe depression progressing to emotional instability and even frank paranoid psychosis. Bone disorders related to hypothyroidism in children may include impairment of linear growth and waddling gait due to maldevelopment of the femoral epiphyses. Radiologic evidence of epiphyseal dysgenesis is virtually pathognomonic for hypothyroidism in infancy and childhood. Although the clinical picture of myxedema is usually clear in non-HCT recipients, it can often be difficult to observe in patients with GVHD of the skin post-HCT.

Hyperthyroidism of Graves disease may present with one or more of the following features: (1) thyrotoxicosis, (2) goiter, (3) ophthalmopathy (exophthalmos), and (4) dermopathy (pretibial myxedema). The clinical signs of hyperthyroidism in younger individuals include palpitations, nervousness, easy fatiguability, hyperkinesias, diarrhea, excessive sweating, intolerance of heat, and preference for cold temperatures, often with marked weight loss without a loss of appetite. Commonly seen manifestations are thyroid enlargement, thyrotoxic eye signs (e.g. ophthalmopathy), and mild tachycardia. Muscle weakness and loss of muscle mass may be severe. Rarely, thyroid dermopathy (thickening of the skin) and hypertrophic osteopathy (subperiosteal inflammation), particularly of the metacarpal and metatarsal bones, may be seen. In children, rapid growth with accelerated bone maturation occurs. In patients over 60 years old, cardiovascular and myopathic signs predominate, with the most common complaints being palpitation, dyspnea on exertion, tremor, nervousness, and weight loss.

Evaluation of functional thyroid derangement post-HCT

The diagnostic laboratory workup of hypo- or hyperthyroidism usually requires one or more of the following values: FT3, FT4, FT4I (FT4I, a calculation that can distinguish the level of T4 present compared with thyroid-binding globulin), and TSH (Figure 97.6). The combination of a low serum FT4 or FT4I and an elevated serum TSH is diagnostic of primary hypothyroidism, whereas the combination of elevated T4 and suppressed TSH confirms the diagnosis of hyperthyroidism. Suppression of both FT4 and TSH, in the absence of ETS suggests secondary hypothyroidsm related to pituitary or hypothalamic deficiencies.

Management of functional thyroid derangement post-HCT

Replacement doses of levothyroxine in adults range from 0.05 to 0.2 mg/day, with a mean of 0.125 mg/day, or 1.7 μg/kg/day. Dosage varies according to patient's age and body weight. In the elderly and in patients prone to cardiac problems, hormone replacement should be initiated at a low dosage (0.025 mg/day), and then be increased at 4–6-week intervals based on FT4 and TSH measurements up to a mean of approximately 1.6 μg/kg/day. Young children require a surprisingly high dose of levothyroxine compared with adults (e.g. 4–5 μg/kg/day in children aged 6–10 years versus 1–2 μg/kg/day in adults), and the initial dosage may be give at half the estimated final dose requirement for 4–6 weeks, and then adjusted based on FT4 and TSH according to the adult schedule. The major toxic symptoms of levothyroxine overdosage are those of hyperthyroidism, particularly cardiac complications (arrhythmia) and osteoporosis, in postmenopausal women.

Hyperthyroidism/Graves disease can be treated with radioactive iodine, antithyroid drugs (propylthiouracil or methimazole), or surgery (subtotal thyroidectomy), but in the Unites States, radioactive sodium iodide (I-131) at a dosage of 80–150 μCi/g thyroid weight estimated on the basis of physical examination and I-123 scan is the treatment of choice in patients without contraindications. The major complication of radioactive iodine is hypothyroidism, which ultimately develops in approximately 80% of patients who are adequately treated, and hormone replacement should begin as soon as hypothyroidism is confirmed. Patients with small, asymptomatic goiters can be monitored with clinical examination and periodic ultrasound evaluations.

Mass lesions of the thyroid post-HCT

In comparison with the general population, the incidence of thyroid nodules in survivors of HCT is fairly high. In one study, 26 of 95 (27.3%) patients who had received high-dose radiotherapy for childhood malignancies had palpable thyroid nodules 5–34 years after therapy [46]. Further, the incidence of nodules found in survivors of Hodgkin disease varies from 2 to 65%, depending on the length of follow-up and method of evaluation [47]. Because these populations are screened more often and more carefully, however, the true relative risk cannot be ascertained easily.

As with thyroid nodules, patients who underwent HCT during childhood harbor an increased risk of developing secondary thyroid carcinoma compared with the general healthy population (aged 0–29 years), with an overall reported incidence of 0.2% of children after HCT [48]. Factors that further increase the likelihood of malignancy are older age (about 45%), male gender (because nodules are less common in men, any given nodule in a male patient has a higher probability of being malignant), and family history of thyroid cancer (Table 97.6). A history of radiation exposure of the thyroid is significant, as irradiation causes subsequent hyperplasia, and thus a greater likelihood for the development of nodules and malignancies. The relative risk of inducing thyroid cancer is estimated as 0.6–14.9 after irradiation, but varies depending on several parameters: in particular, the use of TBI increases the relative risk to 3.6, versus 1.0 without TBI. The irradiation regimen used in HCT ranges from as low as 2–6 Gy up to 8–14 Gy in high-dose therapy. In comparison, as little as 6.5 cGy (1 cGy = 10^{-2} Gy = 1 rad) to the thyroid gland (e.g. during radiation treatment of tinea capitis) reportedly caused cancer in 0.11% of exposed children, versus 0.02% in sibling controls [49].

Increased doses of radiation exposure appear to correlate with a higher incidence of thyroid cancer, while exposure to extremely high doses of radiation (as much as 10,000 cGy) was rarely associated with the development of thyroid malignancy. This latter phenomenon is likely due to the fact that the thyroid gland is largely

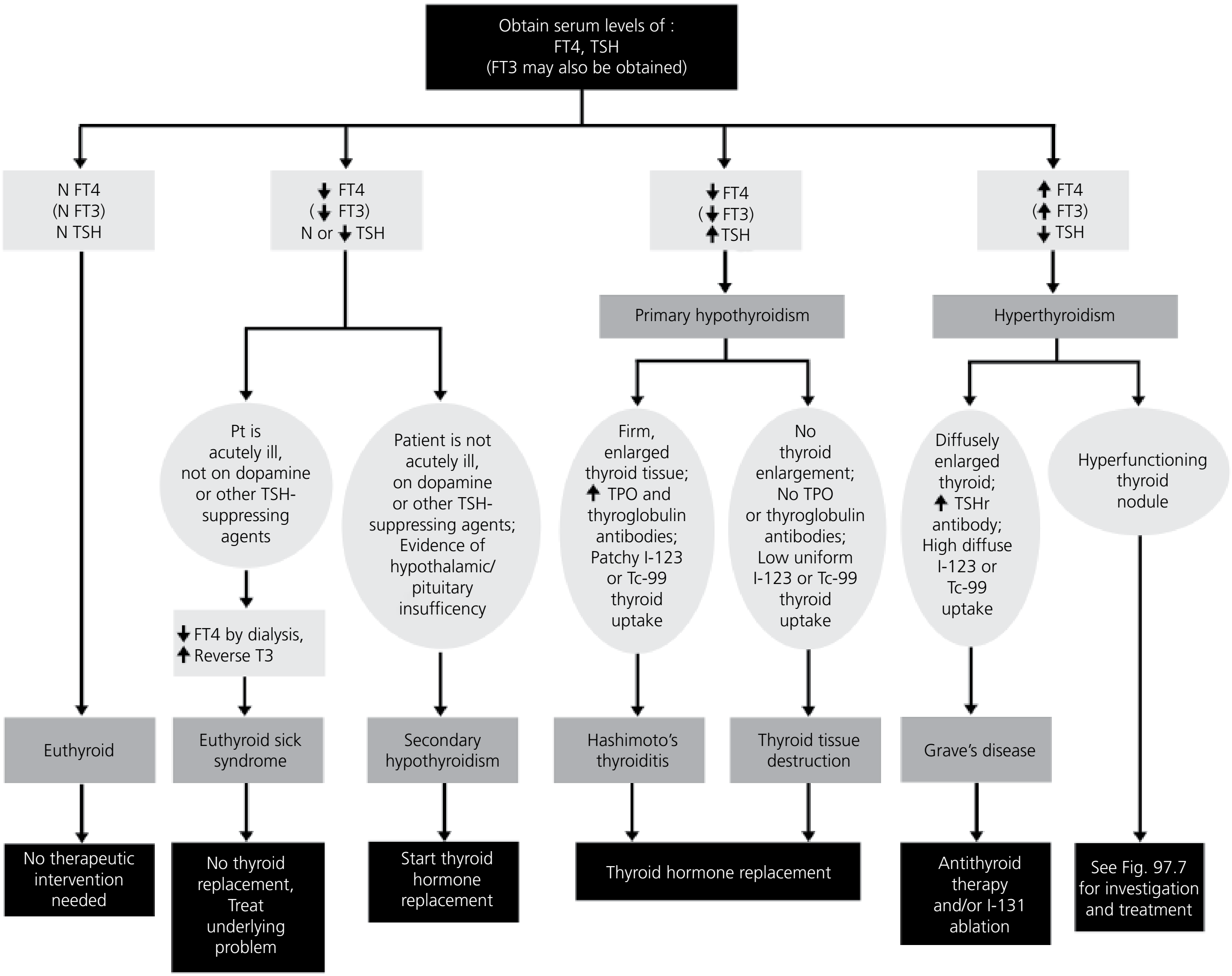
Obtain serum levels of :
FT4, TSH
(FT3 may also be obtained)
N FT4
(N FT3)
N TSH
↓ FT4
(↓ FT3)
N or ↓ TSH
↓ FT4
(↓ FT3)
↑ TSH
↑ FT4
(↑ FT3)
↓ TSH
Primary hypothyroidism
Hyperthyroidism
Pt is acutely ill, not on dopamine or other TSH-suppressing agents
Patient is not acutely ill, on dopamine or other TSH-suppressing agents; Evidence of hypothalamic/ pituitary insufficency
Firm, enlarged thyroid tissue; ↑ TPO and thyroglobulin antibodies; Patchy I-123 or Tc-99 thyroid uptake
No thyroid enlargement; No TPO or thyroglobulin antibodies; Low uniform I-123 or Tc-99 thyroid uptake
Diffusely enlarged thyroid; ↑ TSHr antibody; High diffuse I-123 or Tc-99 uptake
Hyperfunctioning thyroid nodule
↓ FT4 by dialysis, ↑ Reverse T3
Euthyroid
Euthyroid sick syndrome
Secondary hypothyroidism
Hashimoto's thyroiditis
Thyroid tissue destruction
Grave's disease
No therapeutic intervention needed
No thyroid replacement, Treat underlying problem
Start thyroid hormone replacement
Thyroid hormone replacement
Antithyroid therapy and/or I-131 ablation
See Fig. 97.7 for investigation and treatment

Figure 97.6 Algorithm for evaluation of the patient suspected of hypothyroidism or hyperthyroidism following HCT. FT3, free triiodothyronine; FT4, free thyroxine; I-123, radioactive isotope of iodine (gamma emitter); I-131, radioactive isotope of iodine (beta and gamma emitter); N, normal; Reverse T3, isomer of T3, binds to T3 receptors and blocks them; Tc-99, technetium-99; TPO, thyroperoxidase; TSH, thyroid-stimulating hormone; TSHr antibody, thyroid-stimulating hormone receptor antibody.

destroyed by such high doses of radiation, and usually results in hypothyroidism. Notably, the presence or absence of GVHD does not appear to have a significant effect on the development of thyroid carcinoma post-HCT, although some studies have noted a higher relative risk in the presence of chronic GVHD [48].

Another risk factor for the development of secondary thyroid cancer after HCT is a younger age at the time of transplantation, with children 0–9 years old having a relative risk of 12.2 compared with 1.0 between the ages of 10 and 16 years.

Evaluation of mass lesions of the thyroid post-HCT

All patients who have received irradiation, including TBI, should be followed for life with physical evaluations 6 months after HCT, and then annually thereafter, including careful examination of the neck for goiter or nodules, and thyroid function studies (e.g. free T4 with or without TSH to rule out hypothyroidism) 6 months after HCT and annually thereafter. Notably, serum levels of thyroid hormones do not distinguish benign from malignant mass lesions of the thyroid, but may help to establish the presence of a functional thyroid lesion (hyperfunctioning thyroid nodules, also known as Plummer disease), or may reflect hypothyroidism in association with multinodular thyroid goiter or Hashimoto thyroiditis.

If a thyroid nodule >1.0 cm in diameter is found, the workup to rule out malignancy should include a TSH level and fine-needle aspiration (FNA) of the nodule for cytologic assessment and thyroglobulin measurement in needle washout especially if a lymph node is biopsied (Figure 97.7). FNA of thyroid nodules or suspicious neck lymph nodes can be performed blindly or under the guidance of ultrasound.

About 10% of FNA biopsies are inadequate and 20% are indeterminate. Genetic testing of the somatic mutation can be helpful for diagnosis and prognostication of the thyroid tumors, as multiple genes have recently been recognized to be involved in thyroid cancers. Although these mutations can be organized into eight categories, three are most commonly described: BRAF mutation, RET/PTC fusion, and Pax8/PPAR-gamma fusion, all of which converge at the matrix–metalloproteinase pathway associated with invasion and metastasis. The activating mutation in the gene encoding BRAF is the most commonly identified oncogenic abnormality in papillary thyroid carcinoma. In vitro and in vivo models have demonstrated that overexpression of activated BRAF induces malignant

Table 97.6 Clinical presentation of solitary thyroid nodule (>1 cm diameter) suspicious for malignancy

Factors increasing suspicion of thyroid carcinoma:
- ❑ Age <15 years or >45 years
- ❑ Male gender
- ❑ Nodule >4 cm in diameter
- ❑ History of radiation exposure
- ❑ History of diseases associated with thyroid cancer
  - – Pheochromocytoma
  - – Hyperparathyroidism
  - – Gardner syndrome
  - – Familial adenomatous polyposis
  - – Carney complex
  - – Cowden syndrome
- ❑ Suspicious criteria by ultrasound
  - – Central hypervascularity
  - – Irregular border
  - – Microcalcification
- ❑ Incidental focal PET-positive lesion in thyroid

Highly suspicious for thyroid carcinoma if present:
- ❑ Rapid nodule growth (over weeks to months)
- ❑ Very firm nodule
- ❑ Fixed nodule (to adjacent structures)
- ❑ Family history of thyroid cancer
- ❑ Vocal cord paralysis
- ❑ Enlarged regional lymph nodes (particularly low anterior cervical)
- ❑ Symptoms of invasion into neck structures (architectural distortion, e.g. tracheal deviation, muscle involvement, tumor fixation, difficulty swallowing, blockage of internal carotid artery)

transformation and clinically aggressive tumor behavior through constitutive activation, causing a phosphorylation cascade that leads to thyrocyte proliferation along with the loss of differentiation. The BRAF V600E mutation is associated with the presence of extrathyroidal extension and higher clinical stage, and correlates with the worst outcome (persistent disease and lower survival rate). The majority of indeterminate aspirates are of follicular origin, which do not harbor the BRAF V600E mutation; however, detection of the BRAF mutation in an indeterminate sample has 100% specificity and 58% sensitivity for the diagnosis of papillary thyroid carcinoma. The chimeric Pax8/PPAR-gamma gene frequently generates a chromosomal translocation in thyroid follicular carcinoma, but is absent in papillary carcinoma and Hurtle cell carcinoma. Activation of the RET gene via chromosomal rearrangement has been known for almost two decades to be associated with papillary thyroid carcinoma; its two most common variants are RET/PTC1 and RET/PTC3. The prevalence of RET/PTC is higher in patients with a history of radiation exposure, including those subjected to accidental or therapeutic radiation. For example, RET/PTC was present in the majority of thyroid cancer cases in child survivors of the Chernobyl accident. Hence there is clinical relevance in performing genetic analysis of indeterminate FNA aspirates in order to predict tumor behavior. Similarly, targeted disruption of the mutation product (e.g. auto-active BRAF kinase), could serve a therapeutic purpose in halting tumor progression.

A radionuclide thyroid scan can also prove useful in the case of nodules with indeterminate cytology results, as hyperfunctional nodules are almost always benign and can be managed medically with radioactive iodine or surgery. Thus, a radionuclide thyroid scan can assist in evaluating regional uptake or function within the thyroid gland, and is capable of localizing the presence of ectopic thyroid tissue. The radioactive tracers used for thyroid scans are isotopes of iodine (I-131 or I-123) or technetium (Tc-99m). Technetium scanning may be preferred at some diagnostic centers because it can be administered just 2 hours before the study. I-123 tends to be used for thyroid uptake tests and routine thyroid scans, and I-131 is reserved for follow-up evaluations of known thyroid cancer patients, owing to its higher radiation burden.

A normal scan indicates that the iodine uptake is similar in both lobes of the thyroid gland. Nodules may be classified as “cold” (decreased uptake), “warm” (uptake similar to that of surrounding tissue), or “hot” (increased uptake). Although a large proportion of thyroid nodules may be cold on radionuclide scan, only 5–15% of these are malignant in the general population; however, data from several large series suggest that the incidence of cancer in a patient with a history of therapeutic radiation of the head, neck, or chest who presents with a solitary cold thyroid nodule is as great as 50%.

Although MRI is not a primary diagnostic modality in the assessment of patients with thyroid nodules, nodules are often found incidentally during MRI of the neck for non-endocrine indications. The same is true of computed tomography (CT).

With the frequent use of positron emission tomography (PET) in patients with lymphoma and other cancers, an incidental finding of a localized [^{18}F]fluoro-2-deoxy-D-glucose (FDG)-positive lesion in the thyroid gland is associated with one-third of malignancies [50].

Metabolic and electrolyte abnormalities

Patients undergoing HCT are prone to the development of a wide variety of metabolic abnormalities, most of which occur during the early post-transplant period and may vary in severity from mild to life-threatening [51].

One study of 311 patients [51] reviewed the incidence of several metabolic/electrolyte abnormalities following HCT, demonstrating that a high number (269, 86.5%) of patients developed at least one severe metabolic abnormality within 100 days after allogeneic HCT. In all, 196 patients (63.0%) experienced one to three different metabolic abnormalities, whereas 73 (23.5%) had four to seven abnormalities. The most common abnormalities experienced within 100 days after HCT were hyponatremia (185, 59.5%), hypokalemia (149, 47.9%), hypophosphatemia (105, 33.8%), and hypomagnesemia (52, 16.7%). Less frequently encountered abnormalities were hyperkalemia (25, 8.0%), hyperuricemia (22, 7.1%), hypernatremia (13, 4.2%), and hypermagnesemia (9, 2.9%). Calcium abnormalities were found to be relatively rare, with hypercalcemia observed in one patient (0.3%) and hypocalcemia in one other patient (0.3%). One of the unique aspects of children with osteopetrosis undergoing allogeneic BMT is the development of hypercalcemia after bone marrow transplant, which needs to be treated promptly with hydration, furosemide, intravenous bisphosphonates, phosphate, and calcitonin [52]. In addition to these electrolyte abnormalities, derangements in glucose and lipid homeostasis were observed, with hyperglycemia being most common (138, 44.4%), followed by hypoglycemia (12, 3.9%), and hypercholesterolemia (3, 1.0%).

Induction regimens, immune suppression drugs, GVHD, and/or total parenteral nutrition (TPN) supportive therapy of patients undergoing or following HCT are among the factors contributing to the development of the metabolic and electrolyte disturbances seen. For example, the use of cyclosporine and tacrolimus in the prophylaxis and treatment of GVHD has been associated with hyperkalemia, hyperglycemia, hypomagnesemia, and hypertriglyceridemia in HCT recipients. As mentioned elsewhere in this chapter, glucocorticoid therapy may also cause hyperglycemia. GVHD-related gastrointestinal complications may also further the development of metabolic abnormalities. The subsequent occurrence of severe inflammatory or infective processes may lead to

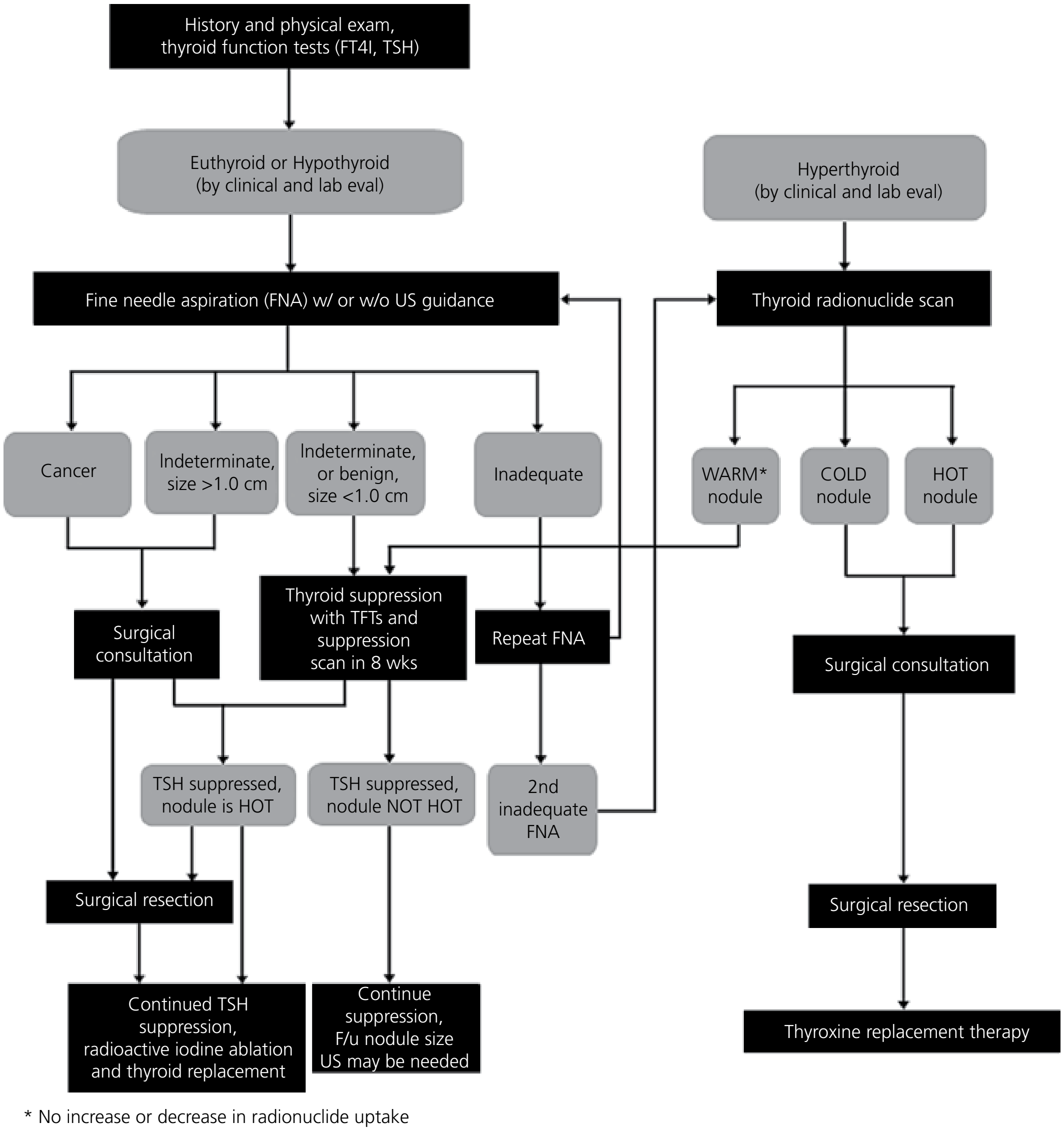
History and physical exam, thyroid function tests (FT4I, TSH)
Euthyroid or Hypothyroid (by clinical and lab eval)
Hyperthyroid (by clinical and lab eval)
Fine needle aspiration (FNA) w/ or w/o US guidance
Thyroid radionuclide scan
Cancer
Indeterminate, size >1.0 cm
Indeterminate, or benign, size <1.0 cm
Inadequate
WARM* nodule
COLD nodule
HOT nodule
Surgical consultation
Thyroid suppression with TFTs and suppression scan in 8 wks
Repeat FNA
Surgical consultation
TSH suppressed, nodule is HOT
TSH suppressed, nodule NOT HOT
2nd inadequate FNA
Surgical resection
Surgical resection
Continued TSH suppression, radioactive iodine ablation and thyroid replacement
Continue suppression, F/u nodule size US may be needed
Thyroxine replacement therapy
* No increase or decrease in radionuclide uptake

Figure 97.7 Algorithm for evaluation of the patient presenting with thyroid nodule following HCT. FNA, fine-needle aspiration; FT4I, free thyroxine index; TFTs, thyroid function tests; TSH, thyroid-stimulating hormone; US, ultrasound.

insulin resistance by increasing levels of tumor necrosis factor-alpha (TNF-α). Long-term treatment with aminoglycoside antibiotics and amphotericin B with concomitant hypomagnesemia and vomiting can cause renal potassium wasting and hypokalemia. Pentamidine, used for the prevention or treatment of pneumocystis pneumonitis, may cause acute release of insulin due to pancreatic beta-cell damage, resulting in severe hypoglycemia followed by hyperglycemia. There was also a case report of hypoglycemia induced by insulin receptor antibodies following HCT [53]. Increased phosphate uptake by replicating neutrophils during the peri-engraftment period may additionally lead to hypophosphatemia in HCT recipients [54].

The occurrence of severe metabolic abnormalities following HCT tends to be associated with inferior clinical outcomes, and the impact of these metabolic disorders may be independent of other complications, including GVHD and hepatic sinusoidal obstructive syndrome (SOS) [51], suggesting that metabolic abnormalities that develop during the early post-transplant period may serve as prognostic indicators for patients undergoing allogeneic HCT. Thus, metabolic parameters should be closely monitored in patients treated with HCT.

In the following section, only HCT-associated hyponatremia will be discussed in detail. The diagnosis and treatment of other electrolyte abnormalities are usually more straightforward and therefore will not be addressed further.

Abnormal water metabolism

Hyponatremia, defined as a decrease in serum sodium concentration to ≤135 mmol/L, is a common electrolyte disorder in hospitalized patients, and is among the metabolic disturbances frequently encountered in oncology. Current data concerning the incidence of hyponatremia following HCT are limited, although one study reported the incidence to be as high as 40% [55]. Risk factors for hyponatremia following HCT include the development of syndrome of inappropriate antidiuretic hormone secretion (SIADH), hypothyroidism, adrenal insufficiency subsequent to discontinuation of glucocorticoid use, chemotherapy, treatment-induced nausea and vomiting, overhydration, pain, narcotic drugs, diarrhea, infection, renal failure, and physical and emotional stress. Diarrhea, in particular, is a relatively frequent complication of HCT because of mucosal damage, infection, and acute GVHD, and hyponatremia due to diarrhea is, therefore, frequently observed following HCT [51].

SIADH is another important, common cause of hyponatremia following HCT, and may result from cytotoxicity to paraventricular and supraoptic neurons (see below) as a side effect of conditioning therapy, especially with cyclophosphamide [51]. The presence of abnormal secretory stimuli, such as intrathoracic infection or the use of positive-pressure ventilation, may also cause SIADH. Inappropriate release of antidiuretic hormone (ADH) leads to retention of free water and hyponatremia. Thus, SIADH is characterized by hyponatremia, low serum osmolality, and an inappropriately high urine osmolality in the absence of diuretics, heart failure, cirrhosis, adrenal insufficiency, and hypothyroidism.

The rate of occurrence of SIADH, which was found in 16 (11.4%) of 140 HCT patients in one study, was unexpectedly high [55]. SIADH has also been associated with several hematologic diseases, such as non-Hodgkin lymphoma (NHL) and Hodgkin disease, although the most common cause of SIADH in NHL is thought to be the use of either vinca alkaloid-containing chemotherapy (see below) or direct invasion into the pituitary gland. The use of an HLA-mismatched donor may be a risk factor for SIADH, as is younger age at transplantation. Because the water content of young children is relatively higher than that of adults, electrolyte imbalances – and, correspondingly, SIADH – may occur more readily in a pediatric patient population. Further, the consequences of acute hyponatremia are more serious in young children.

SIADH-associated hyponatremia has been observed with the use of various antineoplastic agents due to chemotherapy-induced hypothalamic or posterior pituitary damage. Vinca alkaloids (vincristine, vinblastine) are thought to induce SIADH due to paraventricular or supraoptic cell microtubular damage. Vincristine additionally causes gastrointestinal sodium and water loss, resulting in appropriate ADH secretion. When vincristine is used in combination with cyclophosphamide, doxorubicin, methotrexate, and prednisolone (CHOP-M), the incidence of hyponatremia is about 17% [56]. In another series in which high-dose vinblastine, cisplatin, and bleomycin therapy were combined, eight of 12 patients developed hyponatremia and hypoosmolality [57].

High-dose cyclophosphamide therapy causes SIADH via a primarily direct effect on the hypothalamus. Post-mortem examination of a case of fatal cyclophosphamide-induced (1800 mg/m^2) hyponatremia revealed infundibular necrosis, decreased intra-axonal secretory granules, and depletion of posterior pituitary ADH [58]. Other mechanisms have also been invoked: in a small series of 19 chemotherapy courses with lower dosages of cyclophosphamide, there was development of hyponatremia, plasma hypotonicity, and urinary hypertonicity without an increase in plasma ADH levels [59]. This suggests that damage to the renal tubules and resultant defects of salt and water transport are the major cause of hyponatremia associated with low-dose therapy.

The mechanism of cisplatin-induced hyponatremia is unclear, but the renal toxic effects of cisplatin, rather than a direct effect on ADH secretion, are probably the major factor. This drug likely induces renal salt wasting, leading to hyponatremia, hypoosmolality, elevated urinary sodium, and urinary osmolality. Such an SIADH-like syndrome is difficult to distinguish from true SIADH when signs and symptoms of fluid volume depletion are subtle or absent.

It is not clear if melphalan has a direct effect on the development of hyponatremia or if it merely potentiates the effects of other agents. Melphalan-induced hyponatremia and urinary sodium loss, thought to be SIADH, have been reported in two patients who received high-dose melphalan (2 mg/kg) in combination with vincristine and cyclophosphamide [60]. Melphalan was implicated in the causation because challenge with the same regimen but a lower dose of melphalan (0.5 mg/kg) in one patient several weeks later did not cause hyponatremia.

Although not directly related to HCT, the presence of solid tumors may also be responsible for hyponatremia, either through ectopic secretion of ADH by the tumor itself, or through secretion of atrial natriuretic peptide, which causes renal salt wasting.

Among tyrosine kinase inhibitors, imatinib has been reported to cause SIADH [61]. The use of newer tyrosine kinase inhibitors, however, has not reportedly been associated with SIADH, but rather hyponatremia, of which cediranib had the highest (29%) risk of hyponatremia [62], versus patients on axitinib, who had a 9% risk of hyponatremia [63].

Evaluation of abnormal water metabolism post-HCT

As the clinical presentation may be highly variable and dependent on the cause, degree, and the chronicity of hyponatremia, the evaluation of these patients should be directed toward identifying the particular cause of hyponatremia (Figure 97.8) [64]. Establishing the hydration status of the patient is a significant first step. A history of fluid loss (e.g. vomiting, diarrhea, diuretic therapy) or one that is consistent with the development of SIADH, such as intake of an offending drug (see above), should be elucidated. On physical examination, pulmonary rales, S_3 gallop, jugular venous distention, peripheral edema, or ascites suggest hypervolemic hyponatremia. Dry mucous membranes, tachycardia, diminished skin turgor, and orthostasis suggest hypovolemic hyponatremia. Patients who lack findings of hypovolemia or hypervolemia are considered to have euvolemic hyponatremia, although identification of subtle degrees of volume depletion or edema may be difficult. As a result, laboratory testing is almost always required to establish the diagnosis of hyponatremia. Nevertheless, a history of corticosteroid use, or signs and symptoms suggestive of adrenal insufficiency or hypothyroidism, and also other metabolic abnormalities commonly observed post-HCT, such as hyperglycemia, hyperlipidemia, and hyperuricemia, should not be neglected. Other non-specific signs include muscle weakness and cramping; rhabdomyolysis is an occasional consequence of hyponatremia, and should be considered in patients with muscle pain or tenderness.

Standard laboratory testing for the evaluation of hyponatremia should include a chemistry panel, plasma osmolality, urine osmolality, and urine sodium concentration. Most cases of hyponatremia are associated with reduced plasma osmolality, but plasma osmolality may be normal, or even high, in hyponatremia that occurs in association with hyperlipidemia or hyperproteinemia, renal failure, hyperglycemia, or after the infusion of sucrose and maltose-containing immunoglobulin g (IgG) formulations. A urine osmolality of >100 mosmol/kg is consistent with an inability to excrete free water normally, which is generally due to continued secretion of ADH. A urine osmolality of <100 mosmol/kg may be due to primary polydipsia, malnutrition, or a reset osmostat. The urine sodium concentration is used to help distinguish between effective volume depletion and other causes in which euvolemia or renal salt wasting is present, and should be less than 20 meq/L with hypovolemia, unless salt wasting is occurring (e.g. adrenal

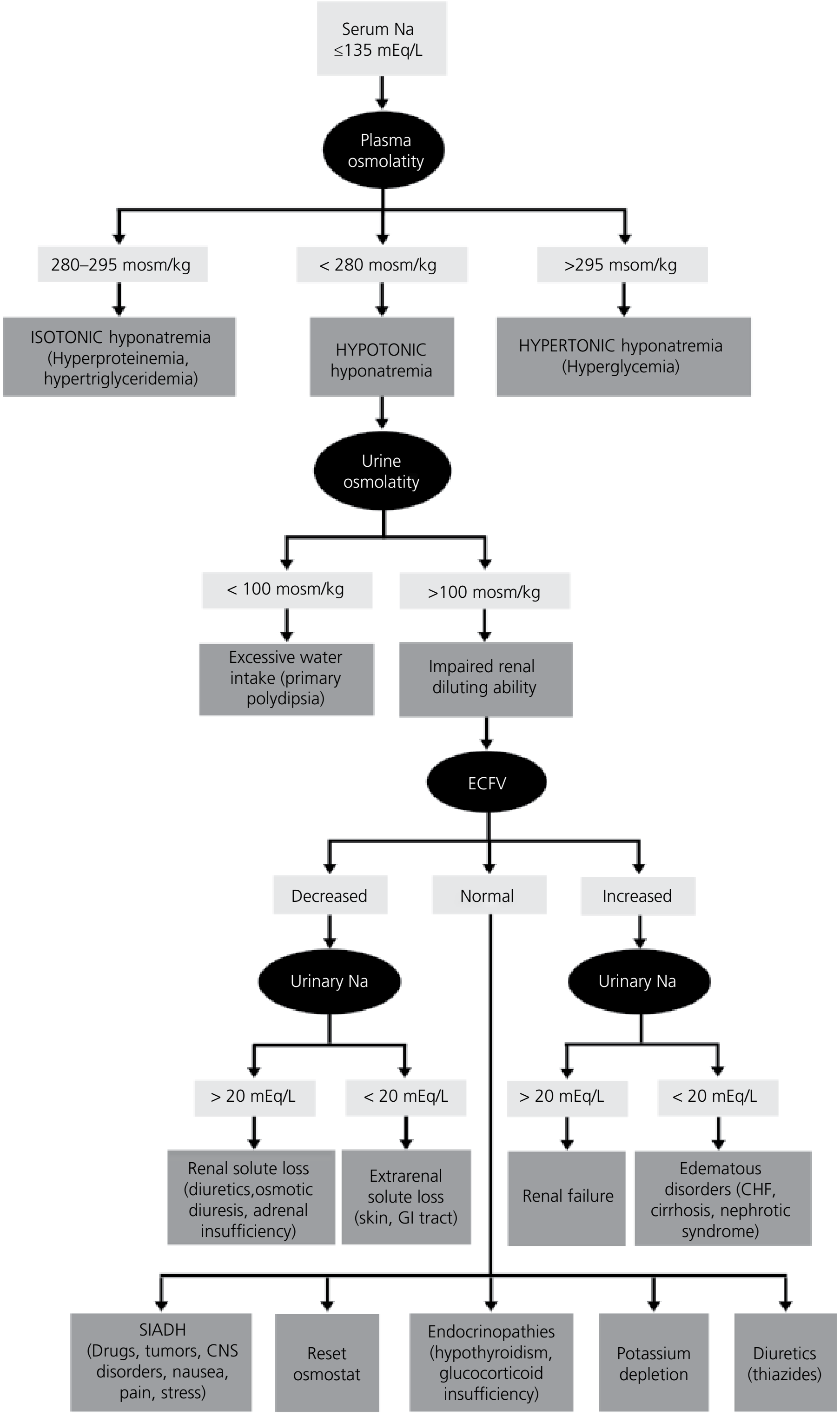
Serum Na
≤135 mEq/L
Plasma osmolatity
280–295 mosm/kg
< 280 mosm/kg
>295 msom/kg
ISOTONIC hyponatremia (Hyperproteinemia, hypertriglyceridemia)
HYPOTONIC hyponatremia
HYPERTONIC hyponatremia (Hyperglycemia)
Urine osmolatity
< 100 mosm/kg
>100 mosm/kg
Excessive water intake (primary polydipsia)
Impaired renal diluting ability
ECFV
Decreased
Normal
Increased
Urinary Na
Urinary Na
> 20 mEq/L
< 20 mEq/L
> 20 mEq/L
< 20 mEq/L
Renal solute loss (diuretics,osmotic diuresis, adrenal insufficiency)
Extrarenal solute loss (skin, GI tract)
Renal failure
Edematous disorders (CHF, cirrhosis, nephrotic syndrome)
SIADH (Drugs, tumors, CNS disorders, nausea, pain, stress)
Reset osmostat
Endocrinopathies (hypothyroidism, glucocorticoid insufficiency)
Potassium depletion
Diuretics (thiazides)

Figure 97.8 Algorithm for evaluation of the patient with suspected hyponatremia following HCT. CHF, congestive heart failure; CNS, central nervous system; ECFV, extracellular fluid volume; GI, gastrointestinal; Serum Na, serum sodium concentration; SIADH, syndrome of inappropriate ADH secretion; Urinary Na, urine sodium concentration. Source: adapted from Weisberg et al. 1992 [64].

insufficiency). Urine sodium concentrations are generally above 40 meq/L in SIADH.

The diagnosis of SIADH is usually a diagnosis of exclusion, made from a combination of results from the history, physical examination (no edema), and laboratory tests, where the following results are generally observed: low plasma osmolality, an inappropriately elevated urine osmolality (above 100 and usually above 300 mosmol/kg), a urine sodium concentration usually above 40 meq/L, low blood urea nitrogen (BUN) and serum uric acid concentration, a relatively normal plasma creatinine concentration, normal acid–base and potassium balance, and normal adrenal and thyroid function. Patients with SIADH may have a low urine sodium concentration if they are also volume depleted, or if their sodium intake is extremely low. In such patients, the diagnosis of SIADH is made by observing the response to a saline load: the urine sodium rises but the urine osmolality remains high.

In patients for whom the diagnosis is not apparent after the initial evaluation, measurement of the plasma uric acid and urea concentrations, the fractional excretion of sodium, and adrenal and thyroid function tests may be helpful.

Management of abnormal water metabolism post-HCT

The choice of therapy is primarily governed by the cause and severity of the hyponatremia. Treatment should be directed first towards correcting any underlying causes of hyponatremia, such as cessation of a drug that induces SIADH, glucocorticoid or thyroid hormone replacement in patients with adrenal insufficiency or hypothyroidism, and control of hyperlipidemia and/or hyperglycemia. In these circumstances, the rate of correction of the underlying condition results in a relatively slow correction of the hyponatremia.

Rapid correction of hyponatremia is generally not indicated in asymptomatic hospitalized patients, particularly if the baseline plasma sodium is above 120 meq/L. Such patients are treated conservatively with free water restriction (e.g. restriction to 50–60% of daily fluid requirements to induce a negative water balance). If correction of volume depletion is indicated, intravenous isotonic saline and/or increased dietary salt (NaCl, 2–3 g/day) is administered. Salt therapy is usually contraindicated in edematous patients (e.g. heart failure, cirrhosis, renal failure) since it will exacerbate the presence of cerebral edema. Notably, the response to isotonic saline differs in states of volume depletion and SIADH. Isotonic saline is typically sufficient in true volume depletion, but ineffective in SIADH. Hypertonic saline is recommended only for patients with symptomatic or severe hyponatremia. To avoid the possibility of overcorrection, the therapeutic goal over the first 24 hours should be to increase serum Na^+ by 8 meq/L, with a maximum rate of correction of ≤10–12 meq/L in the first 24 hours, and ≤18 meq/L over 48 hours.

If the cause of SIADH cannot be corrected, and if water restriction is poorly tolerated or ineffective, demeclocycline (a tetracycline antibiotic that increases electrolyte-free water excretion by inhibiting ADH-mediated water reabsorption in the collecting duct) 300–600 mg in divided doses can be used. If the thirst sensation is intact, patients treated with demeclocycline rarely become hypernatremic. This drug is contraindicated, however, in patients with renal disease, hepatic cirrhosis, or congestive heart failure.

The arginine vasopressin (AVP)-receptor antagonists, a new class of agents, may correct hyponatremia by directly blocking the binding of AVP with its V2 receptors. V2 receptors are expressed in basolateral membranes of renal cortical and medullary collecting ducts and distal tubules. Binding of ADH to V2 receptor in the membranes of cells expressing the V2 receptor enhances production and trafficking of the water channel aquaporin 2 to the luminal membranes to facilitate water reabsorption into the hypertonic medullary interstitium to maintain plasma tonicity. By blocking the V2 receptor, the effect of ADH is blunted and the loss of water in the urine permits dilution of solutes in the urine and increase of urine osmolality. In clinical trials, conivaptan, lixivaptan, tolvaptan, and satavaptan have increased serum osmolality and normalized the serum $[Na^+]$ in hyponatremia associated with SIADH, cirrhosis, or congestive heart failure. These drugs may also have potential in cancer-related hyponatremia. At present, both intravenous conivaptan and oral tolvaptan are available, and are approved for the treatment of euvolemic hyponatremia (i.e. SIADH). The data that are currently available point towards easier management of hospital hyponatremia not only in the setting of SIADH, but also of hyponatremia associated with liver cirrhosis, nephrosis, and congestive heart failure, where the state of upregulated ADH is independent of serum osmolality.

Carbohydrate and lipid abnormalities

The prevalence of frank type 2 diabetes in HCT survivors appears to vary from 3.3 to 17% [65,66]. Impaired glucose tolerance was seen in 3.3–26% of adult patients treated with both TBI and non-TBI regimens prior to HCT. Hyperinsulinemia has also occurred post-HCT, with the highest insulin levels noted in patients receiving preparations of both cytotoxic drugs and TBI [67], but the connections between irradiation, busulfan, and insulin resistance are not clear.

Dyslipidemia of any type has been observed in approximately 27.9% of HCT recipients [65,66]. In general, of the several abnormalities in lipoprotein metabolism associated with insulin resistance, hypertriglyceridemia is the most common. A number of immunosuppressive medications have been documented to cause dyslipidemias, including cyclosporine, tacrolimus, sirolimus, mycophenolate, and high-dose administration of glucocorticoids. Glucocorticoids stimulate hepatic gluconeogenesis, diminish glucose utilization, oppose insulin action, increase protein breakdown, and activate lipolysis in the periphery; if patient insulin levels are inadequate to overcome insulin resistance and increased hepatic glucose output, steroid-induced diabetes mellitus will develop. Older age is a risk factor for steroid diabetes, and also for diabetes mellitus in general [68]. Iwamoto et al. did not find evidence for increased risk of long-lasting steroid-induced diabetes with cumulative doses of glucocorticoids [69], although long-term therapy has been associated with hypercholesterolemia.

It has also been shown that prolonged (4 days') ingestion and infusion of a hypercaloric carbohydrate diet (hyperalimentation) produced chronic elevations in both plasma insulin and glucose concentration, with plasma triglyceride (TG) concentration elevated fourfold compared with the values in the basal state [70]. The hyperalimentation formulas used post-HCT are often high in glucose and lipids, which may contribute to hyperglycemia, insulin resistance, and hyperlipidemia. Severe hyperglycemia early (first 10 days) post-allogeneic bone marrow transplantation has been shown to be associated with increased risk of acute GVHD in the following 90 days [71].

GVHD commonly affects the liver, first presenting with elevated liver transaminase levels, and then with intrahepatic cholestasis and dyslipidemia. This condition frequently manifests between 2 months and 2 years post-transplantation, and may occur in up to 40% of recipients from HLA-matched related donors [72].

Although few cases of severe hypercholesterolemia associated with GVHD of the liver in HCT patients have been reported in the literature, this clinical presentation may occur more frequently than previously perceived [73]. Consequently, there may be insufficient awareness of this important complication.

The patient's diagnosis at transplant was found to be the most significant risk factor for the development of type 2 diabetes post-HCT, with leukemic patients at greater risk than patients with other hematologic diseases, possibly due to greater exposure to pre-transplantation chemotherapy agents, and also to islet cell damage as the result of leukostasis or leukemic infiltration of the pancreas [67]. In this study, the prevalence of type 2 diabetes was found to be 9% among those with leukemia, versus 2% with aplastic anemia, compared with the prevalence of physician-diagnosed diabetes in the general population based on the 1988–1994 National Health and Nutrition Examination Surveys (NHANES) III survey: 1.1% among all 20–39-year-olds and 3.9% among all 40–49-year-olds. Additional evidence suggests that cancer survivors in general, and survivors of childhood acute lymphoblastic leukemia (ALL), are also at increased risk for metabolic syndrome [74].

Autoimmune type 1 diabetes may develop post-HCT due to the transfer of type 1 diabetes in a bone marrow allograft donor to a transplanted recipient, or because of recipient HLA susceptibility [67,75]. Further, as with type 2 diabetes, the prevalence of type 1 diabetes was approximately three times higher in HCT survivors than in the general population, at 0.52% versus 0.17% [67]. One case report, however, described a successful allograft for aplastic anemia in which the donor had type 1 diabetes at the time of transplant, but after a 21-year follow-up period, the recipient had not developed diabetes, although persistent antibodies were developed against pancreatic islet cells [76].

Pediatric HCT survivors seem more likely to develop either type 1 or type 2 diabetes than the general population [67]. Development of type 2 diabetes can also be causally related to the administration of GH therapy. In one study, GH therapy was associated with a significant sixfold increase in new cases of type 2 diabetes, including increases in insulin concentrations and other evidence of insulin resistance [77]. The occurrence of growth hormone deficiency itself may be a consequence of metabolic syndrome [74] (see the section on growth dysfunction).

Evaluation of carbohydrate and lipid abnormalities post-HCT

Clinical signs and symptoms of diabetes mellitus are related to the degree of hyperglycemia and the presence or absence of ketoacidosis or other hyperosmolar states, and may include polyuria, polydipsia, polyphagia, weight loss, and blurred vision, and may be associated with fluid and electrolyte disturbances, including elevated BUN and creatinine, low-normal or mildly reduced sodium levels, elevated serum potassium, low serum bicarbonate (<10 mg/dL), and elevated serum triglycerides. Uncomplicated presentations may reveal normal fluid and electrolyte measures, but if diabetic ketoacidosis is present, laboratory values will represent acidosis and more severe dehydration.

Management of carbohydrate and lipid abnormalities post-HCT

Regardless of whether or not the patient has a known history of diabetes, it should be emphasized that management of the hyperglycemic hospitalized patient must be intensive, particularly in the presence of severe illness. Acute metabolic derangements related to diabetic ketoacidosis (DKA) or hyperosmolar coma require urgent management in order to stabilize the patient and restore normal acid–base balance and volume status, and abrogate electrolyte abnormalities. Although hyperglycemia in itself may often be given secondary consideration in this setting relative to the development of other acute post-HCT complications, the onset and persistence of suboptimal glycemic control post-HCT could significantly prolong the length of hospital stay, and also increase the risk for morbidity and mortality.

The currently available classes of insulin preparations may be divided into (1) those intended for prandial use: regular insulin and rapid-acting insulin (lispro, aspart, and glulisine, onset of action within 1 hour or less), and (2) those for control of fasting blood glucose and background hyperglycemia: intermediate-acting insulin (NPH, onset of action within 1–3 hours and duration of action up to 24 hours, peak action at 6–8 hours) and long-acting insulin (glargine and detemir, peak action within 2–6 hours, duration up to 24 hours). In the acute care setting, continuous intravenous rapid-acting or regular insulin with frequent monitoring of plasma or serum glucose may be required in order to control hyperglycemia. Once stabilized, typical treatment may include multiple daily injections of premixed intermediate- and rapid-acting products, or freshly mixed or separately injected short- and intermediate-acting or long-acting preparations. The most serious complication of insulin replacement is that of hypoglycemia, the risk of which can be reduced in the inpatient setting by the careful monitoring and adjustment of insulin doses, and in the outpatient setting by teaching patients to recognize the signs of hypoglycemia, and also the measures that should be taken to reverse hyperglycemia once these symptoms are experienced.

Hyperalimentation with high lipid content is often prescribed in HCT patients, but in view of the potential metabolic complications from hyperlipidemia, particularly when combined with the presence of GVHD, hyperalimentation should be used for the shortest period possible, and oral or enteral feeding should be initiated as soon as is clinically feasible. Hyperlipidemia may also be seen as the result of GVHD of the liver. If such a patient with GVHD and hepatic damage is also receiving hyperalimentation, the lipid content should be reduced, and insulin may be given. Caution should be exercised when using the antihyperlipidemic medications in patients with hepatic damage, which can limit management options. Low doses of fibrates (i.e. amphipathic carboxylic acids for the treatment of hypercholesterolemia, such as gemfibrozil), niacin (vitamin B_3), and omega-3 fatty acids may be used, particularly when statins are contraindicated. Coenzyme Q10 (Co-Q10) may also be prescribed, but may have little to no therapeutic effects. Regardless of the treatment options utilized, liver enzymes should be closely monitored to avoid added hepatocellular toxicity related to the therapeutic agents themselves. Plasmapheresis may be employed in severe cases of hypertriglyceridemia to avoid pancreatitis.

Adrenal dysfunction

Cortisol secretion is tightly regulated by ACTH (Figure 97.9), and plasma levels of cortisol will parallel those of ACTH. The neuroendocrine mechanisms of the HPA axis normally permit (1) episodic secretion as related to circadian rhythms, and (2) cortisol secretion in large quantities in response to stressful stimuli. Endogenous glucocorticoids cause insulin resistance, increasing blood glucose concentrations via their actions on glycogen, protein, and lipid metabolism. Additionally, glucocorticoids can promote catabolic changes in muscle, skin, and connective tissue, inhibit osteoblast function, increase blood pressure, suppress immunologic responses, affect mood by increasing the manifestations of depression, euphoria, psychosis, apathy, and lethargy, increase the risk of peptic ulcer disease, inhibit linear skeletal growth, and suppress the thyroid axis.

Primary hypoadrenalism (Addison disease) is rare in the general population, with an estimated incidence of 0.8 cases per 100,000 [78]. As with the general population, transplant recipients have a low incidence of primary adrenal failure after HCT. Secondary hypoadrenalism, however, is fairly common. Although the adrenal gland is known to be relatively radioresistant, TBI has been implicated in adrenal

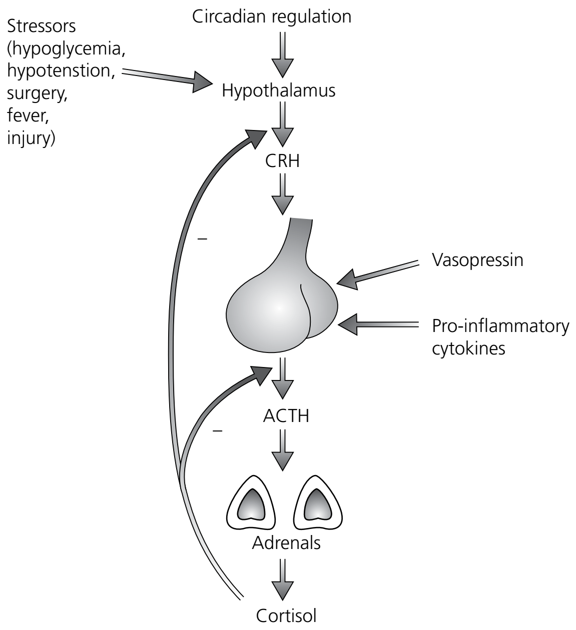

Figure 97.9 Neuroendocrine control of adrenal glucocorticoid secretion. Adrenocorticotropic hormone (ACTH) is secreted from the anterior pituitary under the influence of two principal secretagogues, corticotropin-releasing hormone (CRH) and arginine vasopressin; other factors, including cytokines, also play a role. CRH secretion is regulated by Circadian rhythm and additional stressors operating through the hypothalamus. Secretion of both CRH and ACTH is inhibited by cortisol, highlighting the importance of negative feedback control. Source: Stewart 2008 [78]. Reprinted with permission from Elsevier.

suppression post-HCT. Low-dose cranial irradiation, however, has not demonstrated disruption of spontaneous ACTH or cortisol secretion in several studies [2]. In general, however, secondary adrenal insufficiency is mainly the result of long-term, exogenous corticosteroid therapy, such as in HCT patients being treated for GVHD. Chronic administration of glucocorticoids suppresses the function of the HPA axis through a process that is dependent on both the dose and duration of treatment. Greater length and intensity of exposure are generally associated with a longer persistence of adrenal suppression; adrenal atrophy and subsequent deficiency should be anticipated in any patient who has taken more than the equivalent of 30 mg/day oral hydrocortisone (7.5 mg/day prednisolone or 0.75 mg/day dexamethasone) for more than 3 weeks [78]. High doses of glucocorticoids for as little as 3 days can also result in suppression of the HPA axis. The timing of the dose administration may also influence the severity of adrenal insufficiency, as larger evening doses will block circadian morning surges of endogenous ACTH. Because the HPA axis seems to remain intact, with any defects appearing to occur at the target organ level, adrenal function usually recovers gradually once exogenous corticosteroid exposure ends [2]. After chronic adrenal suppression has been established, however, sudden cessation of glucocorticoid therapy may result in adrenal crises, and care must be taken during steroid withdrawal. Failure to provide adequate glucocorticoid replacement in times of stress, such as with surgery or infection, may also precipitate deterioration in the general condition of the patient related to relative glucocorticoid deficiency.

The secretion of adrenal aldosterone is mainly mediated by angiotensin II, and also by ACTH and local potassium levels. Aldosterone acts on the kidneys to cause active reabsorption of sodium and passive reabsorption of water, and also the active secretion of potassium and protons in the collecting tubule, resulting in increases in blood pressure, blood volume, hypokalemia, and hyperchloremic metabolic alkalosis.

Adult adrenal androgen production is also regulated by ACTH. The most important adrenal androgens are dehydroepiandrosterone (DHEA) and its sulfated form (DHEAS) and 4-androstene-3,17-dione. These hormones also exhibit circadian periodicity in synchrony with ACTH and cortisol, as plasma DHEA and androstenedione increase rapidly with ACTH administration, and are suppressed by the presence of glucocorticoids. Although the adrenal androgens have low androgenic activity, they can be converted into the potent androgens testosterone and 5α-dihydrotestosterone in both peripheral and target tissues. In normal males, this conversion accounts for less than 5% of testosterone, hence the physiologic effect is negligible. In contrast, adrenal androgens contribute to 50% or more of circulating, biologically active androgens in adult women; therefore, the adrenal glands may play important roles in the anabolic status and sexual function of females. Hence lack of DHEA and DHEAS in women may result in the loss of pubic and axillary hair and osteoporosis, and their excess will manifest with acne, hirsutism, and virilization. Thus, any HCT-related adrenal dysfunction is more likely to impact female patients. The significance of this postulation has yet to be clarified.

Evaluation of adrenal dysfunction post-HCT

Patients with primary adrenal failure usually will demonstrate signs of both glucocorticoid and mineralocorticoid deficiency. In both sexes, cortisol deficiency produces symptoms of weakness, fatigue, anorexia, nausea, vomiting, hypotension, hyponatremia, and hypoglycemia. Mineralocorticoid deficiency produces renal sodium wasting and potassium retention, and can lead to severe dehydration, hypotension, hyponatremia, hyperkalemia, and acidosis. Patients with secondary hypoadrenalism will have an intact renin–angiotensin–aldosterone system, which accounts for differences in clinical presentation between these two groups of patients with regard to salt and water balance. The most obvious feature that differentiates primary from secondary hypoadrenalism is the presence of hyperpigmentation of the skin and mucous membranes, which is not present in secondary forms of the disease.

Clinical suspicion should be confirmed with definitive diagnostic tests. Although a basal cortisol value >15 μg/dL normally suggests an intact HPA axis, low basal levels cannot be relied upon to exclude the diagnosis. Therefore, in practice, all patients suspected of having adrenal insufficiency should have an ACTH stimulation test [or Short Syntachten Test (SST)], which involves the administration of tetracosactin (which contains the first 24 amino acids of ACTH, and has the same physiologic properties) 250 μg IV or IM (Figure 97.10). Plasma cortisol levels are measured at 0 and 30 minutes after tetracosactin administration, and a normal response is defined by a peak plasma cortisol level >20 μg/dL. Incremental responses (i.e. the difference between peak and basal values) are of limited value other than determining the level of relative adrenal insufficiency. Response is unaffected by the time of day tested, and the test can still be performed in patients currently receiving corticosteroid replacement therapy, provided that the agent used is not measurable by that particular laboratory method, and the treatment is of short duration.

If necessary, there are several dynamic methods of differentiating primary from second hypoadrenalism. The first is the prolonged ACTH stimulation test (intravenous infusion of cosyntropin over

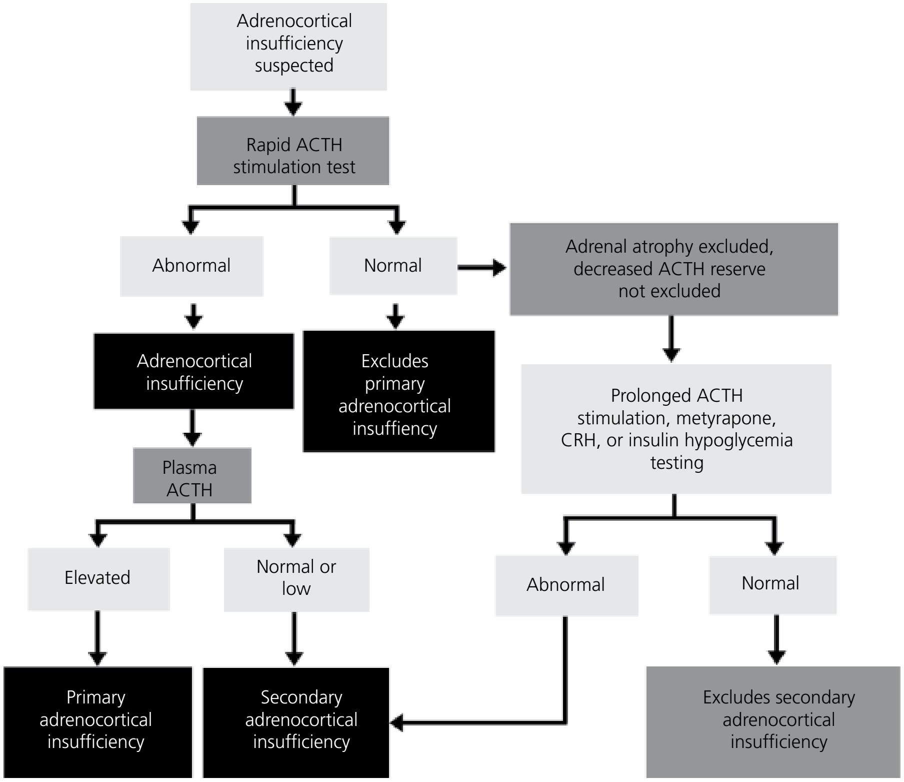
Adrenocortical insufficiency suspected
Rapid ACTH stimulation test
Abnormal
Normal
Adrenal atrophy excluded, decreased ACTH reserve not excluded
Adrenocortical insufficiency
Excludes primary adrenocortical insuffiency
Prolonged ACTH stimulation, metyrapone, CRH, or insulin hypoglycemia testing
Plasma ACTH
Elevated
Normal or low
Abnormal
Normal
Primary adrenocortical insufficiency
Secondary adrenocortical insufficiency
Excludes secondary adrenocortical insufficiency

Figure 97.10 Algorithm for evaluation of the patient with suspected primary or secondary adrenocortical insufficiency following HCT. ACTH, adrenocorticotropic hormone. Source: adapted from Aron et al. 2001 [79].

8 hours or over 24 hours on two or three consecutive days). In normal subjects, plasma cortisol will be >36 μg/dL after 4 hours of continuous infusion of cosyntropin, with no further increases observed over the remaining course of infusion. In primary adrenal insufficiency, no change is seen in cortisol or 17-hydroxycorticosteroid concentrations after prolonged ACTH stimulation. In secondary adrenal insufficiency, an incremental increase occurs during the course of the infusion, with much higher values observed at 24 and 48 hours than at 4 hours.

Many factors complicate the investigation of the HPA axis during critical illness, however. Cortisol levels vary greatly according to disease severity, and it may be difficult to define responses to testing. Decreases in corticosteroid-binding globulin (CBG) in the face of severe disease will alter the free to bound ratios of serum cortisol, further altering the results of testing. Some evidence suggests that a random cortisol value of <15 μg/dL is suggestive of corticosteroid insufficiency, whereas a level greater than 33 μg/dL would be unlikely to occur with compromised HPA axis function [78].

Management of adrenal dysfunction post-HCT

Clinically significant suppression of the HPA axis rarely occurs in patients who have taken steroid doses for less than 3 weeks' duration, and withdrawal of the steroids rarely causes any ill effects. HPA-axis suppression is inevitable, however, in patients taking the equivalent of 15 mg/day or more of prednisolone over the long term. Variable suppression of the HPA axis is seen with doses of prednisolone between 5 and 15 mg/day. To avoid the precipitation of adrenal failure, steroids should be withdrawn cautiously over a period of months (Figure 97.11).

All patients receiving long-term corticosteroid therapy should be treated similarly to patients with chronic ACTH deficiency. The aim is to give replacement doses of hydrocortisone to mimic normal cortisol secretion rates. Most patients are given hydrocortisone 15–25 mg/day in two doses, usually two-thirds in the morning and one-third in the evening. In the event of concurrent stressors, such as infection or surgery, supplemental steroid cover should be given, equivalent to 100–150 mg/day of hydrocortisone. Parental therapy may be required if the patient is unable to consume oral medications. In primary adrenal failure, mineralocorticoids also require replacement in the form of fludrocortisone 0.05–0.2 mg/day.

Administration of DHEA, normally secreted in large amounts by the adrenal glands, has been reported to have positive effects on the wellbeing and sexuality of women with adrenal insufficiency [80]. DHEA also increases bone density, muscle strength, and the sense of wellbeing in normal elderly men and women [81,82]. Prolonged high-dose glucocorticoid therapy may be a possible indication for DHEA therapy, especially in diseases that bear a high risk of osteoporosis [83].

Gonadal dysfunction

The provision of good quality of life in addition to the treatment of the primary disease has become an increasingly important goal, as life expectancy post-HCT continues to expand. Although the majority of young patients who have not yet had children themselves are concerned with subsequent fertility, a retrospective study conducted by the European Group for Blood and Marrow Transplantation (EGBMT), however, determined that the overall post-transplantation pregnancy rate is as low as 0.6% [84], indicating that HCT survivors rarely become parents [85]. Therefore, the high incidence of irreversible gonadal dysfunction observed after

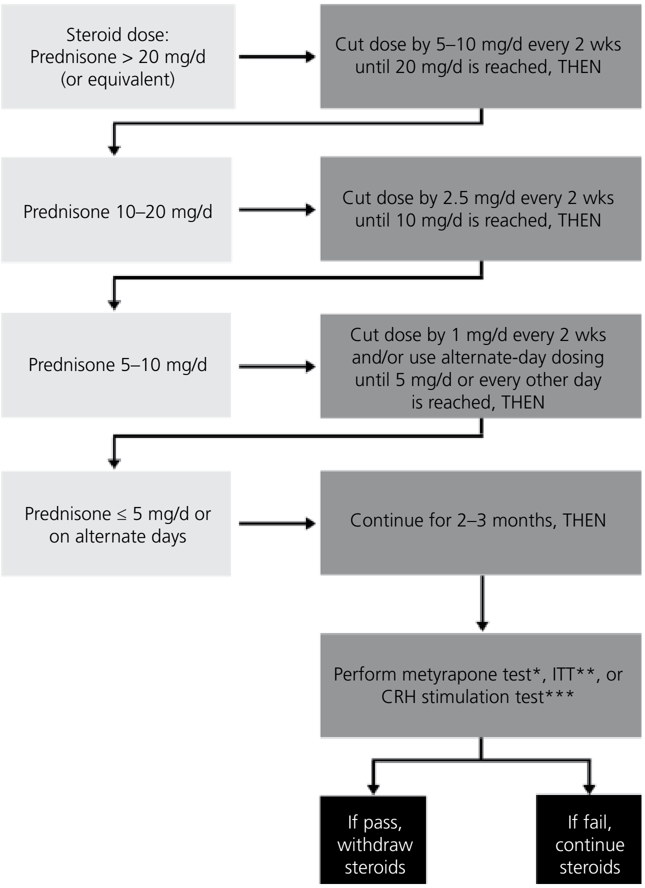
Steroid dose:
Prednisone > 20 mg/d
(or equivalent)
Cut dose by 5–10 mg/d every 2 wks until 20 mg/d is reached, THEN
Prednisone 10–20 mg/d
Cut dose by 2.5 mg/d every 2 wks until 10 mg/d is reached, THEN
Prednisone 5–10 mg/d
Cut dose by 1 mg/d every 2 wks and/or use alternate-day dosing until 5 mg/d or every other day is reached, THEN
Prednisone ≤ 5 mg/d or on alternate days
Continue for 2–3 months, THEN
Perform metyrapone test*, ITT**, or CRH stimulation test***
If pass, withdraw steroids
If fail, continue steroids

Figure 97.11 Algorithm for steroid withdrawal for patients on glucocorticoid therapy of greater than 3 weeks' duration. Relative steroid potency (equivalence): 1 mg prednisone (or prednisolone) ≈ 4 mg cortisol or 5 mg cortisone; 1 mg dexamethasone ≈ 8–10 mg prednisone or 30–35 mg cortisol. *Overnight metyrapone test: 2–3 g (30 mg/kg) at bedtime, measure levels of 11-deoxycortisol, cortisol, and ACTH the following morning. Normal response: 11-deoxycortisol >7 μg/dL, ACTH >250 pg/mL; may compare cortisol level with baseline on previous day (expect inhibition). **ITT = insulin-induced hypoglycemia test: regular insulin 0.05–0.15 U/kg, intravenous push. Measure levels of ACTH and cortisol at 30-minute intervals for up to 2 hours. Physician must supervise. Normal response: cortisol >20 μg/dL, ACTH >250 pg/mL. ***CRH test = corticotropin-releasing hormone test: bovine or synthetic human CRH, 100 μg intravenous push. Measure ACTH and cortisol every 15–30 minutes up to 2 hours. Normal response: cortisol >13 μg/dL, ACTH peak level 10–120 pg/mL. Vasopressin may be administered to potentiate response to CRH.

Table 97.7 Risk factors for gonadal dysfunction following HCT

Female gender
Males undergoing allografted HCT
Older age at transplantation (particularly male patients, over the long term)
Use of TBI
Single-dose radiation
Cranial irradiation
Increased or cumulative doses of alkylating agents

allogeneic or autologous HCT has become a crucial issue for both male and female patients, as gonadal failure often results in incomplete sexual development at puberty (secondary sex characteristics), and a decrease in physical wellbeing, bone density, quality of life, and sterility and sexual dysfunction in adulthood.

Risk factors for the development of gonadal function post-HCT are given in Table 97.7, and the incidence of complications in Table 97.8.

Male

Most preparative conditioning regimens for HCT include high-dose combination alkylating agents and TBI, which are well known to induce gonadal dysfunction directly – namely, primary testicular failure (hypergonadotrophic hypogonadism), with a 50% reduction in testicular volume, severe germ cell injury, and even Leydig cell insufficiency [85]. Interestingly, Leydig cells are more resistant to both the toxic effects of chemotherapy and radiation (up to 24 Gy) than the germinal epithelium [86], which is completely destroyed at radiation doses of 8 Gy [87]. Although less common, gonadal dysfunction may also be initiated by central damage to the hypothalamic–pituitary region as a result of cranial irradiation, which causes impaired gonadotropin secretion; in children, this can lead to delayed onset of puberty, although premature sexual development may also occur in some cases. Hypopituitarism or hyperprolactinemia with subsequent hypogonadism has been reported in recipients of TBI, again reflecting radiation-induced hypothalamic defects. Although in one study the overall incidence of azoospermia after HCT was found to be 70.3%, the highest incidence of azoospermia following HCT was observed in patients prepared with cyclophosphamide (CY) plus TBI or thoracoabdominal irradiation (TAI) (85.4%), and recovery of spermatogenesis never occurred before the fourth year post-transplant, and as late as nine years [88].

Chemotherapy alone can also produce gonadal failure [37], as high-dose chemotherapy invariably provides direct insults to both germ cells and Leydig cells, with concomitant elevation in gonadotrophins. Further, a transient increase in sperm aneuploidy within 100 days after chemotherapy has been documented [88]. Previous administration of chemotherapy also has a cumulative effect on gonadal toxicity. Even monotherapy using a non-high-dose protocol, such as fludarabine alone, does not spare the gonads [85], although melphalan-based protocols are less gonadotoxic than TBI-based, BU–CY, or CY-alone regimens. Other drugs associated with germinal depletion include chlorambucil, nitrogen mustard, procarbazine, and nitrosoureas. Recovery of fertility, however, was found to be more likely after chemotherapy-alone conditioning regimens than after TBI-containing regimens [89], as recovery was seen in 90% of patients conditioned with CY alone, in 50% of patients with CY plus BU or thioTEPA, and in just 17% of patients with CY plus TBI or TAI (Figure 97.12).

Further, sperm quality was found to be within the normal range in the majority of patients following CY, whereas it was consistently severely impaired in patients who received irradiation or two alkylating agents [87]. Temporary falls in testosterone levels in the first 6 months post-transplant are thought to be insignificant.

A widely utilized non-TBI regimen prior to allografting is busulfan–cyclophosphamide (BU–CY), but when compared with BEAM (carmustine, etoposide, cytarabine, melphalan) and BECYM (carmustine, etoposide, cyclophosphamide, melphalan), it also appears to be the most toxic. Current recommendations after BU–CY have been to advise sexually active heterosexual male patients that spermatogenesis and fertility may occur within 2–5 years post-transplant; however, sperm cryopreservation prior to transplant is still recommended. In the minority of patients who remained persistently azoospermic at 5 years, semen analysis should be offered twice per year to detect late recovery.

In addition to the type of pre-transplant therapies employed, the degree of gonadal dysfunction after HCT is dependent on

Table 97.8 Gonadal function in male and female patients following HCT

	Females		Males	
	Auto (*n* = 22)	**Allo/GVHD (*n* = 19)**	**Auto (*n* = 6)**	**Allo/GVHD (*n* = 25)**
Ovarian failure	21 (95%)	19 (100%)		
Clinical hypergonadotropic hypogonadism			1 (17%)	5 (20%)
Subclinical hypogonadism			1 (17%)	9 (36%)
Seminiferous tubule damage (elevated FSH)			3 (50%)	18 (72%)

Allo, allogeneic bone marrow/peripheral blood stem cell transplantation; Auto, autologous bone marrow/peripheral blood stem cell transplantation; FSH, follicle-stimulating hormone. Source: adapted from Somali et al. 2005 [86].

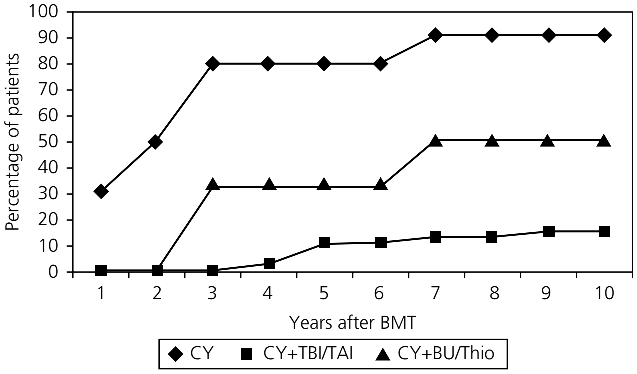

Figure 97.12 Recovery of spermatogenesis after bone marrow transplantation. Percentage of patients according to the conditioning regimen. CY,= cyclophosphamide; CY + Bu/Thio, cyclophosphamide plus busulfan or thioTEPA; CY + TBI/TAI = cyclophosphamide plus total body irradiation or thoracoabdominal irradiation. Source: Anserini et al. 2002 [88]. Reproduced with permission of Nature Publishing Group.

patient-related factors. For example, older age at transplantation appears to worsen gonadal outcome after HCT in men; however, the majority of published studies describing the impact of high-dose chemotherapy plus TBI on gonadal function have evaluated patients who received transplants as adolescents or young adults. Although the data on gonadal function after HCT performed in childhood remain limited, most reports suggest that prepubertal boys experience spontaneous, normal puberty and sexual development, and further demonstrate age-appropriate plasma testosterone levels following HCT, indicating that younger male patient age at transplantation exerts a protective effect; some reports, however, showed impaired Leydig cell function in approximately half of boys who underwent HCT prior to puberty. The reasons for these differing results remain unclear, but could be related to variations in exposure to alkylating agents, and the total dose and/or fractionation schedule of irradiation. Other studies have suggested that the young Leydig cell, may have increased vulnerability to radiation-related damage, as evidenced by elevated luteinizing hormone (LH) levels, although overt Leydig cell failure was rare [87].

Germ cell dysfunction, as indicated by elevated follicle-stimulating hormone (FSH) levels, however, is more common [87], given the known sensitivity of the germinal epithelium to chemotherapy and radiation.

Long-term gonadotropin elevation after HCT despite prepubertal age at transplantation was thought to reflect a probability of future gonadal dysfunction (i.e. oligospermia and infertility) [87]; however, further studies are needed to support this conclusion.

The type of HCT itself also seems to exert an influence on gonadal outcome, since a higher incidence of gonadal toxicity (such as elevated FSH, indicative of seminiferous tubule damage, and elevated LH, indicative of Leydig cell damage) is more frequently seen in allogeneic than autologous transplantation.

The degree to which GVHD may contribute to or sustain gonadal toxicity independent of conditioning regimen remains to be evaluated, as sperm counts in long-term HCT survivors tend to be lower in GVHD patients. It has been suggested that intratesticular damage may occur during an acute graft-versus-host reaction due to infiltration of alloreactive donor T cells and autoantibody production against germinal epithelium. Evidence to support these hypotheses, however, is currently lacking. Additional studies are needed to clarify the role of GVHD in the development of gonadal dysfunction after HCT.

Evaluation of male gonadal dysfunction post-HCT

Semen analysis currently remains the only reliable method of assessing spermatogenesis.

In adult men, the most common symptoms of hypogonadism are diminished libido and erectile dysfunction (ED). Gynecomastia may also be a presenting feature; however, estimates of the incidence of gynecomastia in the general population are variable, and the incidence in the HCT setting is unknown. Primary testicular failure with hypergonadotrophic hypogonadism is likely the key cause of gynecomastia, but additional etiologies include secondary hypogonadism with hyperprolactinemia and drug-induced gynecomastia. For example, histamine (H2) blockers, such as cimetidine and ranitidine, are noted to be associated with sexual dysfunction and gynecomastia. Cyclosporine can cause transient breast enlargement through altered target organ sensitivity, but if present, it tends to be concurrent with hypertrichosis (not hirsutism). Although admittedly uncommon in recipients of high-dose chemotherapy or chemoradiotherapy, the occurrence of gynecomastia should be considered pathologic outside of newborns, puberty, and old age.

In general, gonadal testing and consideration for treatment are recommended based on symptoms (Figure 97.13). A male fertility evaluation should include a careful history and physical examination, and also a minimum of two semen analyses, which are recommended at the time of presentation, even if there has been less than 1 year of unprotected intercourse—particularly in the case of advanced female partner age (greater than 35 years), or the presence of known male risk factors (such as exposure to chemotherapy or radiation). Semen analysis is a common, convenient measure of assessing the male, and should precede any invasive tests of the female. Ejaculate volumes between 2 and 6 mL and sperm concentrations of greater than 15–20 million/mL with more than 50% motile sperm and more than 50% oval forms are considered normal.

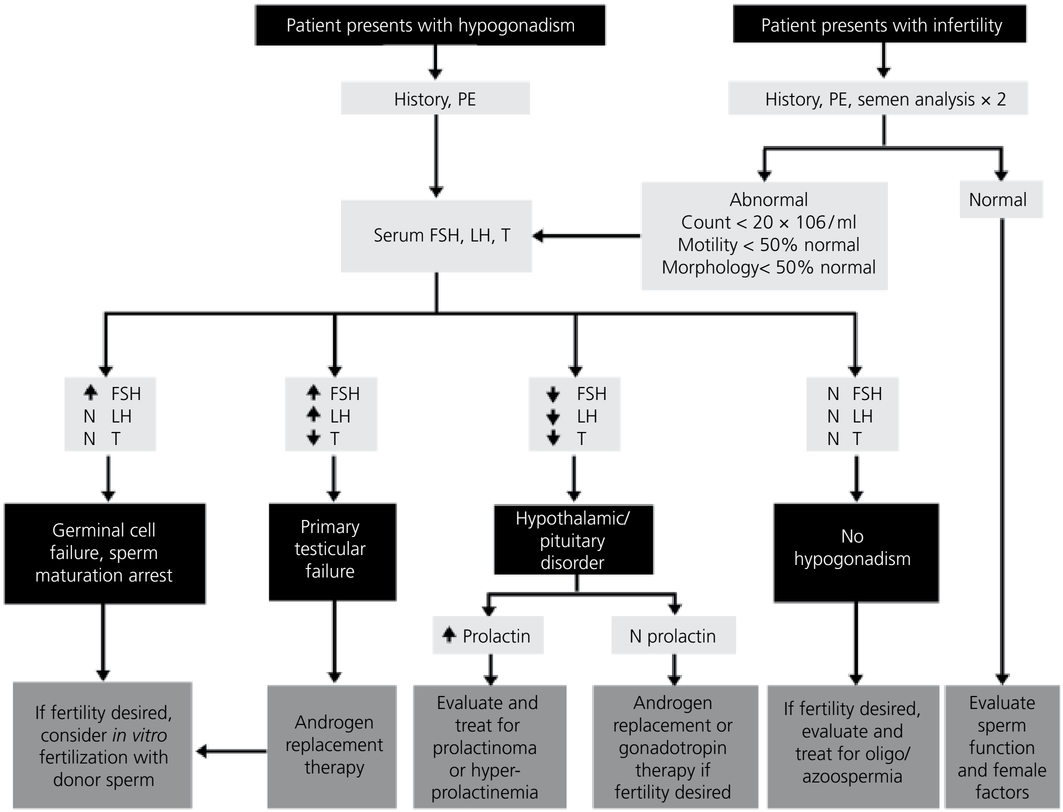
Patient presents with hypogonadism
Patient presents with infertility
History, PE
History, PE, semen analysis × 2
Abnormal
Count < 20 × 106/ml
Motility < 50% normal
Morphology< 50% normal
Normal
Serum FSH, LH, T
↑ FSH N LH N T
↑ FSH ↑ LH ↓ T
↓ FSH ↓ LH ↓ T
N FSH N LH N T
Germinal cell failure, sperm maturation arrest
Primary testicular failure
Hypothalamic/ pituitary disorder
No hypogonadism
↑ Prolactin
N prolactin
If fertility desired, consider in vitro fertilization with donor sperm
Androgen replacement therapy
Evaluate and treat for prolactinoma or hyper-prolactinemia
Androgen replacement or gonadotropin therapy if fertility desired
If fertility desired, evaluate and treat for oligo/ azoospermia
Evaluate sperm function and female factors

Figure 97.13 Algorithm for the evaluation of the male patient presenting with hypogonadal symptoms and/or infertility following HCT. FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; N, normal; PE, physical examination; T, testosterone.
Source: Adapted from Anunta and Kandeel FR 2007 [90].

An initial endocrine evaluation should include at least a plasma testosterone (T) and FSH, but frequently, LH is also included. This should be performed if there is (1) an abnormally low sperm concentration, especially if less than 10 million/mL, (2) impaired sexual function, or (3) other clinical findings suggestive of hypogonadism. A reduction in testosterone levels is usually a reflection of lower testosterone secretion by the testis. Elevated FSH typically indicates significant seminiferous tubule damage, and elevated LH indicates Leydig cell damage. If these hormone measurements are borderline, and the patient has clinical symptoms or signs of hypogonadism, then a free or bioavailable testosterone can be measured to exclude patients with low free T and elevated sex hormone-binding globulin (SHBG) as in elderly men.

Management of male gonadal dysfunction post-HCT

In its simplest form, the management of gonadal dysfunction following HCT should be directed at preventing potential damage by reducing injury wherever possible, such as with the use of less toxic conditioning regimens. TBI and alkylating agents should be avoided if possible.

Patients should be counseled prior to transplant to discuss possible and appropriate strategies to preserve fertility, including cryopreservation of sperm.

Failing preventive measures, hormone replacement therapy (HRT) may be offered to men to relieve some of the side effects of hypogonadism, and also for the preservation of bone mass density. In children, there are two phases to this treatment: a low dose to accelerate height growth during the growing period, without excessive bone-age progression, and then increasing the dose to the adult level at the end of the growth spurt. Sex steroid replacement therapy should be stopped for 2 months at regular intervals in order to check for gonadal recovery. Annual long-term follow-up is necessary, even for those few patients with normal hormonal basal values after HCT, owing to the possibility of future gonadal dysfunction due to diminished gonadal reserve.

The risks of HRT in men include prostate hypertrophy and prostate carcinoma. Side effects of treatment include edema, male pattern baldness, nausea, tachycardia, low HDL cholesterol, and cholestatic liver enzyme abnormalities. Follow-up evaluations should include annual digital rectal exams and measurements of prostate-specific antigen (PSA), liver function, lipid profile, and hemoglobin.

Female

Although the risk of gonadal failure is high in all individuals who undergo HCT, women generally experience higher rates of failure than do men; radiation doses as small as 4 Gy have been observed to destroy approximately 50% of oocytes [91]. As with men, women have a high risk of developing hypergonadotropic hypogonadism after HCT, but the risk of irreversible gonadal toxicity after BU–CY, with no resumption of menses, is almost inevitable in women.

In contrast, there is a higher incidence of fertility recovery in females than in males after TBI, BEAM, cyclophosphamide, BCNU, and etoposide (CBV), and high-dose melphalan. The greatest chance of recovery was observed in cases of non-high-dose therapy, e.g. mustine, vincristine, procarbazine, prednisone, or MOPP [mustargon, Oncovin (vincristine), prednisone, procarbazine], for Hodgkin disease. Similarly, the risk of permanent infertility becomes lower if cyclophosphamide is used alone.

In general, fractionation of radiation reduces the risk of infertility compared with single-dose radiation. Over the long term, when the use of fractionated TBI was considered alone, one study found that the ovaries are actually less sensitive to radiation than the testes are, with only 37% of females demonstrating permanent ovarian dysfunction more than 10 years after TBI administration, compared with 87% of males with at least indirect signs of germinal dysfunction [92].

Older age at HCT is associated with nearly 100% ovarian failure, likely due to the decreased number of primordial follicles. In general, endocrine ovarian failure is irreversible in adult women, but younger women – particularly prepubescent girls treated with chemotherapy and hyperfractionated TBI – have a better opportunity for recovery of gonadal function, and about half may experience spontaneous puberty and menarche. Whereas doses of 600 cGy may produce irreversible ovarian failure in women older than 40 years of age, doses in excess of 2000 cGy have to be given before the same result is seen in girls treated during childhood and adolescence. The parameter of age, however, seems to be less significant in female patients over the long term, as follow-up beyond 10 years has demonstrated a similar probability of ovarian dysfunction in females who were administered TBI prior to puberty as well as post-menarche [36]. Faraci et al. documented the development of amenorrhea in 100% of females receiving TBI at some time after puberty [36]. Some patients were able to recover from the injury, sometimes more than 10 years after TBI; this has been corroborated by other studies that demonstrated recovery of ovarian function in 10–25% of young women after TBI. Regardless of recovery, however, a higher risk of earlier menopause has been suggested with exposure to both alkylating agents and radiation below the diaphragm, even in girls treated during a prepubertal phase. It is unclear what allows some patients to remain fertile when so many other patients become sterile under similar conditions.

Shielding of the gonads is generally not performed after HCT, since the gonads often serve as sanctuaries for disease, particularly in lymphoid disorders such as ALL. Even if shielding is employed, it is less successful in preserving female fertility than male fertility. No effective means of protecting the ovaries during high-dose chemoradiotherapy has yet been developed.

In conclusion, larger studies are required in order to evaluate the incidence of recovery of fertility in females after various conditioning regimens and the effects of variables such as prior treatment and age at transplantation. Until these results become available, it is recommended that post-transplant ovarian function be evaluated in all premenopausal women prior to transplant, particularly if BU–CY will be used. Counseling regarding fertility preservation prior to transplant is necessary; options may include cryopreservation of fertilized embryos, ovarian tissue, and oocytes.

Fertility and offspring

Many animal studies have investigated the possible adverse effects of radiation and chemotherapy on the offspring of treated patients [88]. The risk of genotoxicity is more significant in cases when assisted reproductive technology (ART) with intracytoplasmic sperm injection (ICSI) is used. Although the incidence of congenital anomalies recorded in live-born infants spontaneously conceived after treatment for lymphohematologic malignancies is comparable to that reported in the general population [93], possible long-term radiation-related genetic damage of the spermatozoa and oocytes cannot be excluded [88]. Long-term follow-up of both pregnancies and children of HCT survivors is needed, particularly with regard to the occurrence of miscarriages, low birth weights, congenital malformations, and delayed developmental milestones [93], as the evidence seems to be conflicting. Holm et al. [94] were the first to report a reduction in both uterine and ovarian size shortly after TBI and HCT administered in female children, despite HRTy or spontaneous pubertal development, citing that prescribed doses of HRT, although sufficient to induce bleeding and suppress the onset of premature menopause, were not adequate to generate normal uterine growth; this is significant, as these smaller, irradiated uteri may not be able to grow and expand as required during pregnancy. Further, decreased ovarian volume was found to be reflective of decreased markers of ovarian follicles compared with ovarian follicles found in healthy females entering puberty; small ovaries have been associated with poor response to IVF.

Although there is a theoretical risk of immunocompromise and relapse, the fact of pregnancy is unlikely to alter the history of the primary disease, and is therefore not contraindicated in SCT recipients for hematologic, endometrial, or even breast cancers; however, scattered case reports have shown evidence of leukemic relapse during pregnancy, although it is unknown to what extent these patients were already at risk for recurrence independent of the pregnancy. Nevertheless, it is recommended that patients with CML should delay pregnancy for at least 2 years after HCT, provided that they remain Philadelphia chromosome- and bcr/abl transcriptase-negative.

Evaluation of female gonadal dysfunction post-HCT

Failure to progress through puberty in a timely fashion should prompt the referral for a full endocrinologic evaluation. Prepubertal girls should be monitored closely for the onset of puberty and gonadal dysfunction suspected if not experienced by the age of 12–13 years. In contrast, the association between premature sexual development and both low- and high-dose CNS irradiation in both boys and girls is now well established.

In adult women, the assessment of gonadal function after HCT is difficult, as the ovaries and ova are not easily accessible for study, and E2 levels are highly variable across individuals and even within the same individual. The implicit markers of female fertility are regular menses and the ability to become pregnant; however, the initial investigation should start by ruling out thyroid hormone abnormalities and the possibility of current early pregnancy. Once this has been achieved, then the pursuit of further evaluations will depend upon whether the patient is presenting with complaints of infertility or with oligomenorrhea and symptoms of estrogen deficiency (Figure 97.14). Patients presenting with infertility should be evaluated with measurement of serum inhibin B during the mid-follicular phase (days 5–8) and/or progesterone during the mid-luteal phase (days 19–22) of the menstrual cycle. Low inhibin B levels suggest poor ovarian follicular reserve, and low progesterone levels could indicate poor luteal phase function. Such subjects and those with oligomenorrhea should be further evaluated with measurements of serum E2, FSH, and prolactin. Ovarian failure is clinically represented by amenorrhea and menopausal symptoms, and also low levels of serum estrogen and high levels of serum FSH and normal or low levels of prolactin. In women presenting with decreased sexual libido (hypoactive sexual desire), serum levels of testosterone and DHEA should also be measured. Reduced serum levels of total and/or free testosterone may be associated with

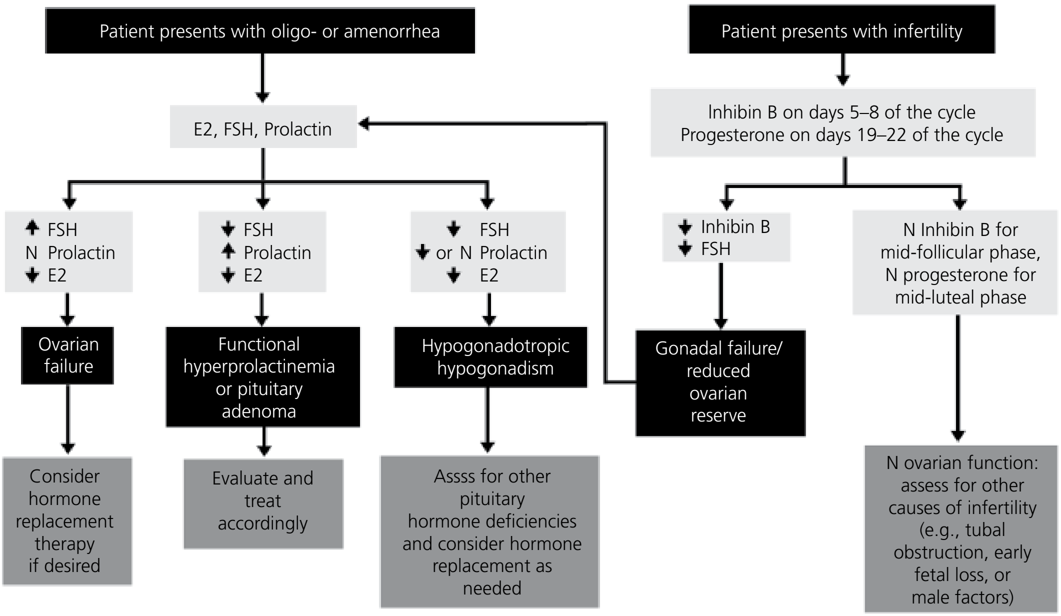
Patient presents with oligo- or amenorrhea
E2, FSH, Prolactin
↑ FSH
N Prolactin
↓ E2
↓ FSH
↑ Prolactin
↓ E2
↓ FSH
↓ or N Prolactin
↓ E2
Ovarian failure
Functional hyperprolactinemia or pituitary adenoma
Hypogonadotropic hypogonadism
Consider hormone replacement therapy if desired
Evaluate and treat accordingly
Assss for other pituitary hormone deficiencies and consider hormone replacement as needed
Patient presents with infertility
Inhibin B on days 5–8 of the cycle
Progesterone on days 19–22 of the cycle
↓ Inhibin B
↓ FSH
N Inhibin B for mid-follicular phase, N progesterone for mid-luteal phase
Gonadal failure/ reduced ovarian reserve
N ovarian function: assess for other causes of infertility (e.g., tubal obstruction, early fetal loss, or male factors)

Figure 97.14 Algorithm for the evaluation of the female patient presenting with hypogonadal symptoms and/or infertility following HCT. E2,= estradiol; FSH, follicle-stimulating hormone; N, normal.

ovarian failure and, in some cases, reduced DHEA levels may reflect adrenal insufficiency.

In vitro fertilization (IVF) using superovulation and embryo cryopreservation is a common treatment for infertility in female HCT recipients, as it has the most promising results, despite some limitations: requirements include the presence of a functional ovary and the time to complete the necessary number of IVF cycles to harvest sufficient eggs prior to the commencement of the cytotoxic regimen, which may be difficult to achieve in some patients without delaying life-saving treatment. Oocyte cryopreservation and gonadotropin-releasing hormone (GnRH) treatment for the suppression of the pituitary–gonadal axis remain more experimental treatments, and further studies are needed to ascertain their usefulness.

Management of female gonadal dysfunction post-HCT

As with men, the management of gonadal dysfunction following HCT in women should be aimed at preventing potential damage by reducing injury wherever possible, such as with the use of less toxic conditioning regimens. TBI and alkylating agents should be avoided if possible.

Patients should be counseled prior to transplant to discuss possible and appropriate strategies to preserve fertility, including cryopreservation of oocytes, ovaries, and embryos, and the use of a surrogate uterus.

As with men, HRT may be offered to females to relieve some of the side effects of hypogonadism, and also for the preservation of bone mass density. The phases of HRT in girls follow those of young boys, described above. In girls, however, the latter phase is given cyclically in order to induce regular menstruation. HRT may be given to stimulate normal uterine growth. Sex steroid replacement therapy should be stopped for 2 months at regular intervals in order to check for gonadal recovery. Annual long-term follow-up is necessary, even for those few patients with normal hormonal basal values after HCT, owing to the possibility of future gonadal dysfunction due to diminished gonadal reserve.

Sexual dysfunction

Although sexual dysfunction may be considered a secondary issue in comparison with the primary malignant disease, sexual activity is a major contributor to quality of life in these patients. HCT patients have been found to be similar to conventional chemotherapy patients with regard to psychosexual functioning, and both groups exhibited significant differences in all aspects of sexuality compared with healthy control subjects – particularly with regard to body image [95]. When given the opportunity, 35% of the sample detailed negative changes in sexual function after transplantation [96]. Wingard et al. reported that 22% of male HCT survivors were dissatisfied with their sexual lives, with 24% reporting ED and 13% ejaculatory dysfunction [97], whereas Molassiotis et al. noted that half of patients were sexually dissatisfied to some extent [98]. Across both sexes, lack of sexual interest is reported at 3–11%, arousal problems 9–15%, and orgasm infrequency or inability 4–12% [96]. Unmarried and single patients seem to be at greatest risk for sexual dissatisfaction [98], and women appear to be affected more frequently than men [99], with 17–35% reporting sexual problems [96].

Sexual difficulties in HCT survivors typically have acute onset, and occur immediately after therapy or during the recovery process as a result of physical gonadal dysfunction due to treatment with TBI and alkylating agents. Adrenal suppression by corticosteroids may also cause a reduction in DHEA levels. Sexuality may be further influenced by the occurrence of fatigue, decreased stamina, alopecia, skin changes due to GVHD, weight changes, anxiety, depression, cognitive interference, and other social changes. Other stress factors, such as financial, familial, or occupational concerns, may further contribute to sexual dysfunction.

Male

As noted above, male HCT survivors are typically less affected with regard to sexual function than female HCT survivors, and if affected, their problems appear to resolve spontaneously. One study noted that men who had no known sexual complaints prior to transplantation who did report sexual dysfunction at 1 year after transplant were found to be problem-free 3 years after transplant [96]. Only 15% of HCT patients in this study reported arousal problems prior to transplantation.

Evaluation of men with sexual dysfunction post-HCT

There are many reasons why HCT in particular may cause sexual dysfunction in male patients. The underlying hematologic disease (e.g. sickle cell anemia) may cause local penile abnormalities and subsequent ED (sickle cell), as can microvascular disease from treatment-associated complications, such as hyperlipidemia. Neuropathy as the result of TBI and chemotherapy or the development of diabetes may additionally cause sexual dysfunction. Glucocorticoid treatment may cause weakness and fatigue, and many other HCT-related pharmaceutical agents are known to cause sexual dysfunction. The development of psychologic issues related to self-esteem that are specific to HCT may precipitate dissatisfaction with one's sexual prowess and create partner discord, including despair regarding the underlying disease and its treatment, a resultant loss of work and livelihood, possible disfigurement due to GVHD of the skin, the loss of body/muscular mass, and the occurrence of new and unpleasant body odor related to medications.

Decreased libido should alert the clinician to three probable causes: endocrinopathy, affective disorder, or relationship discord. A history of frequent strong erections under any circumstances (during foreplay, fantasy or masturbation, with another partner, or upon awakening) likely indicates that the endocrine, vascular, and neurologic systems are intact, and that the ED is predominantly psychogenic. Conversely, historical data relating the presence of decreased erectile turgidity during non-coital activities are highly suggestive of an organic etiology. A report of firm sustained erections during foreplay that are lost after intromission or upon initiation of pelvic movements, however, might suggest either a psychogenic etiology or a vascular problem (pelvic steal syndrome). A history of delayed or retrograde ejaculation may imply a neuropathy or an adverse drug effect. Premature ejaculation, on the other hand, is more compatible with a psychogenic dysfunction. Finally, it must be remembered that the absence of orgasmic sensations in patients with normal erectile and ejaculatory functions is almost always due to psychogenic etiology, whereas failure of detumescence is usually organic in nature; the latter should direct investigations towards ruling out local penile etiologies (neurologic or hematologic) [100].

During the physical examination, every effort should be made to elicit physical signs of pathology that may be suspected based on information obtained during the clinical history. Evidence of chronic, systemic diseases (hepatic, renal, cardiovascular, endocrine, granulomatous, neoplastic) must be ruled out, and if present, the current state of disease control should be determined. In addition to the general and systemic evaluations, a detailed assessment of gonadal function, vascular competence, neurologic integrity, and genital organ normalcy should be performed on every patient.

Laboratory testing should focus on the endocrine aspects of male sexual dysfunction (i.e. the state of gonadal hormones and thyroid hormones), as these are particularly vulnerable to derangement after HCT (see Figure 97.13). Aging, growth hormone deficiency, hyperthyroidism, and liver disease can all be associated with an increase in SHBG, and consequently a greater reduction in bioavailable than in total testosterone. On the other hand, obesity, hyperinsulinemia, hypercortisolemia, and hypothyroidism may be associated with decreased SHBG and thus more suppression in total testosterone than in bioavailable or testosterone [100].

Management of men with sexual dysfunction post-HCT

As sexual dysfunction after HCT is frequently multifactorial in origin, these different etiologies should be distinguished, and treatment should given to address each of the underlying problems accordingly.

If hypogonadism is the cause, hormone replacement should be given (see the previous section). Androgen replacement therapy is usually not required with sexual dysfunction associated with normal bioavailable or free but depressed total testosterone. Phosphodiesterase-5 (PDE-5) inhibitors have been found to be very effective for men with ED in the general population. Several non-surgical treatment options for ED have proven to be safe and efficacious, regardless of disease etiology, and may prescribed in the primary care setting (e.g. oral or intraurethral medications and vacuum constriction devices) [101]. Antioxidant agents such as vitamin E, Korean red ginseng, and α-lipoic acid (ALA) have shown promise in animal studies, but human research is needed.

Psychiatric/psychosocial support may improve a patient's situation and allow for a better quality of life. Unmarried and single HCT survivors should be targeted for early prevention or therapy. Appropriate intervention will involve identifying and addressing all relevant predisposing, precipitating, and perpetuating factors, and thus evidence for the presence of any of these psychologic or situational conditions should be carefully assessed. Moreover, it should not be forgotten that the presence of an organic dysfunction does not preclude the possibility of a coexisting psychogenic factor, and vice versa. Such omissions could lead to diagnostic difficulties, and also to therapeutic failures.

Female

Sexual dysfunction in women treated with HCT may predate their treatment or occur de novo following therapy. In women without sexual problems prior to transplantation, up to 40% developed lubrication difficulty, problems achieving orgasm, and painful intercourse. Dysfunction is often pervasive and severe, and the problems did not seem to improve over time, but rather seemed to consolidate and generalize to other aspects of sexual function [96]. Of women with arousal difficulty prior to transplantation, 71% had this problem after 3 years, whereas 42% without arousal problems prior to transplant had developed it by 3 years after transplant. Particularly for women, if sexual problems were not prevented or resolved at 1 year after transplant, there was an 87% probability that the problems would still be present at 3 years after transplant, despite HRT; however, a substantial number (63%) of women entered transplantation with one or more sexual problems [96]. After HCT, specific sexual complaints in female survivors include dyspareunia (2–8%) and insufficient vaginal lubrication. Prevalence rates for these particular problems vary widely in the general population, depending on the sample.

As with male patients, self-image issues that arise out of the fact of HCT and its treatment may contribute significantly to decreased psychologic and sexual wellbeing.

Evaluation of women with sexual dysfunction post-HCT

In women, the onset of early menopause has been noted as a major cause of sexual dysfunction, including symptoms of vasomotor instability, vaginitis, dry vaginal mucosa, dysphoria, osteoporosis, hot flashes, insomnia, irritability, decreased libido, and vaginal atrophy [96]. The evaluation of gonadal function in women presenting with menstrual irregularities with estrogen deficiency symptoms should be carried out as described in Figure 97.14.

Several methods of testing for female sexual dysfunction exist, including instruments to measure the level of vaginal arousal, lubrication, and blood flow, but the use of these modalities is mainly limited to the research setting.

Management of women with sexual dysfunction post-HCT

Although there are fewer available treatment options for female sexual dysfunction than for ED, for example, a report suggests that sildenafil (Viagra) may improve sexual arousal disorder in premenopausal women [102]. Management of lower urinary tract syndrome (LUTS), such as bladder control/continence and the prevention and treatment of infections, can also improve sexual function for both men and women.

The treatment for gonadal dysfunction and possible infertility post-HCT may have a large impact on patient sexual behavior. It is recommended that counseling regarding the potential for reduced sexual satisfaction and other sexual difficulties should occur prior to transplantation. Clinicians should be aware of the additional possibility of late-occurring problems with psychosexual maladjustment post-HCT. HRT should be offered, although, as noted above, libido is only partially corrected by standard doses of estrogen and progesterone in women; further studies are needed to address the true endocrinologic requirements of these patients.

The needs of women who cannot take HRT, or those who choose not to take HRT, must also be addressed. Many alternative therapies (such as botanical products) are popularly recommended, but controlled clinical trials on their efficacy are entirely lacking. Improvised vaginal lubricants have been offered as a possible treatment.

As with male HCT survivors, psychiatric/psychosocial counseling may significantly improve self-esteem and quality of life.

Conclusion

The widespread implementation of HCT and increasing long-term survival rates have warranted greater concern with regard to long-term quality of life for transplanted patients. Endocrine complications of HCT are often notoriously late in onset, even manifesting decades after the initial transplant. Dysfunction of the endocrine system can severely impact both the morbidity and mortality of HCT recipients. It is hoped that awareness of the various types of endocrine complications of HCT and regular monitoring of transplant survivors for the signs and symptoms of these problems will further improve the long-term quality of life for HCT recipients.

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CHAPTER 98

Common Potential Drug Interactions Following Hematopoietic Cell Transplantation

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Introduction

Drug interactions in hematopoietic cell transplantation (HCT) patients occur owing to the complexity of the medications employed, patients' comorbid conditions, and transplant complications. Often, the pharmacologic effectiveness or safety profile of one drug is altered by the concurrent administration of another drug, food, or herbal product. Pharmacokinetic, pharmacodynamic, and pharmaceutic interactions are possible.

Pharmacokinetic interactions occur when the addition of one drug leads to a change in the concentration of another. These may happen during the absorption, distribution, metabolism, and elimination phases of drug administration, with a majority of them occurring in the latter two phases [1]. Many factors affect a drug's pharmacokinetic profile, including route of administration, affinity for protein binding, and patient variables, such as age, ethnicity, and organ function. Pharmacodynamic interactions occur when two drugs of similar or opposing pharmacologic actions result in an altered (antagonistic, synergistic, or indirect) response by the body [2]. Pharmaceutic interactions occur as a result of chemical or physical incompatibilities [2]. Some pharmacokinetic interactions are beneficial whereas others are harmful. Knowledge of commonly encountered pharmacokinetic drug interactions is critical to mitigating any deleterious impact on HCT patients. These interactions, their management, and the pathways associated with them, such as the P-glycoprotein (P-gp), organic anion-transporting polypeptide (OATP), cytochrome P450 (CYP450) isoenzyme, and uridine 5′-diphosphate glucuronosyltransferase (UGT) systems are reviewed in this chapter. A brief discussion of the role of therapeutic drug monitoring (TDM) is also presented.

P-glycoprotein (P-gp) system

P-gp is an ATP-dependent plasma membrane transport system encoded by human multidrug resistance genes [1]. Originally discovered in cancer cells, P-gp is also found in normal human tissues, including the intestines, blood–brain barrier, placenta, liver, and kidney [3]. P-gp modulation plays an integral role in the overall systemic disposition of drugs. Drugs can be substrates, inhibitors, and inducers of P-gp. The net effect of P-gp-based interactions depends on the location of P-gp, the normal function of P-gp at that location, and a drug's overall effect or dependence on P-gp. In the gastrointestinal (GI) tract, blood–brain barrier, and placenta, P-gp normally functions as an efflux pump to limit drug entry, whereas in the liver and kidneys, it functions to promote drug removal [3]. P-gp-mediated drug interactions in the GI tract alter drug absorption whereas similar drug interactions in the liver and kidneys alter drug metabolism and excretion, thereby affecting the overall systemic concentration of drug in the bloodstream [4]. For example, with the administration of a P-gp substrate and inhibitor, systemic substrate concentrations are increased in the GI tract because normal P-gp function to limit cellular entry is inhibited whereas with P-gp inducers, systemic substrate concentrations are reduced since normal P-gp function to limit cellular entry is enhanced [4,5]. Similar outcomes in terms of systemic substrate concentrations are seen when enzyme inhibition or induction occurs in the liver and kidneys, whereby administration of P-gp inhibitors increases systemic bioavailability of P-gp substrates through decreased metabolism and reduced excretion whereas inducers do the opposite [5]. The extent of these interactions is unpredictable, especially when multiple P-gp substrates, inhibitors, or inducers are administered. Additionally, since drugs, especially those with extensive first-pass metabolism, must go through the liver prior to its entry into systemic blood circulation, they are further susceptible to the effects of other drugs that alter the enzymatic environment within the liver. This also applies to P-gp-mediated interactions since P-gp is more concentrated in the liver [6]. Commonly encountered P-gp substrates in HCT include fentanyl, morphine, ondansetron, phenytoin, and methylprednisolone. Azole antifungals, atorvastatin, and midazolam are examples of P-gp inhibitors and rifampin, phenytoin, and insulin are examples of P-gp inducers. Some drugs, such as amiodarone and sirolimus, can inhibit and induce P-gp. Erythromycin, calcineurin inhibitors, and diltiazem are P-gp substrates and can also inhibit and induce P-gp [5].

Organic Anion-Transporting Polypeptide (OATP) system

Like P-gp, OATP is a transport system found in the GI tract, brain, liver, and placenta [7]. In contrast, OATP is a drug influx pump, transporting drugs intracellularly. Human OATP consists of 11 members; the roles of OATP1B1, OATP1A2, OATP1B3, and OATP2B1 are best characterized. OATP1A2 facilitates drug entry from the duodenum into circulation whereas OATP1B1, OATP1B3, and OATP2B1 mediate the transport of drug from the circulation into hepatocytes for metabolism and elimination [8]. Modulation

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Table 98.1 Selected CYP isoenzymes and common inducers, inhibitors, and substrates [1,9,10]

Isoenzyme	Inducers	Inhibitors	Substrates
CYP1A2	Insulin, omeprazole, phenobarbital, phenytoin, rifampin, tobacco	Amiodarone, cimetidine, ciprofloxacin, erythromycin, fluoxetine, grapefruit juice, paroxetine, sertraline	Amitriptyline, caffeine, duloxetine, haloperidol, mirtazapine, ondansetron, propranolol, verapamil, (*R*)-warfarin
CYP2B6	Phenobarbital, rifampin	ThioTEPA	Bupropion
CYP2C9	Phenobarbital, rifampin	Amiodarone, fluconazole, fluoxetine, fluvastatin, isoniazid, itraconazole, ketoconazole, lovastatin, metronidazole, paroxetine, sertraline, trimethoprim, voriconazole	Fluoxetine, fluvastatin, NSAIDs, phenytoin, sertraline, voriconazole, (*S*)-warfarin
CYP2C19	Carbamazepine, norethindrone, phenobarbital, prednisone, rifampin	Cimetidine, fluoxetine, ketoconazole, lansoprazole, omeprazole, sertraline, voriconazole	Citalopram, cyclophosphamide, diazepam, escitalopram, omeprazole, phenobarbital, phenytoin, sertraline, voriconazole
CYP2D6	Dexamethasone, rifampin	Amiodarone, bupropion, cimetidine, citalopram, duloxetine, escitalopram, fluoxetine, haloperidol, metoclopramide, mirtazapine, paroxetine, sertraline, venlafaxine	Citalopram, duloxetine, escitalopram, fluoxetine, haloperidol, metoclopramide, metoprolol, mirtazapine, ondansetron, paroxetine, venlafaxine
CYP2E1	Ethanol, isoniazid		Acetaminophen, ethanol
CYP3A4/5	Barbiturates, carbamazepine, dexamethasone, phenobarbital, phenytoin, rifampin	Amiodarone, cimetidine, ciprofloxacin, clarithromycin, diltiazem, erythromycin, fluconazole, fluvoxamine, grapefruit juice, itraconazole, ketoconazole, verapamil, voriconazole	Alprazolam, amiodarone, amlodipine, atorvastatin, clarithromycin, cyclosporine, dasatinib, diazepam, diltiazem, erythromycin, escitalopram, ethinylestradiol, felodipine, fentanyl, imatinib, lovastatin, midazolam, mirtazapine, nifedipine, ondansetron, sertraline, simvastatin, sirolimus, tacrolimus, verapamil, venlafaxine, voriconazole

of the OATP system results in altered systemic drug bioavailability. For example, inhibition of OATP in the GI tract results in decreased systemic absorption whereas inhibition in the liver and kidneys results in decreased metabolism and excretion. Agents affecting P-gp also have similar effects on OATP. Some OATP inhibitors include grapefruit juice, cyclosporine, and rifampin. HMG-CoA reductase inhibitors (statins) and ACE inhibitors are examples of OATP substrates [7].

Cytochrome P450 (CYP450) isoenzyme system

The CYP450 isoenzyme system is a family of heme-containing enzymes responsible for phase I (the oxidative phase) of drug, endogenous steroids, and toxin metabolism [1,2]. These enzymes are found in the liver, lungs, kidneys, intestines, and brain [1]. Many enzyme families have been identified in humans, but only seven of them (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5) are responsible for drug metabolism [1,9]. Enzyme induction or inhibition changes the mechanism by which drugs undergo phase I metabolism. Drugs that induce CYP450 activity increase the production of enzymes or decrease their breakdown. This leads to increased drug clearance and decreased drug concentrations. In the case of prodrugs requiring metabolic activation, CYP450 induction leads to increased drug concentrations. The time of onset for enzyme induction is difficult to predict, but generally occurs within days to weeks following administration, and is usually determined by the inducing drug's half-life and rate of enzyme turnover. In contrast, enzyme inhibition results in decreased drug clearance and increased drug concentrations. CYP450 inhibition of prodrug activation results in decreased drug concentrations. Enzyme inhibition usually begins immediately upon the administration of the inhibitor, and the duration corresponds to its half-life.

The net balance of a drug interaction depends on the inhibitory and inducing potency of each agent and on the available routes of substrate elimination. For example, if a drug is a weak inhibitor, the risk of an interaction occurring is relatively low when a substrate of the same family is coadministered. Likewise, if a drug has multiple pathways for elimination, the risk of a major drug interaction is low when an inhibitor or inducer of only one enzyme family is given [10]. Additionally, like the P-gp-mediated pathway, the magnitude of the interaction is higher if both drugs are administered orally and if the substrate undergoes significant first-pass metabolism. Selected inducers, inhibitors, and substrates of the CYP450 system are listed in Table 98.1 [1,9,10].

Uridine 5′-diphosphate glucuronosyltransferase (UGT) enzyme system

Enzymes belonging to the UGT family play a major role in phase II or the conjugative phase of metabolism. They are responsible for 35% of all drugs metabolized by phase II enzymes. During this phase, drug metabolites are made more hydrophilic to facilitate excretion [10,11]. UGT enzymes are primarily found in the liver, but also exist in the GI tract, lungs, nasal epithelium, and kidneys [12]. As with the CYP450 system, modulation of these enzymes can change a drug's concentration and result in altered pharmacologic responses. Tacrolimus, cyclosporine (CSP), and fluconazole are examples of UGT inhibitors and rifampin, phenytoin, and phenobarbital represent potent UGT inducers [11]. Examples of UGT substrates include posaconazole and mycophenolate [11].

Azole antifungals

Fluconazole

Fluconazole is a substrate of CYP2C9 and CYP3A4. It is a potent inhibitor of CYP2C9 but only weakly inhibits CYP3A4, when compared with other azole antifungals such as ketoconazole, itraconazole, and voriconazole [13]. Concurrent administration of fluconazole with CSP, statins, or warfarin results in increased levels of these drugs and thus an increased risk of adverse effects such as renal dysfunction, myopathy, and bleeding [14]. Close monitoring of drug concentrations, use of alternative agents, and frequent dosage adjustments should be used to minimize the severity of these interactions with fluconazole. Administration of potent inducers of CYP2C9 or CYP3A4 with fluconazole can lead to decreased

Table 98.2 Selected drug interactions with fluconazole

Drug	Mechanism	Effect	Management
Benzodiazepines [13,14]	(–) CYP3A4	↑ Benzodiazepine conc.	Monitor for ↑ sedation. Adjust dose accordingly
Calcineurin inhibitors (see Tables 98.5 and 98.6)			
Dihydropyridine calcium channel blockers [13,14]	(–) CYP3A4	↑ Dihydropyridine conc.	Monitor blood pressure and pulse. Adjust dose accordingly
Ergot alkaloids [15]	(–) CYP3A4	↑ Ergot alkaloid conc.	Contraindicated
Ethinylestradiol/norethindrone [14]	(–) Estradiol metabolism	↑ Estradiol conc.	Monitor for toxicity
Fentanyl [16]	(–) CYP3A4	↑ Or prolonged opioid effects	Monitor for toxicity. Adjust dose accordingly
Losartan [13]	(–) CYP2C9	↓ Losartan efficacy	Monitor for efficacy
Phenytoin [13,14]	(–) CYP2C9	↑ Risk of phenytoin toxicity	Monitor phenytoin conc. and toxicity. Adjust dose accordingly
Rifabutin [13,14]	(–) CYP3A4	↑ Rifabutin conc. 80%	Avoid concomitant use. Monitor for toxicity
Rifampin [13,14]	(+) CYP3A4	↓ Fluconazole efficacy	Monitor for efficacy
Sirolimus (see Table 98.8)			
Sulfonylureas [13]	(–) CYP3A4	↑ Risk of hypoglycemia	Monitor blood glucose. Adjust dose accordingly
Warfarin [13,14]	(–) CYP2C9	↑ Prothrombin time 2-fold	Monitor prothrombin time. Adjust dose accordingly

(–) = Inhibits; (+) = induces; ↑ = increases; ↓ = decreases; conc. = concentration; CYP3A4 = cytochrome P450 3A4 isoenzyme; CYP2C9 = cytochrome P450 2C9 isoenzyme.

fluconazole serum levels and less effective antifungal therapy [13]. Selected drug interactions reported with fluconazole are described in Table 98.2 [13–16].

Itraconazole

Itraconazole is a substrate and potent inhibitor of CYP3A4. As a consequence, the interactions with it may be greatly enhanced compared with fluconazole. An example of this difference in magnitude of effect is seen when either azole is given with cyclosporine. Little or no change in CSP concentrations has been reported when fluconazole is given concurrently, but during coadministration with itraconazole, one study showed CSP levels to be elevated by a mean of 80%, necessitating dosage reductions [17]. Since the cost of CSP can be significant, this interaction has been viewed by some as beneficial and even prompted clinicians to use it as a cost-saving measure [18]. An important interaction between itraconazole and sirolimus is described later in this chapter.

Itraconazole is also a substrate and inhibitor of P-gp [19]. One of the interactions affected by this system involves digoxin. In a study of healthy subjects, itraconazole increased the digoxin area under the curve (AUC) by approximately 50% and decreased renal clearance by about 20% [20]. This is caused by itraconazole interfering with P-gp-mediated digoxin renal tubular secretion. Therefore, a 50% digoxin dose reduction is recommended when starting itraconazole. Digoxin concentrations and signs or symptoms of toxicity should be monitored. Other inhibitors of P-gp, like posaconazole, can increase the levels of digoxin when given together [19].

Cardiovascular drug effects can be enhanced via interactions with itraconazole. For instance, the AUC of felodipine increases sixfold and C_{max} eightfold, leading to rapid heart rate and low blood pressure [21]. Quinidine and dofetilide are contraindicated with itraconazole owing to the potential for QTc prolongation and torsades de pointes [22]. Through CYP2C9 inhibition, itraconazole can potentiate the anticoagulant effect of warfarin. In a case report, the international normalized ratio (INR) exceeded 8, which contributed to severe bruising and bleeding [23]. When used in conjunction with itraconazole, it is imperative to follow INRs closely and watch for signs of toxicity.

The efficacy of itraconazole is related to its serum concentration [17]. If its metabolism is induced by drugs such as phenytoin and rifampin, serum levels may be suboptimal, leading to inadequate control or progression of a fungal infection [24,25]. It may be useful to monitor itraconazole levels if such combinations are required, although some would advise against using them concomitantly since treatment failure may result.

Voriconazole

Voriconazole is also extensively metabolized by the CYP450 system and its inhibitory potency of CYP450 is between those of fluconazole and itraconazole. Specifically, voriconazole is a moderate inhibitor and substrate of CYP3A4, a moderate inhibitor but a weak substrate of CYP2C9, and a moderate inhibitor but strong substrate of CYP2C19. It is not an inhibitor or substrate of P-gp [11]. Since voriconazole affects multiple CYP pathways, it is reasonable to expect that the potential for drug–drug interactions is high.

Among the many drug interactions associated with voriconazole, its effects on cyclosporine, tacrolimus, and sirolimus are the most clinically relevant in HCT. Inhibition of CYP3A4-mediated metabolism of these immunosuppressants resulted in increases in AUC and blood concentrations in solid organ and HCT patients receiving concurrent voriconazole therapy [11]. More details regarding these interactions are provided in the respective immunosuppression sections and tables of this chapter. Close monitoring of immunosuppressive levels and dosage adjustments are imperative to avoid toxicity or therapeutic failures when voriconazole is being added or discontinued.

Voriconazole is contraindicated with pimozide, quinidine, and ergot alkaloids because of the concern for QTc prolongation and ergotism [26]. Increased incidence of voriconazole treatment failures is common when combined with potent inducers such as rifampin, rifabutin, and carbamazepine. Consequently, the use of these agents with voriconazole is also contraindicated [26].

Pharmacokinetic studies of voriconazole's effect on chemotherapy have been conducted with various agents [26]. Although voriconazole's theoretical interaction with cyclophosphamide has not been formally evaluated, it is of particular interest in HCT. Additional details of this interaction are described in the cyclophosphamide section.

Voriconazole concentrations are decreased by phenytoin and human immunodeficiency virus antivirals, whereas drugs such as cimetidine, erythromycin, and indinavir have negligible effects on its metabolism [27]. Voriconazole also decreases the metabolism of statins, phenytoin, and warfarin [28]. It has minor to negligible effects on prednisolone, digoxin, and mycophenolic acid derivatives. Table 98.3 further describes the clinically relevant drug interactions with voriconazole [1,27].

Table 98.3 Selected drug interactions with voriconazole

Drug	Mechanism	Effect	Management
Benzodiazepines [1]	(–) CYP3A4	↑ Benzodiazepine conc.	Monitor for ↑ sedation. Adjust dose accordingly
Calcineurin inhibitors (see Tables 98.5 and 98.6)			
Calcium channel blockers (diltiazem, verapamil) [26]	(–) CYP3A4	↑ Calcium channel blocker conc.	Monitor BP and HR. Adjust dose accordingly
Carbamazepine [26]	(+) CYP3A4	↓ Voriconazole conc.	Contraindicated
Ergot alkaloids [26]	(–) CYP3A4	↑ Ergot alkaloid conc.	Contraindicated
Ethinylestradiol/norethindrone [26]	(–) CYP2C19	↑ Voriconazole C_{max}, AUC ↑ Ethinylestradiol/ norethindrone C_{max}, AUC	Monitor for voriconazole toxicity and for menstrual bleeding
HMG-CoA reductase inhibitors (see Table 98.9)			
Macrolide (clarithromycin, erythromycin) [26]	(–) CYP3A4	↑ Macrolide conc.	Monitor EKG for QT_c prolongation. Consider alternative macrolide (e.g. azithromycin)
Methadone [26]	(–) CYP2C19, CYP2C9, CYP3A4	↑ Active (*R*)-methadone C_{max} 31%, AUC 47%	Monitor for toxicity. Adjust dose accordingly
Phenytoin [26]	(–) CYP2C9	↑ Phenytoin C_{max}, AUC 2-fold	Monitor phenytoin conc. and toxicity
	(+) CYP3A4	↓ Voriconazole C_{max} 50%, AUC 70%	↑ Voriconazole maintenance to 5 mg/kg IV BID (from 4 mg/kg) or to 400 mg PO BID (from 200 mg)
Proton pump inhibitors (PPIs) [26]	(–) CYP2C19, CYP2C9, CYP3A4	↑ PPI conc.	↓ Omeprazole 50% if dose >40 mg/day
Rifabutin [26]	(+) CYP3A4	↓ Voriconazole C_{max} 93%	Contraindicated
Rifampin [26]		↓ Voriconazole conc.	Contraindicated
Sirolimus (see Table 98.8)			
Sulfonylureas [26]	(–) CYP2C9	↑ Sulfonylurea conc.	Monitor blood glucose. Adjust dose accordingly
Warfarin [26]	(–) CYP2C9	↑ PT, INR	Monitor for bleeding, PT, INR. Adjust dose accordingly

(–) = Inhibits; (+) = induces; ↑ = increases; ↓ = decreases; conc. = concentration; AUC = area under the curve; C_{max} = maximum concentration; CYP3A4 = cytochrome P450 3A4 isoenzyme; CYP2C19 = cytochrome P450 2C19 isoenzyme; CYP2C9 = cytochrome P450 2C9 isoenzyme; PT = prothrombin time; INR = international normalized ratio

Posaconazole

Like all azoles, posaconazole inhibits CYP3A4, and like fluconazole, it is not a substrate [11]. The primary metabolism of posaconazole is through glucuronidation by UGT enzymes. It is also a P-gp inhibitor and substrate like itraconazole. Posaconazole is contraindicated with pimozide and quinidine because of the potential for QTc prolongation and torsades de pointes [29]. Ergot alkaloids are contraindicated since they can precipitate ergotism [29]. Some drugs metabolized through CYP3A4, such as diltiazem and verapamil, have not been formally studied, but are likely to have an interaction with posaconazole [29]. Other benzodiazepines and statins listed in Table 98.4 may also have their concentrations elevated by concomitant posaconazole administration [29]. Inducers reduce posaconazole plasma levels and should be avoided unless the benefit exceeds the risk [30]. Relevant interactions occurring with posaconazole are summarized in Table 98.4 [19,29–32].

Similarly to itraconazole and voriconazole, posaconazole has inhibitory effects on the calcineurin inhibitors, requiring dosage adjustments and frequent monitoring of their levels. When the combination of posaconazole and CSP was analyzed in allogeneic transplant patients, the CSP levels increased, requiring about a 50% decrease in the dose [31]. Despite the makers of posaconazole recommending a cyclosporine dose reduction of 25% when posaconazole is added, this was not done preemptively in this study. The authors advised not to reduce the CSP dose empirically at the start of coadministration, but instead, to follow levels closely with subsequent adjustment since potential subtherapeutic levels may adversely affect the outcome of transplant, including increased rates of graft-versus-host disease (GVHD). The same might also be true for sirolimus. A description of this interaction can be found in the sirolimus section.

Echinocandins

In comparison with other antifungals, echinocandins are the least dependent on Pg-p, OATP, and CYP pathways for drug transport and metabolism. Micafungin in vitro was found to be a substrate and weak inhibitor of CYP; however, this was not demonstrated with in vivo studies [33]. Caspofungin is a substrate for OATP transport. Anidulafungin is not affected by these pathways [33,34]. Consequently, serious drug interactions with echinocandins are minimal.

The interaction between echinocandins and calcineurin inhibitors warrants some elaboration. In two studies, CSP inhibited OATP-mediated transport of caspofungin. This resulted in a 35% increase in the caspofungin AUC and transient elevations in transaminases [35,36]. However, subsequent retrospective reviews found negligible hepatotoxicity when this combination was employed [37,38]. Regardless, the manufacturer has cautioned against using these agents together unless the potential benefits outweigh the risks and, if used, monitoring of liver function tests is imperative. Conversely, tacrolimus concentrations, as described by AUC, maximum concentrations (C_{max}), and 12-hour blood levels decreased by 20, 16, and 26%, respectively [35,36]. Routine monitoring of tacrolimus concentrations with appropriate adjustments is advised. Micafungin mildly inhibited CSP metabolism in 20% of healthy volunteers; however, this was not observed in a study with HCT patients [39,40]. Careful monitoring for signs of CSP toxicity and adjustment of levels may be necessary when micafungin is initiated or discontinued. Anidulafungin concentrations were increased by 22% when given with CSP, but this interaction does not require any dosing modification [33]. Clinically significant interactions between tacrolimus and micafungin or anidulafungin have not been reported [33].

Other relevant echinocandin interactions include caspofungin with rifampin and micafungin with sirolimus and nifedipine. Concurrent administration of caspofungin and rifampin resulted in

Table 98.4 Selected drug interactions with posaconazole

Drug	Mechanism	Effect	Management
Calcineurin inhibitors			
Cyclosporine (CSP) [19,31]	(–) CYP3A4, P-gp	↑ CSP conc.	↓ CSP dose 25%. Monitor CSP conc. Adjust dose accordingly
Tacrolimus [19]		↑ Tacrolimus AUC 358%, C_{max} 121%	↓ tacrolimus dose 66%. Monitor tacrolimus conc. Adjust dose accordingly
Cimetidine [19]	Alteration of gastric pH	↓ Posaconazole AUC and C_{max} 39%	Avoid concomitant use
Digoxin [19,29]	(–) P-gp	↑ Digoxin conc.	Monitor digoxin conc.
Esomeprazole [19]	Alteration of gastric pH	↓ Posaconazole AUC 32%, C_{max} 46%	Avoid in lower absorption states (e.g. GVHD, mucositis)
Midazolam [19]	(–) CYP3A4	↑ Midazolam AUC up to 10-fold, C_{max} up to 3-fold	Monitor for toxicity
Phenytoin [19]	(–) CYP3A4 (+) UGT	↑ Phenytoin AUC and C_{max} 16% ↓ Posaconazole AUC 50%, C_{max} 41%	Avoid concomitant use. If used, monitor for therapeutic failure and phenytoin conc.
Rifabutin [19,30]	(–) CYP3A4 (+) UGT	↑ Rifabutin AUC 72%, C_{max} 31% ↓ Posaconazole AUC 49%, C_{max} 43%	Avoid concomitant use. If used, monitor for therapeutic failure and rifabutin toxicity
Rifampin [32]	(+) UGT	↓ Posaconazole conc. up to 80.3%	Avoid concomitant use. If used, monitor for therapeutic failure
Simvastatin (see Table 98.9)			
Sirolimus (see Table 98.8)			

(–) = Inhibits; (+) = induces; ↑ = increases; ↓ = decreases; conc. = concentration; CYP3A4 = cytochrome P450 3A4 isoenzyme, P-gp = P-glycoprotein; UGT = uridine 5′ diphosphate glucuronosyltransferase; AUC = area under the curve; C_{max} = maximum concentration.

Table 98.5 Selected drug interactions with cyclosporine (CSP)

Drug	Mechanism	Effect	Management
Azoles			
Fluconazole [42]	(–) CYP3A4	↑ CSP conc. 21%	Monitor CSP conc. Adjust dose accordingly
Itraconazole [17]		↑ CSP conc. 80%	↓ CSP dose ~50%. Monitor CSP conc. Adjust dose accordingly
Posaconazole [29]		↑ CSP conc.	↓ CSP dose 25%. Monitor CSP conc. Adjust dose accordingly
Voriconazole [43]		↑ CSP AUC 1.7-fold, conc. 2.5-fold	↓ CSP dose 50%. Monitor CSP conc. Adjust dose accordingly
Diltiazem [44]	(–) CYP3A4	↑ CSP AUC 28–62%, conc. 49–88%	Monitor CSP conc. Adjust dose accordingly
Carbamazepine [45]	(+) CYP3A4	↓ CSP conc. 50%	Monitor CSP conc. Adjust dose accordingly
Caspofungin [35]	(–) CYP3A4	↑ Caspofungin AUC 35%	Avoid concomitant use. Monitor liver function tests
Digoxin [46]	Unknown	↑ Digoxin conc., ↓ Cl 53%	Consider ↓ digoxin dose ~50%. Monitor digoxin conc.
HMG-CoA reductase inhibitors (see Table 98.9)			
Imatinib [47]	(–) CYP3A4	↑ CSP conc. 1.7-fold	Monitor CSP conc. Adjust dose accordingly
Metronidazole [48]	? (–) CYP3A4	↑ CSP conc. 2-fold	Monitor CSP conc. Adjust dose accordingly
Mycophenolic acid derivatives (see Table 98.7)			
Phenobarbital [49]	(+) CYP3A4	↓ CSP conc.	Monitor CSP conc. Adjust dose accordingly
Phenytoin [49]	(+) CYP3A4	↓ CSP conc.	Monitor CSP conc. Adjust dose accordingly
Rifampin [50]	(+) CYP3A4	↓ CSP AUC	Avoid concomitant use. Monitor CSP conc. Adjust dose accordingly
Sirolimus (see Table 98.9)			

(–) = Inhibits; (+) = induces; ↑ = increases; ↓ = decreases; conc. = concentration; CYP3A4 = cytochrome P450 3A4 isoenzyme; ALT AUC = area under the curve; Cl = clearance.

a transient 61% increase followed by a 14–30% reduction in caspofungin concentration at the steady state [33]. It is believed that this interaction is OATP mediated since both drugs are OATP substrates and rifampin has both inductive and inhibitory effects on OATP transport [33]. To avoid possible therapeutic failure, the daily caspofungin dose should be increased to 70 mg. This higher dose should also be considered when other known inducers are added to caspofungin [33]. Coadministration of micafungin with sirolimus and nifedipine resulted in 12 and 18% increases in sirolimus and nifedipine concentrations, respectively [33]. Dose adjustments of both agents may be necessary if given together with micafungin.

Immunosuppressants

Calcineurin inhibitors

The metabolisms of both CSP and tacrolimus are mediated predominantly through CYP3A4. They are CYP3A4 substrates and inhibitors and substrates of P-gp [41]. Therefore, they have similar drug interaction profiles. These are depicted in Table 98.5 [17,29,35,42–50] and Table 98.6 [17,29,35,42,51–58]. Differences may be attributed to the relative contributions of liver or intestinal metabolism, affinity to bind to specific CYP isoenzymes, and involvement of other transporter systems [41]. Also, published data may be lacking in some instances, but the potential for interaction may still exist, as in the case of imatinib with tacrolimus.

Knowing which agents may affect the concentrations of these medications is critical because the calcineurin inhibitors have a narrow therapeutic window. Too much of either drug results in toxicity (nephrotoxicity, neurotoxicity, etc.), whereas too little will cause inadequate immunosuppression and possibly lead to increased risk of GVHD or graft rejection.

In a study of calcineurin inhibitors and voriconazole in allogeneic HCT patients, the median increase in tacrolimus concentration to dose (C/D) ratio was 115.6% [59]. In those who received CSP, the C/D ratio was 82.1%, suggesting a smaller interaction. Considerable interpatient variability was observed in the magnitude of the

Table 98.6 Selected drug interactions with tacrolimus

Drug	Mechanism	Effect	Management
Azoles			
Fluconazole [42]	(–) CYP3A4	↑ Tacrolimus conc. 16%	Monitor tacrolimus conc. Adjust dose accordingly
Itraconazole [17]		↑ Tacrolimus conc. 83%	↓ Tacrolimus dose ~50%. Monitor tacrolimus conc. Adjust dose accordingly
Posaconazole [29]		↑ Tacrolimus AUC 358%, C_{max} 121%	↓ Tacrolimus dose 66%. Monitor tacrolimus conc. Adjust dose accordingly
Voriconazole [51]		↑ Tacrolimus conc. 10-fold	↓ Tacrolimus dose 66%. Monitor tacrolimus conc. Adjust dose accordingly
Calcium channel blockers			
Diltiazem [52]	(–) CYP3A4	↑ Tacrolimus conc.	Monitor tacrolimus conc. Adjust dose accordingly
Felodipine [53]		↑ Tacrolimus conc. 3-fold	
Caspofungin [35]	Unknown	↓ Tacrolimus AUC 20%, C_{max} 16%, conc. 26%	Monitor tacrolimus conc. Adjust dose accordingly
Clarithromycin [54]	(–) CYP3A4	↑ Tacrolimus conc.	Monitor tacrolimus conc. Adjust dose accordingly
Metronidazole [55]	? (–) CYP3A4, P-gp	↑ Tacrolimus conc. 3-fold	Monitor tacrolimus conc. Adjust dose accordingly
Phenobarbital [56]	(+) CYP3A4	↓ Tacrolimus conc. 73%	Monitor tacrolimus conc. Monitor for ↑ risk of GVHD. Adjust dose accordingly
Phenytoin [57]	(+) CYP3A4	↓ Tacrolimus conc.	Monitor tacrolimus conc. Monitor for ↑ risk of GVHD. Adjust dose accordingly
Rifampin [58]	(+) CYP3A4	↓ Tacrolimus conc., requiring 10-fold ↑ in tacrolimus dose	Avoid concomitant use
Sirolimus (see Table 98.8)			

(–) = Inhibits; (+) = induces; ↑ = increases; ↓ = decreases; conc. = concentration; CYP3A4 = cytochrome P450 3A4 isoenzyme; AUC = area under the curve; C_{max} = maximum concentration; P-gp = P-glycoprotein.

interaction, leading the authors to recommend not uniformly reducing the dose of calcineurin inhibitors. In another trial examining the concomitant use of tacrolimus and voriconazole in transplant recipients, a preemptive dose reduction strategy was utilized to maintain tacrolimus levels successfully within the targeted goal [60]. Doses were lowered by 60–80% compared with those at baseline. Although the manufacturer's recommendations suggest preemptively decreasing the immunosuppressant doses when azoles are initiated, these came from limited data in renal transplant recipients and healthy volunteers [26]. In clinical practice, some may choose to adhere to the prescribing guidelines or opt to monitor drug concentration closely s and adjust doses as needed.

When orally administered tacrolimus and voriconazole were evaluated in HCT patients, the median increase in the C/D ratio of tacrolimus was 138.8% [61]. Since CYP3A4 enzymes are found in the liver and GI tract, the interaction is greater than when both drugs are given intravenously. Additionally, if the immunosuppressant is a substrate of P-gp and an azole is a P-gp inhibitor, then the bioavailability of the immunosuppressant is further enhanced. Therefore, when changing from intravenous to oral dosing, a dosage reduction may be required [41].

Proton pump inhibitors (PPIs) are metabolized mainly via CYP2C19 and CYP3A4, hence drug interactions with calcineurin inhibitors would be expected. The data, however, are conflicting with both tacrolimus and cyclosporine. In a case report involving a kidney transplant recipient, the tacrolimus concentration was increased to over 20 ng/mL when esomeprazole and omeprazole were used at different times [62]. In contrast, the interaction between omeprazole and tacrolimus was deemed a clinically irrelevant drug interaction in 51 renal allograft patients [63]. Lansoprazole, however, was shown to interact with tacrolimus in several case reports [64,65]. This was particularly evident in those who had CYP2C19 gene mutations and were intermediate or poor metabolizers, which shifted the metabolism of lansoprazole to the 3A4 pathway and, consequently, a greater increase in tacrolimus AUC [66]. Pantoprazole and rabeprazole have not been shown to interact with tacrolimus. Studies and case reports have shown either an insignificant interaction, or both increases and decreases in cyclosporine levels when used concomitantly with PPIs [67]. The package labeling on omeprazole cautions that it may prolong cyclosporine's elimination. A theoretical interaction with rabeprazole also exists. In vitro, it has been shown to inhibit cyclosporine metabolism similarly to omeprazole at equivalent concentrations, but no in vivo clinical reports have been published. Nonetheless, close monitoring and dosage adjustments may be warranted when PPIs are used concurrently with calcineurin inhibitors. Two specific drugs that alter the metabolism of CSP and tacrolimus in different ways are caspofungin and sirolimus. These interactions are further characterized in their respective sections. The cyclosporine–posaconazole interaction is also illustrated in the posaconazole section.

Mycophenolic acid (MPA) derivatives

The metabolism of mycophenolate mofetil (MMF) and mycophenolic sodium is complex; it involves the rapid conversion to mycophenolic acid (MPA), metabolism of MPA to 7-*O*-MPA glucuronide (MPAG) by UGT enzymes, possible MPAG enterohepatic recirculation by intestinal bacteria, and MPAG excretion by active tubular secretion and, to a lesser extent, Pg-p, into the urine [68].

Decreased absorption, inhibition of enterohepatic recycling, and competition for renal tubular secretion are the main mechanisms by which drug interactions occur with these agents. Reductions in MPA exposure can lead to therapeutic failures, whereas increases can result in toxicities such as hypersensitivity, GI disturbances, and cytokine release [68]. Drugs interacting with MPA derivatives and their clinical implications are discussed below and presented in Table 98.7 [68–73].

Coadministration of agents such as oral divalent electrolyte supplements and phosphate binders have been shown to reduce absorption, leading to lower concentrations of MPA [68,69,73]. Reduction in MPA C_{max} and AUC of orally administered MMF, but not mycophenolic sodium, was observed with concurrent

Table 98.7 Selected drug interactions with mycophenolic acid derivatives

Drug	Mechanism	Effect	Management
Antacids [68]	↓ MPA absorption	↓ MPA absorption	Monitor for therapeutic failure
Bile acid binders [68]	(–) Enterohepatic recycling	↓ MPA AUC 40%	Avoid concomitant use
Cyclosporine [69]	(–) Enterohepatic recycling	↓ MPA conc.	Monitor for therapeutic failure
Divalent oral electrolyte supplement [70]	↓ MMF absorption via chelation	↓ MPA absorption	Monitor for therapeutic failure
Ganciclovir [70]	Competes for renal tubular secretion	↑ Ganciclovir conc. in renal impairment	Monitor blood counts
Oral contraceptives [70]	Unknown	↓ Oral contraceptive exposure	Monitor for menstrual bleeding
Proton pump inhibitors [61]	↓ MMF dissolution and MPA conversion	↓ MPA absorption	Use mycophenolic sodium
Rifampin [72]	(+) UGT, (–) enterohepatic recycling	↓ MPA AUC	Monitor for therapeutic failure
Sevelamer [73]	↓ MMF absorption, (–) enterohepatic recycling	↓ MPA AUC 25%, C_{max} 30%	Separate by ≥2 hours

(–) = Inhibits; (+) = induces; ↑ = increases; ↓ = decreases; conc. = concentration; C_{max} = maximum concentration; AUC = area under the curve; MMF = mycophenolate mofetil; MPA = mycophenolic acid; UGT = uridine 5′-diphosphate glucuronosyltransferase.

Table 98.8 Selected drug interactions with sirolimus

Drug	Mechanism	Effect	Management
Azoles [41]			
Fluconazole	(–) CYP3A4, P-gp	↑ Sirolimus conc. 3-fold	Monitor sirolimus conc. Monitor for toxicity.
Itraconazole		↑ Sirolimus conc. > 8-fold	Adjust dose accordingly. See text
Posaconazole		↑ Sirolimus AUC 8.9-fold, C_{max} 6.7-fold	Contraindicated. See text
Voriconazole		↑ Sirolimus AUC 7-fold, C_{max} 11-fold	
Calcium channel blockers [76]			
Diltiazem	(–) CYP3A4, P-gp	↑ Sirolimus C_{max} 1.4-fold, t_{max} 1.3-fold, AUC 1.6-fold	Monitor sirolimus conc. Monitor for toxicity. Adjust dose accordingly
Verapamil		↑ Sirolimus C_{max} 2.3-fold, AUC 2.2-fold ↓ Active (*S*)-(–)-enantiomer of verapamil C_{max}, AUC 1.5-fold	
Calcineurin inhibitors			
CSP [78]	(–) CYP3A4, P-gp	↑ Sirolimus C_{max} 116%, AUC 230%.	Administer sirolimus 4 hours after CSP
Tacrolimus [79]		↓ Tacrolimus conc.	Monitor tacrolimus conc.
Erythromycin [76]	(–) CYP3A4, P-gp	↑ Sirolimus C_{max} 4.4-fold, AUC 4.2-fold, t_{max} 0.4 h ↑ Erythromycin C_{max} 1.6-fold, AUC 1.7-fold, t_{max} 0.3 h	Monitor sirolimus conc. Monitor for toxicity. Adjust dose accordingly
Micafungin [76]	Unknown	↑ Sirolimus AUC 21%	Monitor sirolimus conc. Monitor for toxicity. Adjust dose accordingly
Rifampin [76]	(+) CYP3A4, P-gp	↓ Sirolimus AUC 82%, C_{max} 71%	Monitor sirolimus conc. Monitor for toxicity. Adjust dose accordingly or use alternative agent

(–) = Inhibits; (+) = induces; ↑ = increases; ↓ = decreases; C_{max} = maximum concentration; t_{max} = time to maximum concentration; AUC = area under the curve; CYP3A4 = cytochrome P450 3A4 isoenzyme; P-gp = P-glycoprotein.

treatment with PPIs. This difference is due in part to the dependence of an acidic environment for MMF dissolution and conversion to MPA, and consequently MPA absorption in the stomach. In contrast, dissolution of mycophenolic sodium to MPA is not acidic pH dependent [71].

CSP but not sirolimus or tacrolimus administration was shown to decrease MPA concentrations through P-gp inhibition and consequently enterohepatic recycling of MMF [69,74,75]. Other inhibitors of enterohepatic recycling include bile acid sequestrants, rifampin, and to some extent sevelamer [69,72,73].

Competition for renal excretion of MPA is also a possible mechanism for drug interactions. Ganciclovir is an example of a drug that interacts via this mechanism [70].

Sirolimus

Sirolimus is a substrate and inhibitor of P-gp and CYP3A4 [76,77]. Consequently, concomitant administration of sirolimus with inducers of CYP3A4 and P-gp leads to increased sirolimus metabolism, decreased concentration, decreased efficacy, and potentially increased GVHD. Likewise, the converse is true when sirolimus is given with CYP3A4 or P-gp inhibitors. Monitoring sirolimus trough levels and signs of toxicity and treatment failure is recommended when combined with agents mediated by CYP3A4 or P-gp. Sirolimus interactions with azole antifungals and calcineurin inhibitors are discussed below. Additional interactions are presented in Table 98.8 [41,76,78,79].

Decreased sirolimus metabolism and supratherapeutic levels are well documented with the azole antifungals. Empiric dosage reduction of ketoconazole (by 80–90%) and daily doses of fluconazole ≥200 mg (by 50–70%) are recommended to mitigate the magnitude of the interaction. Caution should be exercised , however, when reducing the doses of these agents since interpatient variability can widely affect the incidence, magnitude, and significance of the interaction [41]. The interaction of sirolimus with itraconazole exists in case reports. In two solid organ transplant reports, an 8.5-fold increase in sirolimus levels was reported when itraconazole was added and a twofold increase in sirolimus dose was necessary to maintain similar immunosuppressant levels when itraconazole was discontinued [80]. Similarly, in an HCT setting, a doubling of sirolimus concentration was noted following 1 week of concurrent therapy when added to itraconazole. This resulted in the need to withhold sirolimus for 8 days followed by a 3.5-fold sirolimus dosage reduction. The authors of this report recommended caution when adding either agent to or removing it from existing sirolimus or itraconazole therapy. In addition, sirolimus concentration monitoring and judicious use of sirolimus dose adjustments were suggested [80]. Voriconazole labeling contraindicates its use with sirolimus due to a sevenfold increase in C_{max} and an 11-fold increase in the AUC of sirolimus [76]. Case reports, however, have described the safe administration of this combination [81]. Mathis et al. cited a 75–87% dosage reduction in sirolimus during therapy with voriconazole in two renal transplant patients [81]. Similarly, Marty et al. reported the use of sirolimus and voriconazole in 11 patients undergoing HCT [82]. In this case series, the sirolimus dose was empirically reduced by 90% upon initiation of voriconazole. The investigators of both studies concluded that the combination is feasible and safe. Frequent monitoring of sirolimus levels upon initiation and discontinuation of voriconazole was also recommended. Posaconazole increased sirolimus exposure 6.7-fold in C_{max} and 8.9-fold in AUC when given together. The authors of this study and posaconazole labeling contraindicate the concomitant use of these agents [11,83]. Evaluation in 15 HCT patients, however, showed that this combination is feasible in the setting of an empiric 33–50% sirolimus dose reduction [77]. In general, close monitoring of sirolimus levels and adjustments in dosage are recommended to optimize therapy during and upon the discontinuation of azole use.

Systemic sirolimus exposure in healthy volunteers is affected by the timing of sirolimus administration in relation to CSP through CSP-mediated inhibition of P-gp and CYP3A4. Statistically significant increases in the bioavailability, C_{min}, C_{max}, time to C_{max}, and AUC of sirolimus were observed when both agents were taken together. In contrast, when sirolimus was administered 4 hours after CSP, these measures were modestly increased. The pharmacokinetics of CSP were unaffected by sirolimus. The authors advised that sirolimus should be administered 4 hours after CSP to avoid toxic sirolimus levels [78]. Changes in sirolimus pharmacokinetics were not demonstrated with tacrolimus. However, a reduction in tacrolimus levels occurred with this combination compared with tacrolimus alone [79].

Chemotherapy

Busulfan

Busulfan has a narrow therapeutic window and, as a result, interactions involving this drug are noteworthy. It is primarily metabolized via glutathione conjugation, and subsequently oxidative transformation in the liver [84]. Acetaminophen depletes glutathione, which in turn reduces busulfan clearance. Hence it should not be administered concomitantly with or within 72 hours of busulfan [84]. Itraconazole, but not fluconazole, reduced the clearance of busulfan by 20%, resulting in elevated busulfan levels [85]. Theoretically, voriconazole could interact with busulfan by decreasing its clearance through inhibition of glutathione conjugation [86]. Therefore, the combination of itraconazole and voriconazole with busulfan should be avoided. Currently, interaction data regarding posaconazole and busulfan have not been reported.

Metronidazole increases busulfan levels, thus contributing to severe toxicity and even mortality [87]. Although the exact mechanism is unclear, it is possible that metronidazole inhibits CYP3A4-mediated metabolism of busulfan. Metronidazole and busulfan should not be used concurrently.

Phenytoin reduces busulfan concentrations by inducing its metabolism [84]. In one study, a decline of 15% or more in busulfan levels was noted in the phenytoin group versus subjects who received diazepam for seizure prophylaxis [88]. Based on these findings, the use of alternative anticonvulsants has been promoted. Options such as benzodiazepines and levetiracetam may be considered. If continued use of phenytoin is desired, the interaction can be managed by adjusting busulfan doses based on busulfan pharmacokinetics. Of the other antiepileptics, levetiracetam may be preferred to benzodiazepines given its favorable pharmacokinetic profile such as a short half-life, excellent bioavailability, and low potential for drug interactions [89,90]. Cyclophosphamide may also interact with phenytoin, but the extent of the interaction is unpredictable given the complexity of cyclophosphamide's metabolism and the relatively short duration of phenytoin use in busulfan/cyclophosphamide conditioning regimens. Phenytoin has been employed as busulfan-induced prophylaxis for over 20 years [89]. In addition, head-to-head studies comparing phenytoin with alternative antiseizure agents looking at long-term outcomes are absent. Furthermore, the outcome data in HCT have historically been based on the use of phenytoin as seizure prophylaxis. Therefore, for these reasons, many transplant centers have not opted to change their prophylactic strategy.

Cyclophosphamide

When itraconazole was given as prophylaxis to HCT patients conditioned with cyclophosphamide, higher serum creatinine and bilirubin levels were seen in the subset who received itraconazole as opposed to fluconazole [91]. Those who were assigned to itraconazole had greater exposure to toxic metabolites linked to hepatic venocclusive disease (VOD). Fluconazole, however, showed a protective benefit by inhibiting the formation of a harmful metabolite, 4-hydroxycyclophosphamide (4-HC). Inhibition of CYP3A4 and CYP2C9 by voriconazole could potentially lead to altered cyclophosphamide activation, thereby reducing cyclophosphamide's efficacy or producing undesirable toxicity analogous to that of itraconazole. Alternatives to voriconazole, such as echinocandins or amphotericin-based products, should be employed to avoid the potential interaction. If temporary cessation of voriconazole is possible, it should be discontinued at least 30 hours prior to starting cyclophosphamide and resumed when five cyclophosphamide half-lives have elapsed [1].

The use of aprepitant, a neurokinin antagonist antiemetic with moderate CYP3A4 inhibitor activity, could potentially interact with high-dose cyclophosphamide used in conditioning; however, a study has shown no meaningful influence on cyclophosphamide pharmacokinetics [92].

An interaction between cyclophosphamide and cyclosporine has also been considered. In a review of allogeneic HCT patients, factors affecting cyclosporine concentrations up to 14 days after transplantation were elucidated [93]. It was found that cyclosporine levels were much lower in the group who received cyclophosphamide-based conditioning, as opposed to the others who did not. They postulated that cyclophosphamide autoinduction caused an increase in hepatic enzymes, leading to a decline in cyclosporine concentrations. Therefore, levels of the calcineurin inhibitor should be closely monitored, especially early after transplant and when cyclophosphamide is part of post-transplant GVHD prophylaxis.

Tyrosine kinase inhibitors (TKIs)

Imatinib is a P-gp, OATP, organic cation-transporting polypeptide (OCTP), and CYP3A4 and, to a lesser extent, CYP2C9 and CYP2D6 substrate [94,95]. It is also a potent inhibitor of these pathways [96]. Dasatinib is a CYP3A4 and P-gp substrate and a weak CYP3A4 inhibitor [94,97]. Nilotinib is a CYP3A4 and P-gp substrate but also competitively inhibits CYP3A4, CYP2C8, CYP2C9, CYP2D6, P-gp, and OCTP [94,95]. In contrast, sorafenib is excreted 95% unchanged. The remainder undergoes hepatic metabolism, where it is metabolized by CYP3A4 followed by glucuronidation by UGT1A9 [94]. It has been found to inhibit CYP2B6, CYP2C8, CYP2C9, CYP2D6, P-gp, and UGT1A1 in in vitro studies [96].

Table 98.9 Selected drug interactions with HMG-CoA reductase inhibitors

Drug	Interacting drug	Mechanism	Effect	Management
Lovastatin, simvastatin, atorvastatin [99,100]	Amiodarone	(–) CYP3A4	↑ Simvastatin, lovastatin, atorvastatin conc.	Simvastatin NTE 10 mg/day Lovastatin NTE 40 mg/day
	Amlodipine			Simvastatin NTE 20 mg/day
	Carbamazepine	(+) CYP3A4	↓ Simvastatin, lovastatin AUC 70–95-fold ↓ Atorvastatin AUC 60–90-fold	Monitor for therapeutic failure
	Clarithromycin	(–) CYP3A4	↑ Simvastatin, lovastatin AUC 4–12-fold ↑ Atorvastatin AUC 1.5–5-fold	Contraindicated with lovastatin, simvastatin
	Cyclosporine		↑ Simvastatin AUC 6–8-fold ↑ Lovastatin AUC 5–20-fold ↑ Atorvastatin AUC 6–15-fold	Contraindicated with simvastatin. Avoid with lovastatin
	Diltiazem		↑ Simvastatin, lovastatin AUC 3–8-fold	Simvastatin NTE 10 mg/day Lovastatin NTE 20 mg/day
	Gemfibrozil	(–) OATP1B1	↑ Simvastatin and lovastatin AUC 2–3-fold ↑ Atorvastatin AUC <1.5-fold	Contraindicated with simvastatin. Avoid with lovastatin
	Itraconazole	(–) CYP3A4	↑ Simvastatin, lovastatin AUC 5–10-fold ↑ Atorvastatin AUC 2–4-fold	Contraindicated with lovastatin, simvastatin
	Phenytoin	(+) CYP3A4	↓ Simvastatin, lovastatin, atorvastatin conc.	
	Posaconazole	(–) CYP3A4	↑ Simvastatin AUC 5–11-fold	Contraindicated with lovastatin, simvastatin
	Rifampin	(+) CYP3A4	↓ Simvastatin and lovastatin AUC 70–95-fold ↓ Atorvastatin AUC 60–90-fold	
	Verapamil	(–) CYP3A4	↑ Simvastatin, lovastatin, atorvastatin conc.	Simvastatin NTE 10 mg/day Lovastatin NTE 40 mg/day
	Warfarin	(–) Warfarin metabolism	↑ INR	Monitor for bleeding. Adjust dose accordingly
Fluvastatin [100,101]	Amiodarone Cyclosporine Fluconazole	(–) CYP2C9	↑ Fluvastatin conc. ↑ Cyclosporine AUC 2–4-fold	Monitor for toxicity
	Phenytoin Voriconazole	(+) CYP2C9	↓ Fluvastatin conc.	
	Warfarin	(–) Warfarin metabolism	↑ INR	Monitor for bleeding. Adjust dose accordingly
Pitavastatin [100]	Cyclosporine Gemfibrozil	(–) OATP1B1	↑ Pitavastatin AUC 5-fold ↑ Pitavastatin AUC <1.5-fold	Monitor for toxicity
Pravastatin [100,101]	Clarithromycin Cyclosporine Gemfibrozil	(–) OATP1B1	↑ Pravastatin AUC 2-fold ↑ Pravastatin AUC 5–10-fold ↑ Pravastatin AUC 2-fold	Monitor for toxicity
	Warfarin	(–) Warfarin metabolism	↑ INR	Monitor for bleeding. Adjust dose accordingly
Rosuvastatin [103]	Antacids	(–) Absorption	↓ Rosuvastatin conc. 50%	Administer 2 hours after rosuvastatin
	Cyclosporine	(–) CYP2C9, CYP2C19 (–) OATP1B1	↑ Rosuvastatin AUC 7.1-fold, C_{max} 10.6-fold	Monitor for toxicity
	Fluconazole	(–) CYP2C9, CYP2C19	↑ Rosuvastatin AUC, C_{max}	
	Gemfibrozil	(–) OATP1B1, UGT	↑ Rosuvastatin AUC 2-fold	
	Ursodiol	(–) OATP1B1, hepatic nuclear factor 1-alpha	↑ Rosuvastatin AUC	
	Warfarin	(–) CYP2C9 warfarin metabolism and displaces warfarin from protein-binding sites	↑ INR	Monitor for bleeding. Adjust dose accordingly

(–) = Inhibits; (+) = induces; ↑ = increases; ↓ = decreases; C_{max} = maximum concentration; conc. = concentration; CYP3A4 = cytochrome P450 3A4 isoenzyme; OATP1B1 = organic anion transport peptide 1B1; UGT = uridine 5′-diphosphate glucuronosyltransferase; CYP2C9 = cytochrome P450 2C9 isoenzyme; CYP2C19 = cytochrome P450 2C19 isoenzyme; AUC = area under the curve; INR = international normalized ratio; NTE = not to exceed.

The use of imatinib with P-gp inhibitors, such as pantoprazole, results in increased imatinib systemic exposure [96]. A 70% reduction in imatinib plasma levels was observed when potent CYP3A4 inducers were coadministered. Consequently, imatinib doses of 600–700 mg daily may be needed in the setting of concurrent therapy with CYP inducers. Conversely, concomitant administration of ketoconazole and imatinib resulted in a 40% increase in the AUC and a 26% increase in C_{max} of imatinib[96]. As a result, an imatinib dose of 300 mg daily is recommended with ketoconazole. Caution is advised when using imatinib with other known CYP3A4 inhibitors [96]. Imatinib was shown to increase CSP concentrations 1.7-fold through inhibition of CYP3A4 [47]. Administration of imatinib with other CYP3A4, CYP2D6, and CYP2C9 substrates should also be prescribed with caution [96]. Examples of these substrates include simvastatin, metoprolol, and warfarin, respectively. Avoidance or use of alternative therapies such as pravastatin, atenolol, and low molecular weight heparin, which do not utilize similar metabolic pathways to imatinib, are suggested. Dosage reductions of the substrate may be necessary if the interaction with imatinib is unavoidable.

The AUC and C_{max} of dasatinib are reduced by 60% when it is given concomitantly with antacids and H_2 blockers, and 40% when given with proton pump inhibitors [97,98]. This interaction can be managed by administering the antacid 2 hours before or after dasatinib [97]. Coadministration of dasatinib with CYP3A4 inhibitors may lead to an increase in dasatinib concentration and increased toxicity. Conversely, coadministration of dasatinib with CYP3A4 inducers could lead to a >80% reduction in dasatinib exposure and diminished efficacy [97].

The use of nilotinib with CYP3A4 inhibitors such as azole antifungals is not recommended owing to a 30% to threefold increase in nilotinib concentrations. If concurrent therapy is necessary, the dose of nilotinib should be reduced to 400 mg daily with close monitoring of the QTc interval [95]. Decreased nilotinib levels have occurred with concurrent use of CYP3A4 inducers. Nilotinib dose adjustments should be considered in these patients. Administration of P-gp inhibitors with nilotinib will also increase nilotinib exposure [95].

Like other TKIs, the use of sorafenib with rifampin resulted in 37% lower sorafenib concentrations [96]. Sorafenib doses up to 1000 mg daily are recommended when prescribed with rifampin [96]. A reduction in sorafenib concentration can be expected if it is coadministered with other CYP3A4 inducers. Interestingly, administration of sorafenib with ketoconazole, a CYP3A4 inhibitor, or warfarin, a CYP2C9 substrate, did not result in any alteration of sorafenib levels or coagulation parameters, respectively [96]. Inducers of UGT1A9 such as omeprazole have the potential to decrease the sorafenib AUC [94].

Statins

Atorvastatin, lovastatin, and simvastatin are CYP3A4 substrates whereas fluvastatin is a CYP2C9 substrate [11,99,100]. Atorvastatin, lovastatin, simvastatin, and pitavastatin are P-gp substrates and inhibitors whereas fluvastatin, rosuvastatin, and pravastatin are not [99,100]. All currently available statins are substrates of OATP1B1-mediated transport [99,100]. Administration of statins with drugs that are substrates, inhibitors, or inducers of CYP3A4, CYP2C9, P-gp, and OATP pathways may result in drug interactions. Selected interactions with statins are also described in Table 98.9.

The risk of myopathy or rhabdomyolysis is increased when azole antifungals are administered concurrently with statins. Inhibitors of CYP3A4 cause inhibition of statin metabolism, as evidenced by an increase in atorvastatin, lovastatin, and simvastatin concentrations [11]. In addition, concomitant use of itraconazole significantly increased the AUC of statins except fluvastatin. New package labeling contraindicates the use of lovastatin and simvastatin with itraconazole and posaconazole. [11,99,101]. The effect on the pravastatin AUC with itraconazole is conflicting [99]. In the case of the posaconazole–simvastatin interaction, posaconazole significantly increased the C_{max} and AUC of simvastatin (5–11-fold increase in AUC) [102]. The statin dose reduction should be considered when voriconazole is also prescribed. Fluconazole, a strong inhibitor of CYP2C9 and CYP2C19, causes clinically relevant increases in the AUC and C_{max} of rosuvastatin [103]. The use of statins that do not rely on CYP3A4 for metabolism or an empiric reduction in statin therapy with follow-up monitoring for efficacy are viable approaches to minimizing the risk of azole and statin drug interactions.

Cyclosporine is an inhibitor of P-gp, OATP, and CYP3A4. Inhibition of P-gp and CYP3A4 resulted in an increased AUC when CSP was combined with lovastatin, simvastatin, and atorvastatin [99]. A 20-fold increase in pravastatin and increases in rosuvastatin AUCs with cyclosporine therapy have also been reported. Inhibition of OATP2- and OATP1B1-mediated uptake by cyclosporine was implicated in these interactions [99,103]. A 3.1-fold increase in fluvastatin AUC was also noted with cyclosporine [99]. Vigilant monitoring for statin-related adverse effects, especially myopathy and rhabdomyolysis, is important. Limiting the statin dose or an empiric dose reduction may also be necessary in the setting of cyclosporine and pravastatin, rosuvastatin, or fluvastatin coadministration.

Therapeutic drug monitoring (TDM)

TDM is typically performed on drugs with a narrow therapeutic window, large pharmacokinetic variability, and/or when a direct relationship between plasma concentrations and clinical efficacy and toxicity exists [104]. TDM has historically been used as one method to monitor patient compliance with therapy, efficacy, and toxicity. It may also be useful for managing drug–drug interactions to achieve targeted blood concentrations. TDM is a well-established approach to individualized drug dosing for busulfan, posaconazole, voriconazole, cyclosporine, tacrolimus, and sirolimus. Assays for monitoring trough blood levels of these antifungals and immunosuppressants are available at most transplant centers and routinely used to monitor for therapeutic efficacy and toxicity. AUC monitoring following oral and intravenous busulfan administration is applied as standard in most transplant centers to target blood levels known to affect transplant outcomes, such as cure rates and limit toxicity such as VOD. TDM for cyclophosphamide, mycophenolic acid derivatives, and TKIs have been utilized in various research studies; however, the routine use of these assays in clinical practice is limited owing to the lack of assay availability and unknown clinical significance.

Conclusion

As new drugs are being developed and used in different combinations, the risk for drug interactions will always be a concern. Having a firm understanding of the mechanisms by which drugs are metabolized and eliminated is a powerful tool to help predict and possibly minimize the occurrence of these interactions in the HCT setting.

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CHAPTER 99

Nutrition Support of the Hematopoietic Cell Transplant Recipient

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Introduction

Patients undergoing hematopoietic cell transplantation (HCT) are a highly heterogeneous population in terms of their nutrient needs and requirement for nutrition intervention. The implications of dose-intensive conditioning regimens, coupled with the debilitating effects of graft-versus-host disease (GVHD), can be extremely complex and adversely affect the patient's nutritional status. Decreased oral intake, increased nutrient requirements, and impaired nutrient utilization often necessitate medical nutrition therapy. The use of standard supportive care measures, including parenteral nutrition (PN), enteral nutrition (EN), and other dietary intervention strategies, such as the immunosuppressed diet, have varied over the past four decades and are highly dependent upon the type of transplant being utilized. This chapter highlights nutrition assessment components, appropriate nutrition support practices, and long-term management of nutritional sequelae associated with HCT.

Nutrition assessment

Initial and serial assessment is necessary for all patients undergoing HCT, owing to the anticipated nutrition issues associated with the conditioning regimen and other treatment-related toxicities that alter nutrient intake, absorption, and utilization [1]. A comprehensive evaluation is the foundation that identifies high-risk patients who may require aggressive nutrition intervention [2]. Frequent, ongoing nutrition assessment allows for a rapid response to sudden changes in clinical status.

Pre-transplant assessment

The baseline nutrition assessment may include data obtained from a patient-generated questionnaire, combined with further discussion with the patient and caregiver, and also the medical record, which elicits information about diet, use of nutritional supplements, prior need for nutrition support, subjective information on oral and gastrointestinal (GI) symptoms, and weight history. Components of the nutrition assessment are outlined below [2,3].

Nutrition history

- Oral/GI symptoms, including dysgeusia or hypogeusia, xerostomia, dysphagia or odynophagia, dental health, oral aversions, mucositis or esophagitis, heartburn or reflux, nausea and vomiting, early satiety, anorexia, altered bowel habits;
- current dietary modifications and special diets;
- food allergies or intolerances;
- use and dosage of vitamin, mineral, and herbal supplements;
- integrative medicine therapies;
- current or past nutrition support interventions (i.e. PN, EN) or intravenous fluid (IVF) support;
- infants and young children: if breast-fed, the mother's cytomegalovirus serology must be negative to prevent transmission of virus to the infant (Seattle Cancer Care Alliance, Standard Practice Guideline); if formula-fed, determine infant formula and concentration; assess stage of eating development (e.g., cup versus bottle; purée versus solid foods).

Anthropometry

- Accurate measurement of height and weight, which provide landmark data for serial measurements; for pediatric patients up to 24 months, a recumbent length and occipital frontal circumference are obtained and plotted on the Centers for Disease Control and Prevention growth charts;
- weight history and determination of ideal weight; for patients >120% ideal weight, an adjusted weight should be used; an example of a formula to calculate ideal weight is as follows [4]:

$$\text{adjusted weight} = (\text{actual weight} - \text{ideal weight}) \times 0.25 + \text{ideal weight};$$

- arm anthropometry, as an indicator of somatic muscle protein and adipose reserves, is most useful when obtained sequentially and compared over time [5].

Biochemical indices

- Baseline electrolytes, glucose, renal parameters, liver function enzymes, serum proteins, blood lipids, iron status, and 25-hydroxyvitamin D;
- information from baseline laboratory values serves as a guide to decisions about changes in nutrient and fluid requirements over time, especially if organ function changes [6].

Other assessment tools

- Medical history, including past surgeries and endocrine or cardiac complications;
- physical strength, exercise history, and activity level;

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- organ function, noting any pre-existing alterations in renal or hepatic function;
- drug–nutrient interactions;
- visual assessment, noting presence of obesity, fluid retention, dehydration, or emaciation.

Impact of body weight

Early identification of patients at nutritional risk minimizes the detrimental effects of weight loss or adverse consequences of obesity, both of which have been associated with poor outcome. In a retrospective analysis, Deeg et al. [7] examined the effect of body weight on clinical outcome in 2238 transplant recipients in the first 150 days post-transplant. Patients who were less than 95% of ideal weight had a significantly poor prognosis, with an even poorer prognosis in patients weighing less than 85% of ideal [7]. Using a patient-generated-subjective global assessment tool, Horsley et al. [8] observed that malnourished HCT recipients had a longer length of hospital stay. Obesity also appears to influence outcome in the HCT population, as adult and pediatric transplant recipients with a high body mass index (BMI) have worsened survival outcomes [9,10].

Integrative medicine

The use of complementary or integrative therapies by transplant patients is becoming increasingly prevalent. Patients often pursue these therapies to help their improve sense of wellbeing, reduce anxiety, and provide a sense of control. Bruemmer et al. [11] found that upon arrival for HCT, a high percentage of patients used supplemental vitamins and minerals.

The use of non-conventional therapies, such as herbal and megavitamin therapy, poses great concern for the transplant population. Dietary supplement preparations may alter the therapeutic effect of medications, chemotherapeutic agents, and radiation, thereby impacting treatment effectiveness and adding a variable to the treatment protocol evaluation [12]. Potential contamination of preparations derived from plants poses the risk of bacterial, fungal, or parasitic infections in immunocompromised patients. As supplements are not regulated by the US Food and Drug Administration (FDA), dosages may vary widely and products may contain unlisted contents. Some preparations may be contaminated with toxic metals, drugs, or other botanicals with a drug-like effect, which could result in serious health risks.

Screening for the use of supplements is an important component of the baseline nutrition assessment for all transplant patients; those patients taking supplements should be advised to discontinue all such remedies in the pre-transplant evaluation period. A standard iron-free multivitamin and mineral supplement is recommended for all patients to avoid micronutrient deficiencies that may develop due to either drug-induced vitamin and mineral wasting or changes in eating patterns that may result in a micronutrient deficiency. Supplementation with all antioxidants and other vitamins should not exceed the Dietary Reference Intakes as a precaution to avoid potential interaction with chemotherapeutic agents. Supplements associated with serious adverse effects that are contraindicated during HCT are listed in Table 99.1 [3].

Nutrient and fluid requirements

Guidelines for dosing nutrient and fluid needs are outlined in Table 99.2 [2]. Individual requirements, however, vary in the setting of organ dysfunction. Of paramount importance throughout the transplant course is the provision of adequate oral and/or IVF for maintenance of hydration status.

Table 99.1 Contraindicated supplements during HCT

Alfalfa
Botanicals containing pyrrolizidine alkaloids (borage, coltsfoot, comfrey, groundsel, valerian, maté tea)
Botanicals that react with drugs utilizing the cytochrome P450 enzyme system (echincea, garlic, ginkgo, ginseng, grape seed, kava)
Chaparral
Chinese/oriental herbs
DHEA
Dieter's tea
Ephedra
Laetrile
Licorice root
Lobelia
L-Tryptophan
Pau d'arco
Pennyroyal
Sassafras
St. John's wort
Yohimbe
Yohimbine

Source: adapted from Macris (ed.) 2012 [3]. Reproduced with permission of Seattle Cancer Care Alliance.

Electrolyte and metabolic complications may alter nutrient needs. Table 99.3 [3] lists nutrition intervention strategies for common complications observed during HCT.

Nutrition support

The goal of nutrition support is to provide sufficient exogenous energy substrates, especially during regimen-related toxicities and GVHD, to minimize the loss of lean body mass, and to avert morbidity associated with malnutrition.

Oral diet

Oral feedings are indicated for patients with a functional GI tract. Historically, HCT recipients were treated with GI decontamination in ultraisolation environments and served diets of varying microbial content ranging from "sterile" to "cooked food only," in attempts to minimize the introduction of pathogenic organisms [13]. A survey by Smith and Besser [14], involving 156 institutions belonging to the Association of Community Cancer Centers, reported that most transplant centers still utilize some type of neutropenic diet for their HCT population.

Although several studies have examined the role of diet and infectious risk, the protective benefit of a low microbial diet against infection has not been established [1]. DeMille et al. [15] examined the effect of a neutropenic diet on infection in a group of 28 adult patients and reported no significant differences in rates of febrile admissions or positive blood cultures between compliant and non-compliant patients. A study by Moody et al. [16] compared the neutropenic diet with the FDA-approved food safety guidelines in children undergoing aggressive chemotherapy regimens and observed that infection rates were similar between the groups.

Although more research about the protective benefits of a neutropenic diet for the HCT population is needed before its effectiveness can be established, present practice is to implement a diet that restricts high-risk foods and safe food handling [1]. An example of a diet for immunocompromised transplant patients is presented in Table 99.4. In general, autologous patients follow the diet during the first 3 months after transplantation; allogeneic patients follow the diet until all immunosuppression has been discontinued.

When oral intake is not adequate in meeting nutrient needs, EN in the form of tube feedings or PN support may be indicated.

Table 99.2 Nutrient and fluid requirements

Nutrient	Requirement	Altered requirements
Calories	Adults: Basal needs × 1.3–1.5 *or* 30–35 kcal/kg Adolescents and older children: Basal needs × 1.4–1.6 Young children: Basal needs × 1.6–1.8	Increase: Immediate post-transplant period due to metabolic stress induced by the preparative regimen, fever, infections Decrease: Engraftment; absence of metabolic complications; limited physical activity
Protein	Adults: 1[a]–1.5 g/kg/day 15–18 years: 1.8 g/kg/day 11–14 years: 2 g/kg/day 7–10 years: 2.4 g/kg/day Birth–6 years: 2.5–3 g/kg/day	Increase: Immediate post-transplant period; corticosteroid treatment; CRRT Decrease: Renal or hepatic dysfunction
Carbohydrate	50–60% total energy support Dextrose load: Adults: <5 mg/kg/min Adolescents: 7–10 mg/kg/min Children: ≤15 mg/kg/min	Decrease: Lower dextrose PN or hydration fluid concentration with pre-existing diabetes or hyperglycemia Modify oral carbohydrate intake when insulin required; provide diabetes education
Fat	Minimum dose: 6–8% total calories Maximum dose: 40% total calories	Increase: When providing lower dextrose PN concentrations Decrease: Hyperlipidemia
Fluids	1–10 kg: 100 mL/kg/day 11–20 kg: 1000 mL + 50 mL for every kg >10/day 21–40 kg: 1500 mL + 20 mL for every kg >20/day >40 kg: 1500 mL/m^2 BSA	Increase: fever; excessive GI losses; hypermetabolism; high-output renal failure; nephrotoxic medications Decrease: Compromised liver, cardiac or pulmonary function; iatrogenic fluid overload
Vitamins	Parenteral: standard adult and pediatric packages Extra vitamin C to promote tissue recovery after cytoreductive therapy: <31 kg: additional 250 mg/day ≥31 kg: additional 500 mg/day Oral: after PN discontinued, oral vitamin-mineral supplement without iron	Increase: Vitamin D in presence of low serum 25-OH-vitamin D levels: <20 ng/mL: 50,000 units weekly for 8 weeks, followed by 1000–2000 units/day <30 ng/mL or steroid therapy: 1000 units/day Decrease: Discontinue extra vitamin C if serum ferritin >1000 μg/L CRRT: Oral: Standard renal vitamin Parenteral: ½ dose standard vitamin package + water-soluble vitamins; evaluate need for fat-soluble vitamin replacement
Trace minerals and electrolytes	Parenteral: standard adult and pediatric packages Typical initial PN additives per liter: 30 mEq sodium chloride 25 mEq potassium chloride 15 mEq potassium phosphate 8 mEq calcium gluconate 16 mEq magnesium sulfate Oral: after PN discontinued, oral vitamin–mineral supplement without iron	Electrolyte needs are frequently higher owing to food–drug interactions (Table 99.3); serum electrolytes should be monitored daily during PN and 2–3 times weekly in the ambulatory clinic; adjust IV or oral supplementation as indicated Increase: Calcium during corticosteroid therapy or documented osteoporosis: 1500 mg/day for adults and older children 1000–1200 mg/day for younger children Zinc with large-volume diarrhea: 1 mg/100 mL stool output Decrease: Iron supplementation contraindicated Eliminate copper and manganese from PN in presence of hepatic dysfunction

BSA: body surface area; CRRT: continuous renal replacement therapy; GI: gastrointestinal; IV: intravenous; PN: parenteral nutrition.

[a] Non-myeloablative regimens.

Source: Macris (ed.) 2013 [2]. Reproduced with permission of the Academy of Nutrition and Dietetics.

Enteral nutrition

When the GI tract is functional and intact, EN is the preferred method of nutrition support [1]. During HCT, EN support is appropriate for patients who are malnourished and who are anticipated to be unable to ingest and/or absorb adequate nutrition for a prolonged period. Historically, barriers such as thrombocytopenia, GI complications, mucositis or esophagitis, lack of enteral access, and patient acceptance precluded the use of enteral feedings in the transplant population, so PN was standard supportive care [17]. In recent years, however, EN has attracted more attention owing to the need to reduce costs and the desire to mitigate infection risk, often associated with PN [6]. The benefits of EN support include maintenance of mucosal gut integrity with stimulation of mucosal repair, decreased incidence of hyperglycemia, maintenance of normal gut barrier function, decreased risk of infection, and decreased cost [6,17]. A recently published study by Seguy et al. [18] suggested that provision of EN support may have a protective effect against the development of grade III–IV acute GVHD and also on transplant survival.

Candidates for EN support in the transplant setting include patients who receive non-myeloablative or reduced-intensity conditioning regimens with poor oral intake or malnutrition or those undergoing a myeloablative conditioning regimen with a lower GI toxicity profile [6,19]. The use of EN may also be an option for patients receiving a low-risk transplant (autologous or matched related donor), those with long-term eating problems following engraftment, resolution of conditioning-related mucositis and esophagitis, and adequate platelet count (as defined by the institution) [6,19]. Finally, EN may be appropriate for patients with chronic oral or esophageal GVHD or ongoing weight loss when insufficient nutrients are consumed for weight maintenance.

Although EN may be a viable form of nutrition support, case series or pilot studies suggest formidable challenges in utilizing nasoenteric or enterostomy feeding tubes. Sefcick et al. [20] reported tube displacement by vomiting and aspiration in a pilot study of 15 allogeneic transplant patients. Lenssen et al. [21] observed similar results of vomiting with dislodgement of feeding tubes, despite scheduled antiemetics, in a cohort of eight transplant recipients. Post-transplant gastroparesis was described by Eagle et al. [22], with allogeneic patients at higher risk of delayed gastric emptying compared with the autologous population (26% vs. 0%; $p < 0.0001$). Poor tolerance of rapid advancement of feeds, as indicated by diarrhea and abdominal pain, when transitioning from PN after myeloablative conditioning is another complication associated

Table 99.3 Nutrition intervention strategies for common metabolic complications

Measure	Indicator	Nutritional intervention
Potassium	<3.5 mEq/L related to: Correction of hyperglycemia GI losses Medications: amphotericin, corticosteroids, diuretics, foscarnet Refeeding syndrome/anabolism >5.5 mEq/L related to: Excessive supplementation (e.g. resolving potassium wasting) Medications: cyclosporine, potassium-sparing diuretics, tacrolimus Renal insufficiency Tumor lysis syndrome	Oral: Develop a diet plan that includes high-potassium foods and fluids Oral supplementation IV: Supplement in PN, IVF, or separate IV infusion up to a maximum 20 mEq/h (0.3 mEq/kg/h for children) without a cardiac monitor; maximum 10–15 mEq/h in ambulatory care Develop a diet plan that includes low-potassium foods and fluids Discontinue all potassium supplementation
Magnesium	<1.8 mEq/L related to: Diarrhea/steatorrhea Malabsorption syndromes Medications: amphotericin, cyclosporine, foscarnet, loop diuretics, prior cisplatinum therapy, tacrolimus Refeeding syndrome Severe malnutrition >2.4 mEq/L related to: Discontinuation of a magnesium wasting medication Excessive supplementation Renal insufficiency	Oral: Develop a diet plan that includes high-magnesium foods and fluids Oral supplementation IV: Supplement in PN, IVF, or separate IV infusion, not to exceed 24 mEq/h (1 mEq/kg/h for children) Discontinue all magnesium supplementation; decrease IV before oral supplement
Phosphorus	<2.5 mg/dL (<4.5 mg/dL for children) related to: Medications: corticosteroids, cyclophosphamide, foscarnet, sirolimus Mineralocorticoid deficiency Refeeding syndrome Renal acidosis >4.5 mg/dL (>6.0 mg/dL for children) related to: Excessive phosphorus intake Multiple myeloma Renal failure Tumor lysis syndrome	Oral: Develop a diet plan that includes high-phosphorus foods and fluids Oral supplementation IV: Supplement in PN, IVF, or separate IV infusion up to 7 mEq/h in ambulatory clinic or 5 mEq/h for home administration Oral: Develop a diet plan to limit foods high in phosphorus (*not recommended for extended periods owing to the possibility of decreased intake of other nutrients such as protein and calcium*) Phosphate binders with meals Discontinue all phosphorus supplementation
Calcium	<8.9 mg/dL (corrected for abnormal serum albumin) related to: Diarrhea Hyperphosphatemia Hypoalbuminemia Hypomagnesemia Medications: corticosteroids, foscarnet Vitamin D deficiency >10.2 mg/dL (corrected for abnormal serum albumin) related to: Hyperparathyroidism Multiple myeloma Renal failure Tumor lysis syndrome	Correct serum level in presence of hypoalbuminemia Check ionized serum calcium level Provide IV calcium gluconate at starting dose of 9 mEq (0.45 mEq/kg for children <20 kg) Discontinue all calcium supplementation Provide sodium-containing IVF as necessary If on PN, remove calcium; if alkalotic, add chloride
Sodium	<135 mEq/L (corrected for abnormal serum glucose levels) related to: Diabetes insipidus Excess GI losses Excessive IV or fluid/free water intake Fluid retention Hyperglycemia Medications: cyclophosphamide, cyclosporine, furosemide Syndrome of inappropriate antidiuretic hormone >145 mEq/L related to: Medications: corticosteroids, foscarnet	Oral: If fluid overloaded, develop a diet plan that limits total fluid intake to 1500 mL × BSA or less as indicated IV: If excessive sodium losses, consider normal saline infusion Oral: Decrease dietary sodium intake IV: Adjust sodium additive in PN; change to low-sodium containing IV hydration
Glucose	≥180 mg/dL on two or more episodes related to: Medications: corticosteroids, cyclosporine, tacrolimus Excessive dextrose load Patient or family history of DM Sepsis	Oral: Nutrition education regarding appropriate meal and snack choices, including monitoring intake of simple carbohydrate foods and fluids; if on insulin therapy, assess need for carbohydrate counting IV: During PN: decrease total energy support if in excess of needs; adjust macronutrients to lower carbohydrate load while maintaining adequate energy support by adjusting protein and fat load as indicated; attempt continuous infusion of macronutrients throughout 24-hour period Initiate PN at a lower dextrose concentration Add insulin to PN; an insulin drip may be necessary if serum glucose is labile or refractory in increasing insulin in PN If on IVF: change to dextrose-free hydration Endocrinology consult
Triglyceride	Non-fasting or fasting level of >350 mg/dL related to: DM Excessive dextrose support Familial hyperlipidemia Hepatic dysfunction Medications: corticosteroids, cyclosporine, hormones, contraceptives Obesity Pancreatitis Sepsis	Oral: Develop a diet plan that may include achieving desirable body weight, limit simple carbohydrate and fat intake; increase omega-3 fatty acid intake; no alcohol intake IV: Provide IV lipids at 4–8% total calories If serum triglycerides >1000 mg/dL, hold lipids and cycle PN for mobilization of body stores of essential fatty acids Endocrinology consult

BSA, body surface area; DM, diabetes mellitus; GI, gastrointestinal; IV, intravenous; IVF, intravenous fluid; PN, parenteral nutrition.
Source: adapted from Macris (ed.) 2012 [3]. Reproduced with permission of Seattle Cancer Care Alliance.

Table 99.4 Diet guidelines for immunosuppressed patients

Food restrictions

- Raw and undercooked: meat (including game), fish, shellfish, poultry, eggs, sausage, bacon
- Raw tofu, unless pasteurized or asceptically packaged
- Luncheon meats (including salami, bologna, hot dogs, ham) unless heated until steaming
- Refrigerated smoked seafood typically labeled as lox, kippered, nova-style, or smoke or fish jerky (unless contained in a cooked dish; pickled fish
- Unpasteurized milk and raw milk products, unpasteurized cheese, and unpasteurized yogurt
- Blue-veined cheeses including blue, Gorgonzola, Roquefort, Stilton
- Uncooked soft cheeses including Brie, Camembert, feta, farmer's
- Mexican-style soft cheese, including queso blanco and queso fresco
- Cheese containing chili peppers or other uncooked vegetables
- Fresh salad dressings containing raw eggs or contraindicated cheeses
- Unwashed raw and frozen fruits and vegetables and those with visible mold; all raw vegetable sprouts
- Raw or unpasteurized honey
- Unpasteurized commercial fruit and vegetable juices
- Well water must be boiled for 15–20 minutes and consumed within 48 hours

Source: Reproduced with permission of Medical Nutrition Therapy Services, Seattle Cancer Care Alliance, Seattle, WA, USA.

with enteral feedings [19]. Finally, weight loss [20], decreased body cell mass [23], and electrolyte and mineral deficiencies [24,25], due primarily to difficulties in maintaining access and in delivering adequate support, are other noted implications associated with EN support.

Experience at our institution suggests that when absolute neutrophil count (>1000/mm^3) and platelet count (>50,000/mm^2) are adequate, nasoenteric and enterostomy feeding tubes may safely be placed [19]. A range of enteral formulas have been necessary depending upon the patient's GI tolerance and organ function. Adult and pediatric semi-elemental formulas, renal formulas, and concentrated formulas, when volume restriction has been indicated, have all been used [2,6]. Mulder et al. [26] also suggested that the combined use of EN with PN support may be an acceptable and cost-effective alternative.

Parenteral nutrition

Historically, PN was standard supportive care for HCT recipients due to the multiple adverse effects of regimen-related toxicities and GVHD, which precluded tolerance to oral feedings or EN; the constant availability of central venous access also made the administration of PN fluids possible. Early studies suggested that PN support during transplantation was associated with improved visceral protein status [27], maintenance of body weight [28], and increased disease-free survival in the allogeneic population [29]. More recent studies, however, have reported conflicting evidence supporting the routine use of PN. The use of PN during transplantation has been associated with complications such as hyperglycemia [30], increased infection risk [31], and thrombocytopenia [32], which may result in greater morbidity and poorer outcome.

To limit these complications, shortening the exposure time of PN support may be strategic in limiting infections or other adverse complications once stem cells have engrafted and regimen-related toxicities have resolved [1]. The clinical decision to discontinue PN, however, should consider the patient's overall nutritional status, severity of GI toxicities, and ability to maintain oral intake. Provision of PN can safely be discontinued, without adverse effects, when patients consume approximately 30% of estimated energy needs, without evidence of malnutrition, malabsorption, or other significant GI toxicities, at time of hospital discharge [33,34]. Discontinuation of PN has also been shown to result in an earlier resumption of oral intake after transplantation [34].

Candidates for PN may include transplant recipients receiving high-intensity conditioning regimens with anticipated severe GI toxicity that precludes adequate intake [19]. The use of PN is also indicated for patients who develop severe intestinal GVHD or other GI toxicities such as high-volume infectious diarrhea, patients who fail to receive adequate nutrition from an enteral route (orally or via tube feedings), and malnourished patients with minimal reserves [1,17].

Even with high-intensity preparative conditioning regimens, however, PN is not uniformly indicated for all patients. Iestra et al. [35] applied standard criteria for malnutrition in oncology patients to determine the appropriate use of PN during HCT: (1) severe malnutrition at admission (serum albumin <3 g/dL or BMI <18.5 kg/m^2), (2) a prolonged period (7–10 days) of minimal oral intake, or (3) clinical weight loss of >10%. Indications for the use of PN differed significantly between treatment groups; PN was required in only 37% autologous patients conditioned without total body irradiation (TBI) versus 92% of patients who received a mismatched allograft [35].

With the advent of reduced-intensity and non-myeloablative conditioning regimens, PN is no longer considered standard supportive care as many of these conditioning protocols are associated with less regimen-related toxicities than standard myeloablative regimens. Diaconescu et al. [36] compared 73 non-myeloablative with 73 myeloablative matched related transplant recipients and found that the greatest difference between the groups was seen in GI toxicities, with no severe mucositis among non-myeloablative patients compared with a 70% incidence of mucositis among ablative patients. A similar study by Topcuoglu et al. [37] showed that mucositis was observed less frequently and with decreased severity and duration in a reduced-intensity conditioning regimen compared with a myeloablative regimen (46% vs. 93%; $p < 0.0001$), resulting in a decreased need for PN (21% vs. 77%; $p < 0.0001$). Other investigators have also documented a significant reduction in the need for PN due to decreased GI injury in reduced-intensity and non-myeloablative conditioning regimens [38,39].

Irrespective of the type of transplant, a major complication of PN is hyperglycemia. Fuji et al. [30] reported an association between the degree of hyperglycemia during neutropenia and an increased risk of complications and non-relapse mortality (NRM). Management of PN-induced hyperglycemia is dependent upon the patient's clinical status, etiology, and institutional practice, with the goal of achieving a glucose level as close to normal as safely possible [19]. Provision of intravenous lipids, with a concomitant decrease in dextrose substrate, may also be used to minimize the development of hyperglycemia that can occur in patients who have sepsis, pre-existing diabetes mellitus, or GVHD treated with glucocorticoids [40]. During episodes of PN-induced hyperglycemia, the nutrition support regimen should be continuously evaluated to ensure that the patient is not being overfed and/or receiving excessive carbohydrate [6].

Intravenous lipids are a necessary component of PN to supply energy and essential fatty acids. While lipids have been implicated as being immunosuppressive, there is not an established increased risk of infection in the transplant population. Lenssen et al. [41] compared the rates of bacteremia and fungemia in autologous and allogeneic HCT patients receiving 6–8% (low dose) or 25–30% (standard dose) of total energy as a 20% lipid emulsion. The results showed that there were no treatment-related differences in incidence or time to first infection or incidence of acute or chronic

GVHD [41]. A review by Arfons and Lazarus [42] suggested that because lipids may alter the immunologic responses of prostaglandins and leukotrienes, they may play a role in decreasing the incidence of acute or chronic GVHD. More research is needed to determine the impact of lipids on the development of GVHD [40].

The management and prevention of PN-induced complications require close monitoring by the clinician. Hagiwara et al. [43] suggested that intervention with a nutrition support team may provide more economical and clinical efficacy with respect to managing clinical complications, such as hyperglycemia.

Glutamine-enriched nutrition support

Over the past two decades, considerable research has been focused on the role of glutamine-enriched nutrition support during HCT to ameliorate gut and liver toxicity [40]. Studies assessing the impact of orally administered glutamine have shown no effect on morbidity or mortality in transplant recipients [44,45]. Patients maintained on glutamine-enriched PN solutions have been reported to have decreased hospital stays, reduced incidence of positive blood cultures [46], and improved lymphocyte recovery [47]. Conversely, two randomized double-blind clinical trials described significantly more relapses in autologous patients receiving parenteral glutamine administration [48,49]. More recent data suggest that additional studies are needed to determine the appropriate dose and timing of glutamine supplementation in the HCT population [50].

Special management problems

HCT recipients experience numerous problems that interfere with adequate nutrient intake and nutritional status. These complications may be associated with the pre-transplant conditioning regimen and include oral and GI sequelae and organ or tissue damage resulting in hepatic, renal, or pulmonary impairment; iron overload and glucocorticoid-induced hyperglycemia are also frequently observed. Moreover, GVHD has its own unique nutritional challenges that impact nutritional status.

Oral/gastrointestinal issues

As nutrition-related effects vary widely between myeloablative, reduced-intensity, and non-myeloablative transplant regimens, recommendations are not universal for all transplant recipients. Myeloablative conditioning regimens often result in toxicities, such as oral mucosal ulceration, that develop within the first month and significantly impact the patient's ability to achieve adequate oral intake, thus necessitating nutrition support. Oral mucositis is a frequent complication of HCT with an incidence as high as 75–99% [51].

Robien et al. [52] observed that patients treated with a TBI conditioning regimen who had methotrexate for GVHD prophylaxis, in addition to a BMI >25 kg/m^2, and methylenetetrahydrofolate reductase 677 TT genotype, were found to have a higher oral mucositis index score. Multivitamin supplementation in these patients showed a trend towards statistical significance as a protective factor for the development of severe mucositis [52].

Novel approaches to preventing mucositis during HCT have included the use of gut-protective therapy and cryotherapy. Palifermin, a humanized keratinocyte growth factor that preserves intestinal barrier function, has been shown to decrease the severity and duration of mucositis, in very mucotoxic regimens, resulting in less need for nutrition support [53]. Cryotherapy has been effective in reducing the severity of grades III–IV mucositis, with a statistically lower use of PN in 40 autologous patients treated with melphalan [54].

Conditioning chemotherapy and TBI decrease and destroy taste receptor cells, resulting in dysgeusia or altered taste sensation, which affects food selection and contributes to poor oral intake [19,55]. Medications, including morphine, antibiotics, tacrolimus, and cyclophosphamide, are also associated with taste alterations [19]. Studies examining taste alterations in HCT patients have shown increased taste thresholds for both salt [56,57] and sour [57,58]. Whereas an early study reported recovery of taste changes by day 30–50 after transplantation [58], a more recent study found that patients continued to report dysgeusia as late as day 90–100 after transplantation [59]. Nutrition management of dysgeusia requires a complete clinical and nutritional evaluation with appropriate nutrition counseling.

Nausea and vomiting are most frequently associated with the preparative conditioning regimen, especially those containing alkylating agents, TBI, and upper intestinal GVHD [3]. Antiemetics are routinely prescribed to prevent and control symptoms, with the goal of allowing patients to continue eating and taking oral medications. With severe GI symptoms, nutrition support may be indicated. Diarrhea is common following high-dose cytoreductive therapy, oral antibiotics, intestinal infections, such as cytomegalovirus enteritis or *Clostridium difficil*e colitis, intestinal GVHD, lactose intolerance, gastric motility agents, and magnesium salts [3,19].

Dietary guidelines for managing oral and GI toxicities associated with HCT are described in Table 99.5.

Sinusoidal obstructive syndrome

The condition of sinuosoidal obstructive syndrome (SOS) is characterized by toxic injury to the sinusoidal and venular epithelium [6]. Clinical symptoms include insidious weight gain, ascites, right upper quadrant tenderness and hepatomegaly; hyperbilirubinemia and renal dysfunction follow [60]. Medical nutrition therapy entails management of sodium and water balance through concentration of PN fluids and medication volumes as well as reduction of both oral and intravenous sodium; continuous renal replacement therapy may be necessary if renal function is compromised [3,60,61]. If hyperbilirubinemia (serum bilirubin rises to >10 mg/dl), biliary excreted tace elements, copper and manganese, should be removed from PN solutions [2]. If hypertriglyceridemia (serum triglyceride >350 mg/dL) develops, the dose of intravenous lipid should be modified to provide 4–8% of total calories to prevent essential fatty acid deficiency [3]. The patient's level of nutrition support should be also assessed to minimize the risk of overfeeding and ensure optimal carbohydrate metabolism as a lower dextrose load may be indicated [3].

Renal complications

Acute kidney injury is a recognized complication of HCT with a prevalence as high as 70% following HCT [62]. Renal compromise ranges from prerenal insufficiency to acute renal failure requiring continuous renal replacement therapy and is associated with an increased risk of morbidity and mortality [62,63]. Nutrition support goals during renal impairment are to minimize uremic toxicity and other metabolic derangements while preventing malnutrition. Nutrition intervention involves maximizing nutrition support within fluid allowance, correcting electrolyte imbalances, and maintaining sufficient intravascular volume [19]. During continuous renal replacement therapy, protein and vitamin needs are altered as shown in Table 99.2.

Table 99.5 Nutrition management of common transplant-related oral and gastrointestinal complications

Indicator	Recommended intervention
Lack of interest in eating due to HCT *Related to:* Anxiety Depression Dysgeusia Food aversion Hepatic disease History of prior eating disorder Lack of interest in food Long period of NPO Upper intestinal GVHD/lymphocytic gastritis	Develop a diet plan that may include small, frequent, low-fat feedings and encourage food experimentation to optimize oral intake Evaluate appropriateness of appetite stimulant
Early satiety *Related to:* Hypomotility of GI tract due to prolonged NPO, conditioning, GVHD Medications: cyclosporine, narcotics Severe ascites Splenomegaly PN	Develop a diet plan that may include small, frequent, low-fat feedings and nutritionally dense fluids to optimize oral intake If prolonged course of PN, trial off or cycle infusion at night Gastric motility agents
Vomiting/nausea *Related to:* Altered salivary secretions Anticipatory nausea and vomiting Dehydration Food aversion GVHD Hepatic disease Medications: cyclosporine, trimethoprim + sulfamethoxazole Systemic infections	Develop a diet plan that may include salty or sour foods; cold, clear liquids; cold solids; small, frequent meals; and low-fat, low-fiber foods to optimize oral intake Scheduled antiemetics including GI motility agents If symptoms are severe, begin PN or postpyloric tube feeding
Dysgeusia/hypogeusia *Related to:* Conditioning regimen, especially TBI Medications Oral infections	Encourage good oral hygiene Develop a diet plan that may include a variety of highly spiced, flavorful foods (if mucositis/esophagitis absent) and food experimentation to optimize oral intake
Xerostomia *Related to:* Conditioning regimen Dehydration Healing mucositis Medications: anticholinergics, methotrexate Oral GVHD	Develop a diet plan that may include high-moisture foods, the use of saliva stimulants (e.g. citric acid beverages, sugarless candy, sugarless gum) to optimize oral intake Additional oral or IV fluids as needed Encourage good oral hygiene Saliva substitute
Mucositis *Related to:* Conditioning regimen Methotrexate Oral GVHD Oral viral infections	Develop a diet plan that may include altered texture/temperature and provides adequate vitamin/mineral supplementation to optimize intake If treatment plan includes high-dose melphalan, consider cryotherapy If oral intake is suboptimal, consider EN or PN support
Esophagitis/odynophagia ***Related to:*** Bacterial overgrowth Conditioning regimen GVHD Infection Mucositis Persistent vomiting Prolonged intubation GERD	Develop a diet plan that may include avoidance of alcohol, spicy foods, high-fat foods, and secretagogues; alteration of texture and temperature of foods as appropriate; and maintenance of upright position postprandially If oral intake is suboptimal, consider EN or PN support Initially antacids, then proton-pump inhibitors or H_2 blocker
Dysphagia *Related to:* Chronic GVHD Poor salivary flow Prolonged intubation Reflux/GERD	Speech pathology swallow evaluation Develop a diet plan that may include alterations of texture and temperature If oral intake is suboptimal, consider EN or PN support
Diarrhea *Related to:* Antibiotics Bacterial overgrowth Conditioning regimen Intestinal GVHD GI infection Lactose intolerance Medications: gastric motility agents, magnesium salts Viral enteritis	Adult: if stool exceeds approximately 2000 mL/day, for several days, place patient NPO Child: If stool exceeds approximately 500 mL/day for several days, place patient NPO Initiate GI diet progression (see Table 99.6) If extended NPO, recommend PN Trial with soluble fiber If lactose intolerant, provided low lactose diet and prescribe lactase enzymes If magnesium-related, modify dose and institute IV magnesium supplementation If suspect related to gastric motility agent, discontinue oral medication and institute IV supplementation Antidiarrheals for mild diarrhea Develop a diet plan that may include low-fat, low-insoluble fiber; low-lactose products and lactase enzyme preparation; avoid GI irritants/stimulants and offending disaccharides
Constipation *Related to:* Drug toxicity: narcotics History of GI dysfunction Inactivity Low-fiber diet Low fluid intake	Develop a diet plan that includes dietary fiber with increased insoluble fiber and fluid intake Encourage physical activity as appropriate Assess need for pharmacological intervention

EN, enteral nutrition; GERD, gastrointestinal reflux disease; GI, gastrointestinal; GVHD; graft-versus-host disease; IV, intravenous; IVF, intravenous fluid; NPO, nothing by mouth; PN, parenteral nutrition; TBI, total body irradiation.
Source: Adapted from Macris (ed.) 2012. Reproduced with permission of Seattle Cancer Care Alliance.

Pulmonary compromise

Pulmonary complications account for significant morbidity and mortality in transplant recipients and are related to infections, regimen-related toxicities, and chronic GVHD [64]. Nutrition intervention for patients who develop transplant-related pulmonary compromise include reducing total sodium intake (oral, intravenous, and medications) and restricting fluids by using concentrated dextrose and amino acid solutions and also lipid emulsions. Assessing the patient's level of nutrient support to minimize the risk of overfeeding is also necessary. Finally, for patients with excessive CO_2 production, adjusting the proportion of energy from carbohydrate and fat may be necessary [3]. Pulmonary insufficiency associated with chronic GVHD may raise metabolic requirements resulting in hypercaloric needs to prevent weight loss and debilitation, so close nutrition monitoring is required.

Iron overload

Iron overload, related to multiple red blood cell transfusions and dyserythropoiesis leading to aberrant hepcidin regulation and increased iron transport by the intestine, is frequently observed during transplantation [65,66]. McKay et al. [66] reported an 88% incidence of elevated serum ferritin concentrations, suggestive of iron overload, in a group of 76 autologous and allogeneic transplant survivors. For this reason, iron supplementation is contraindicated during HCT. If baseline serum ferritin is grossly elevated (i.e., >1000 μg/L), no additional vitamin C, which acts as a pro-oxidant in the presence of free iron (other than what is in the standard parenteral or oral vitamin supplement), should be provided to minimize iron-induced organ damage [3].

Glucocorticoid-induced diabetes

Hyperglycemia is a common complication of glucocorticoid therapy used in the treatment of GVHD in allogeneic transplant recipients. A retrospective review by Pidala et al. [67] demonstrated that aberrant glucose levels were associated with increased NRM. Recommendations from this study include routine assessment of glycemic control with management in the setting of a multidisciplinary team [67]. The registered dietitian is a vital team member who can provide appropriate nutrition education, especially when glucocorticoids are tapered, as oral intake and insulin needs vary widely.

Acute graft-versus-host disease

Acute GVHD is an inflammatory disease predominantly targeting the skin, liver, and GI tract. Clinical GI symptoms associated with acute GVHD include nausea, vomiting, anorexia, early satiety, and/or diarrhea. Diarrhea related to GVHD is multifactorial in etiology and includes villous atrophy, mucosal ulceration, secretory dysfunction, osmotic factors, rapid passage, and pancreatic insufficiency [68]. Secretory, voluminous diarrhea and intestinal bleeding occur in advanced disease [69]. Intestinal losses often result in dehydration, loss of electrolytes, fat, and protein and also intolerance of oral and enteral feeding, necessitating bowel rest and PN support. In addition to immunosuppressive therapy, empiric diet guidelines have been developed as detailed in Table 99.6 [70]. The diet progression for refeeding patients includes bowel rest with PN support, followed by introduction of liquids and solids low in lactose, fiber, acid, and fat, and expansion of the diet as tolerated [70].

Rezvani et al. [71] observed that a change in serum albumin concentration from baseline to initiation of acute GVHD treatment was a predictive biomarker of GVHD severity and mortality in 401 allogeneic transplant recipients following a reduced-intensity conditioning regimen. A serum albumin level ≥ 0.5 g/dL decrease from baseline value predicted the subsequent development of grades III and IV acute GVHD with a sensitivity of 69% and a specificity of 73% [71].

Acute GVHD may also involve the oral mucosa, with symptoms ranging from mild pain to frank mucosal ulceration [19]. Patients with severe oral GVHD may require EN or PN support to maintain lean body mass if an adequate oral intake cannot be sustained.

Initial treatment of patients with acute GVHD often includes glucocorticoid therapy. Long-term exposure to glucocorticoids, however, may result in infectious, metabolic, and other adverse complications, thereby increasing transplant-related morbidity and mortality [72]. Lee et al. [73] reported that >40% of patients with acute GVHD treated with high-dose glucocorticoids develop

Table 99.6 Gastrointestinal diet progression: the table is an idealized progression and serves as a guideline rather than a rigid regimen

Phase	Clinical symptoms	Diet	Clinical symptoms of diet intolerance
1. Bowel rest	GI cramping Large-volume watery diarrhea Depressed serum albumin Severely reduced transit time Small bowel obstruction or diminished bowel sounds Nausea and vomiting	Oral: NPO IV: Stress energy and protein requirements	
2. Introduction of oral feeding	Minimal GI cramping Diarrhea <500 mL/day Improved transit time Infrequent nausea and vomiting	Oral: Isotonic, low-residue, low-lactose fluids IV: As for Phase 1	Increased stool volume or diarrhea Increased emesis Increased abdominal cramping
3. Introduction of solids	Minimal or no GI cramping Formed stool	Oral: Allow introduction of solid foods containing minimal lactose, low fiber, low fat, low total acidity, no gastric irritants IV: As for Phase 1	As in Phase 2
4. Expansion of diet	Minimal or no GI cramping Formed stool	Oral: Minimal lactose, low fiber, low total acidity, no gastric irritants; if stools indicate fat malabsorption: low-fat diet IV: As needed to meet nutritional requirements	As in Phase 2
5. Resumption of regular diet	No GI cramping Normal stool Normal transit time Normal serum albumin	Oral: Progress to regular diet; acid foods with meals, fiber-containing foods, lactose-containing foods; order of addition will vary depending on individual tolerances and preferences; patients without steatorrhea should have fat restriction slowly liberalized IV: Discontinue when oral intake meets nutrient needs	As in Phase 2

Source: Gavreau et al. 1981 [70]. Am Diet Assoc 79:673–7. Reproduced with permission of Elsevier.

myopathy. For these patients, a resistive exercise program has been shown to be beneficial in increasing muscle strength [74]. Alterations in nutrient needs during glucocorticoid therapy are outlined in Table 99.2 [2].

Long-term complications and management

As advances in technology and supportive care measures have improved, the number of long-term HCT survivors continues to increase. With this growth, a greater emphasis is now being placed on treating long-term complications [2].

Chronic graft-versus-host disease

Chronic GVHD, a multisystem disease involving inflammation and fibrosis, has a somewhat different presentation to the acute form and often occurs later in the transplant course. However, an "overlap" syndrome has been recognized in which diagnostic or distinctive features of both chronic GVHD and acute GVHD appear together [75].

Chronic GVHD is a separate entity from acute GVHD and, like acute GVHD, can affect nutritional status. A retrospective review of 192 allogeneic transplant recipients found that weight loss, weight gain (due to glucocorticoid therapy), oral sensitivity, xerostomia, stomatitis, anorexia, and reflux symptoms were among the nutritional sequelae observed at 1 year after transplantation [76]. Kyle et al. [77] found that patients with chronic GVHD had a significantly lower lean body mass with a higher body fat index at 6 and 12 months after transplantation.

Oral manifestations, as described above, are common in patients with chronic GVHD, leading to inability to chew and swallow food properly and affecting diet variety. Texture modification, including liquid, puréed, or soft foods, is indicated during dysphagia with referral to a speech pathologist for swallowing evaluation. With severe symptoms, esophageal dilatation may be necessary. If oral and/or esophageal symptoms preclude adequate calorie–protein intake for weight maintenance, gastrostomy tube placement for EN support is appropriate.

Diarrhea and nutrient malabsorption, associated with chronic GVHD, can result from abnormal intestinal mucosa, intestinal bile salt deficiency, pancreatic exocrine insufficiency, and bacterial overgrowth [78]. Histologic changes in pancreatic ductular tissue may result in pancreatic insufficiency with subsequent steatorrhea [65]. Diagnosis of fat malabsorption may be confirmed through screening tests such as fecal elastase (<100 μg/g) or serum trypsinogen (<20 ng/ml) [3,65]. Most patients respond to supplementation with oral pancreatic enzymes and dietary fat restriction; the use of products containing medium-chain triglycerides may also be indicated [3,78].

Growth and development issues

Impaired growth and development frequently occur in pediatric patients following HCT. Myeloablative conditioning regimens influence endocrine function. With cyclophosphamide alone, thyroid function, growth rates, and pubertal maturation are normal. Decreased growth velocity, growth hormone deficiency, and delayed onset of puberty have been observed in children treated with busulfan or TBI [79]. All children undergoing HCT should have regular evaluations to detect endocrine dysfunction. Initiation of appropriate hormonal therapy, when abnormal endocrine function is detected, may improve growth and development.

Metabolic syndrome

Metabolic syndrome, characterized by obesity, hyperlipidemia, hypertension, and glucose intolerance, is becoming more prevalent in both adult and pediatric transplant survivors. Majhail et al. [80] reported a 49% prevalence of metabolic syndrome in 86 transplant survivors more than 1 year after transplantation compared with a prevalence of 30% in controls selected from the 2005–2006 National Health and Nutrition Examination Study database. Annaloro et al. [81] also reported a high incidence of metabolic syndrome in a cohort of 85 patients who were over 5 years after autologous and allogeneic HCT. Chow et al. [82] showed a statistically significant difference in the incidence of cardiometabolic traits, including central adiposity, hypertension, insulin resistance, and dyslipidemia in childhood survivors transplanted for acute lymphoblastic leukemia compared with controls ($p = 0.02$). Increased serum C-reactive protein and leptin levels with decreased adiponectin, suggestive of underlying inflammation and increased visceral fat, were also observed in the survivors [82]. In general, pediatric HCT survivors are more likely to develop diabetes [83] and hypertension [84] than the general population and should be monitored throughout adulthood. Ongoing dietary surveillance and referral to a registered dietitian for nutritional counseling to address contributing dietary factors to metabolic syndrome are appropriate.

Osteoporosis and alterations in vitamin D status

Osteoporosis is a recognized complication of HCT with a prevalence as high as 50% as early as 1 year after transplantation [85,86]. Factors such as chemotherapy, TBI exposure, calcineurin inhibitors, and glucocorticoids all affect bone loss [87]. Prevention and management of osteoporosis includes calcium and vitamin D supplementation. Calcium requirements vary by age and vitamin D needs are typically based on serum levels (Table 99.2). Strategies to improve bone density after transplantation include regular weight-bearing and muscle strengthening exercises [3,87]; bisphosphonate therapy, in conjunction with calcium and vitamin D supplementation, has also been reported to improve bone mineral density [88].

Vitamin D deficiency and insufficiency are common following HCT in both adult [89] and pediatric transplant recipients [90], and current research is examining the relationship between vitamin D status and GVHD. Some researchers speculate that low serum vitamin D levels may play a role in the development of GVHD due to alterations in T-lymphocyte regulation and function [91]. Further studies are needed, however, to determine the impact of vitamin D supplementation on GVHD.

Conclusion

In HCT, an aggressive treatment that results in multiple acute and long-term nutritional challenges, nutrition support has been a life-preserving modality. The registered dietitian plays a key role in recommending and implementing nutrition intervention strategies for these complex patients. Ongoing nutrition assessment and monitoring are essential in this highly technical and rapidly evolving form of therapy.

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CHAPTER 100

Pain Management

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Introduction

Patients who receive hematopoietic cell transplantation (HCT) may suffer pain of varying etiologies and severity (Table 100.1). This chapter defines the neurophysiology and pharmacology of pain; what is meant by "pain;" what factors lead a patient to experience and suffer pain; what physical, psychological, environmental, and pharmacologic factors influence the amount of pain and suffering; how this compendium of pain and suffering can be evaluated; and the different means by which pain and suffering can be relieved in the HCT recipient.

Anatomy and physiology of pain

Classically, pain begins with tissue damage (nociception), the type and location detected by peripheral nerves and then signaled to the brain. Our understanding of the neurophysiology and neurochemistry of nociceptive damage detection, transmission to the central nervous system (CNS), and CNS processing to produce the clinical phenomenon of pain, is far from perfect. Our understanding of how the CNS may be influenced by pharmacologic or non-pharmacologic means to suppress nociception and reduce pain also is imperfect. A summary of the known neurophysiology and neuropharmacology of nociception and pain is helpful in understanding clinical management of the HCT patient.

Ascending nociceptive systems

All body tissues possess nociceptors (neural structures that detect tissue damage) that are activated by physical stimuli or by algogenic (pain-producing) substances released when there is tissue injury. Alternatively, nociceptors may be sensitized by algogenic substances, resulting in noxious activation by normally innocuous stimuli, for example, light touch in the presence of inflammation. Nociceptors signal to the spinal cord through fast-conducting Aδ fibers, or slow-conducting C fibers in peripheral nerves, which enter the neuraxis and synapse through dorsal horn cells (Figure 100.1). Dorsal horn cell axons cross the midline and ascend cephalad in the spinothalamic tracts. Information from the fast Aδ fibers ascends rapidly, with no intermediate synapses, to the thalamus, and hence to the cerebrum. Information from the slow C fibers ascends through a number of different synapses, notably to the limbic system, before reaching the thalamus and cerebrum. The C fiber/limbic system synapses initiate the emotional responses to pain [1].

Modulation of nociceptive traffic

Afferent nociceptive transmission is not "hard wired" – signals do not progress to the cerebrum unimpeded and unimpedable. With evolving neurophysiologic knowledge, it has been recognized that afferent transmission may be modulated, as hypothesized in the "gate control theory" of pain [2,3]. This theory states that there are various "gates" within the nervous system that may be opened, or closed, to nociceptive transmission by a variety of processes. The "gating" concept is a useful tool when teaching patients and relatives about pain relief techniques. A basic understanding of this physiology helps patients and family members understand that active interventions, pharmacologic and non-pharmacologic, are essential to obtain optimal pain relief.

Afferent nociceptive transmission can be enhanced or, more importantly, inhibited by the following:

1. Modification of the peripheral nociceptor signaling. Algogenic substances [prostaglandins, serotonin (5-hydroxytryptamine, or 5-HT), substance P, bradykinin] can activate or sensitize peripheral nociceptors; anti-inflammatory medications [aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs)] can oppose such effects.
2. Inhibition of nociceptive transmission in the dorsal horn by non-noxious peripheral stimulation. Rubbing, heat, cold, or electric stimulation peripherally activates sensory afferent fibers that synapse with dorsal horn cells to inhibit nociceptive transmission.
3. Inhibition of dorsal horn nociceptive transmission by the descending nociceptive modulating system. This process originates in the periaqueductal gray matter of the hindbrain (Figure 100.1). The neurotransmitters of this system include 5-HT, norepinephrine (NE), and endogenous opioid (morphine-like) substances, among others. The descending modulatory system can be activated to produce pain relief by learned behavioral or cognitive strategies, or by pharmacologic agents that either mimic endogenous neurotransmitters (e.g. opioids) or stimulate activity of the system {e.g. tricyclic antidepressants (TCAs) [4] and, probably, serotonin–norepinephrine reuptake inhibitors (SNRIs) [5]}.

The clinical phenomenon of pain

The neurophysiology and neuropharmacology described so far deal only with physiologically demonstrable phenomena. The experience of pain clearly is more than neurophysiology, encompassing the subjective interpretation and expression of the neurophysiologic phenomenon.

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Table 100.1 Common sources of pain in bone marrow transplantation

Etiology	Examples
Infiltration from primary disease	Bone pain Fractures Soft tissue of visceral pain
Hematopoietic stem-cell mobilization	Bone or joint aching
Chemotherapy or irradiation	Mucositis Cutaneous burns Peripheral neuropathies
Graft-versus-host disease	Skin irritation Gut cramps Oropharyngeal pain
Immunosuppressive drugs	Peripheral neuropathies Joint aching Long bone pain Osteopenic bone fractures
Infection or viral activation	Shingles from herpes zoster Postherpetic neuralgia Cytomegalovirus gut pain
Diagnostic and surgical procedures	Bone marrow aspiration Lumbar puncture Postlumbar puncture headache Line placement Tissue biopsies
Pains unrelated to disease	Headache Low back pain Myalgias from inactivity

What is pain?

Pain is defined as a multidimensional experience [6]. The degree to which an individual feels pain, suffers from pain, and complains of pain is influenced by a number of factors, in addition to tissue damage/nociception. The model depicted in Figure 100.2 [7] is helpful in conceptualizing these components. Nociception is the detection of tissue damage by sensory transducers, and the signaling of this information to the brain. Processing of nociceptive information by the CNS results in the human experience of pain, which is both sensory and affective (feeling). Suffering, the negative cognitive and emotional responses to pain, can be heightened by pain-independent emotional factors, such as fear, anxiety, and depression, that may impact the HCT patient [8]. The compendium of nociception, pain, and suffering, which are subjective, personal, and unmeasurable, emerge to be expressed as pain behaviors. Observations of pain behaviors, including report of pain and inhibition of activity, constitute the raw data that allow the diagnosis of the source of pain and estimation of the magnitude of suffering.

Pain and suffering in the HCT patient

When an HCT patient complains of pain, the most common circumstance is one of obvious tissue damage, such as in oropharyngeal mucositis (OPM). Patients may also complain of pain with little evidence of tissue damage (neuropathy). Irrespective of its source, nociceptive information is processed identically by the CNS, and in all cases the patient feels pain, suffers, and exhibits pain behaviors.

Some patients have easily observed pathology, such as an erythematous and ulcerated mucosa in the mouth, but complain of little pain and appear to suffer little. Other patients have little or no evidence of tissue damage yet complain of much pain and suffer a great deal. What can a clinician make of these two disparate situations? Is the first patient really in pain and really suffering, but not telling anyone? Is the second patient imagining or faking pain? Both explanations are plausible, but unlikely. The fact is, the relationship between nociception, pain, suffering, and pain behaviors is

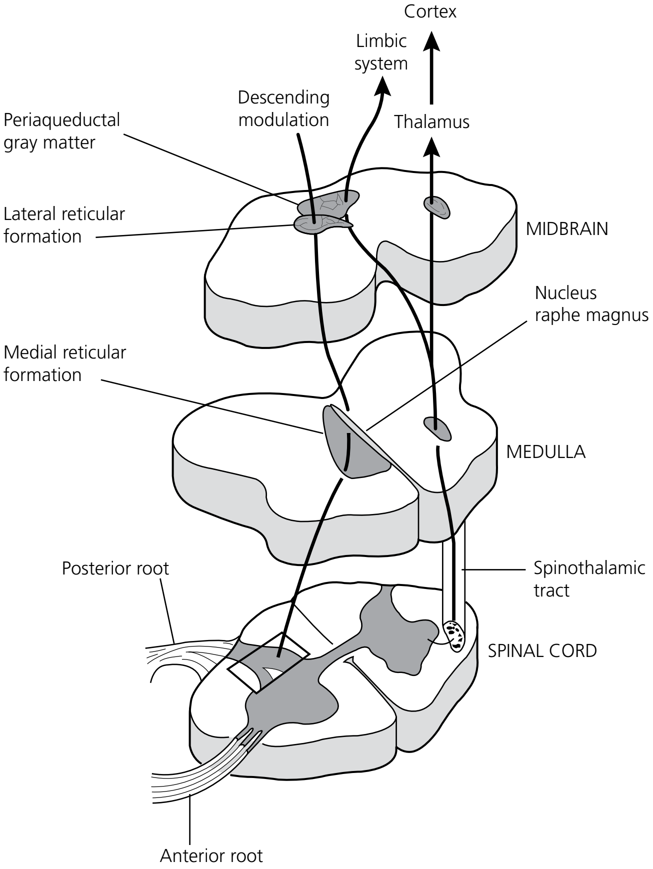

Figure 100.1 Nociceptive information is carried to the spinal cord in Aδ and C fibers. The nerves enter the dorsal horn, where they synapse with second-order neurons that cross the midline of the cord at about the entry level and ascend in the spinothalamic tracts. The Aδ-derived information goes on a fast track directly to the thalamus. The C fiber-derived information reaches the thalamus later, having synapsed in the limbic system. The descending nociceptive afferent modulating system originates in the periaqueductal gray matter. It projects through the nucleus raphe magnus down to the posterior horn, where it has a modulatory effect on the afferent nociceptive traffic.

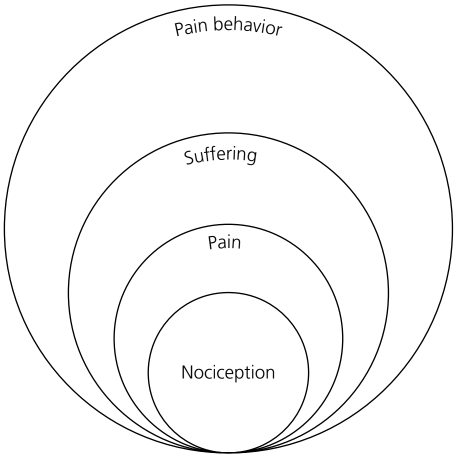

Figure 100.2 A schema of the various aspects of the human pain experience.

not a fixed one. A number of correlates, in addition to physiologic and pharmacologic factors, influence how much patients feel, suffer and complain of pain. Among these correlates are the following:

1 The meaning of the pain. Pain from a known or predictable source often results in less suffering and fewer complaints than pain from an unknown, potentially threatening source.
2 The circumstances in which the pain occurs. A simple pain problem, such as toothache, may result in more suffering and complaints in a patient undergoing HCT than the same pain experienced in normal circumstances.
3 The patient's style of coping with adversity. This style is influenced by social and cultural factors. At one end of the spectrum are those who tough it out and never call for help; at the other end are those who call for help at the first sign of adversity.
4 Emotional state. Fear or anxiety can lead patients to be hyperattentive to sensations. Similarly, depression often reduces activities and increases attention to discomforts.
5 Previous experience with pain. Experience with chemotherapy may serve to reassure patients that current experience is "normal" and will resolve. An inexperienced patient may be thinking, "How can I feel so bad and possibly be doing OK?"
6 Previous exposure to CNS-active pharmacologic agents (analgesics, anxiolytics, or alcohol). These agents may directly influence CNS processing of nociception. If a patient is opioid tolerant or has extensive exposure to alcohol, opioid dosing needs may be higher than average. Usage patterns may also reflect the patient's style of coping with adversity, perhaps to escape into a "pharmacologic fog."

It is well to remember that, regardless of the source, all patients have cognitive and processing components to their pain. Remember the phrase "no brain, no pain." If it is not processed in the brain, it is not felt. Most HCT patients have a nociceptive source of their pain, onto which the cognitive and processing components are grafted.

Assessment of pain and treatment efficacy

History

Some patients will have histories suggesting they are at risk of having significant pain problems or may have difficulty obtaining pain relief. Significant historical factors include:

1 active pain problems related or unrelated to the patient's disease or therapies;
2 previous severe pain after surgery or trauma;
3 difficulty in obtaining satisfactory pain control during events in items 1 and 2;
4 non-pharmacologic pain-relieving strategies that the patient uses;
5 analgesics that the patient has used and any problems with them;
6 previous or current significant emotional or affective disorders;
7 previous or current CNS-active substance use (alcohol, opioids, and anxiolytics);
8 the patient's understanding of the current pain, its significance, and likely duration;
9 the patient's preferred mode of dealing with pain (both non-pharmacologic and pharmacologic).

Pain measurement and monitoring

How is one to assess what the HCT patient is experiencing? Much of the system of nociceptive transmitting, processing, and suffering is subjective and not directly observable (Figure 100.2). It is possible to observe and quantify the system's output (complaints of pain) and use that as an approximation. In clinical practice, an HCT patient's pain is what the patient says hurts, in the amount the patient says it hurts. Those caring for HCT patients should accept pain complaints as real, genuine, worthy of attention, and deserving alleviation.

In practice, pain and its impact are monitored by subjective pain scores and observational function scores. The indices used to monitor pain, the effects, and side effects of therapies and medication use should be observed and recorded frequently using a pain assessment tool. Far from feeling burdened by reporting pain and other symptoms on a daily or per shift basis, patients are often relieved that staff are aware of their symptoms. Consistent evaluation assures patients that their experience is recognized and that evaluation is routine, and therefore normal. Uncertainty and unpredictability about what will happen cause the greatest distress for the greatest number of HCT patients [9–11]. Knowledge that the pain is expected, how it is likely to feel, and how long it is likely to last can help patients to endure even extremely difficult circumstances.

Cancer and HCT patients are reluctant to complain and do not know what experiences are "normal" or what should be reported. They will often answer "fine" or "OK" if asked only, "How are you feeling?" Pain scores and ratings can be used as "objective" quality assurance tools to determine the need for further evaluation or change in treatment. Pain above the equivalent of a score of 3 or 4 (on a scale of 0–10) probably indicates a need to improve pain treatment because the pain likely will substantially disrupt function [12], although in chronic pain patients a more sophisticated rating scale may prove to be more helpful [13]. Even temporary pain should not exceed a score of 6 without mandating further evaluation or additional pain treatment methods [12–15]. The Joint Commission mandates pain assessment as the "fifth" vital sign; pain scores of 5 or above strongly suggest the need for active intervention [16].

Pain scales

Adults and children from about the age of 7 years can report with scales as seen in Figures 100.3, 100.4, and 100.5:

1 Numerical rating scales use scores from 0 = "no pain" to 10 = "pain as bad as can be." It can be helpful the first time with a patient to use a few familiar examples such as: "When you think of a headache you have had in your lifetime, can you remember how it felt?" (Help the person think of a pain he or she remembers.) "When you think of that pain, where would it be on a scale from no pain which is a 0 to pain as bad as anyone could possibly have, which is a 10?" Further help can be offered by saying: "Many people think of 1, 2, or 3 as milder pain; of 4, 5, or 6 as moderate pain; of 7, 8, or 9 as severe pain; and of 10 as being the most extreme pain anyone could ever have." If the patient is still unable to use the scale for cognitive or cultural reasons, the addition of faces, as in Figure 100.4, can be helpful. Numerical scales can be given orally or on paper, as seen in Figure 100.3. In general, these numerical scales are easy to use and record. This scale is favored by clinicians and researchers [14,17].
2 Categorical scales use descriptive words from "no pain" to "extremely severe pain." Such scales are well understood by patients, but are difficult to record or to use to track pain and treatment success.
3 Visual analogue scales. These are 10-cm lines anchored at one end with "no pain" and at the other end with "pain as bad as can be." Patients make a mark at any point along the scale to indicate pain intensity. These scales require measurement of the line in millimeter increments after the patient completes the written scale.

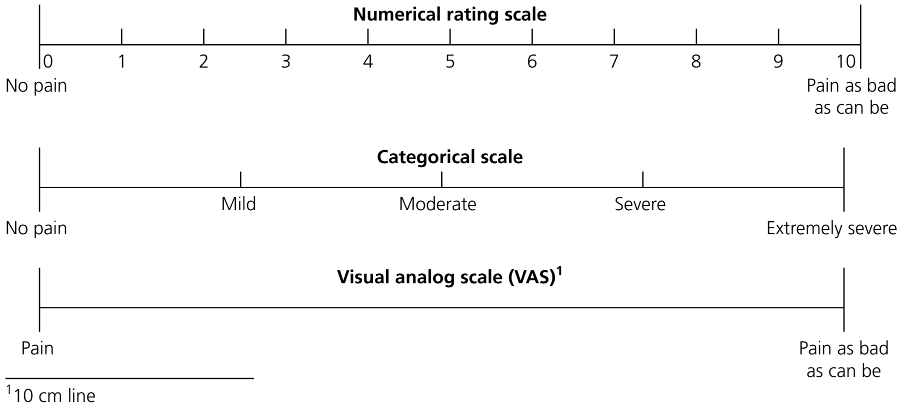

Figure 100.3 Self-report scales for assessing pain in adults. The numerical rating scale is used most frequently.

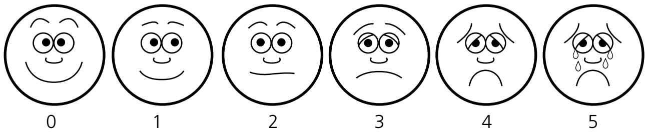

Figure 100.4 A "faces" scale used to monitor pain and pain management efficacy in children between ages 3 and 7 years.

Children below the age of 7 years usually cannot use the above scales. From ages 3 to 7 years, children can usually indicate their pain using one of a variety of "Faces" scales (Figure 100.4) [18]. Children below the age of 3–4 years cannot use such scales, and observational methods from nurses or family members may be used. The most commonly used, and best-validated, observational tool for preverbal children is the Faces, Legs, Activity, Cry, Consolability (FLACC) tool (Figure 100.5) [19]. Although designed specifically for assessment of postoperative pain, this tool has utility for preverbal children in other settings. In children with cognitive impairment, parental observation is a workable proxy measure [20]. A revised form of the FLACC is reliable and valid for cognitively impaired children [21].

Ability to function

The provision of adequate pain control is essential not only for humane reasons, but also to preserve the patient's ability to provide self-care and to maintain health. Function may be directly observed based on specific behaviors necessary to participate in HCT (bathing, exercise, and mouth care). Pain behaviors may be noted, such as requests for medication, grimacing, or refusals to move the affected area of the body [22].

Medication use and side effects

Analgesics reduce the sensation of pain and can improve function, but can also produce unacceptable side effects. The magnitude of drug consumption and the occurrence of common side effects should be monitored and recorded on the same scale format and on the same assessment sheets as pain scores.

Pain diagnostics and therapy selection

A clear diagnosis of the cause of a pain is essential in selecting therapies. To be effective, therapies must be aimed at the specific issues that contribute to the patient's problem. For pain with a large nociceptive contribution from tissue damage, for example OPM, antinociceptive agents (analgesics) are appropriate. For pain with a nociceptive component originating from neural tissue, different agents are appropriate, for example, anticonvulsants. For pain complaints that are concurrent with strong emotional/environmental components, treatments aimed at those components, in addition to pharmacologic agents, are appropriate. The first step in intervention is usually education as described below. Pharmacologic therapies should be pursued in a rational pharmacokinetic and pharmacodynamic fashion, for a period that will give the therapies a reasonable trial.

Treating the patient with difficult pain management problems

Pain may be alleviated by a variety of methods that can be initiated and used effectively by the staff caring for the patient. Sometimes, the magnitude of the problem may be beyond abilities of the primary caregivers. In these circumstances, the assistance of personnel with special expertise in pain management (nurses, neurologists, anesthesiologists, psychologists, and psychiatrists) may be helpful. The assistance can be provided in a variety of forms, ranging from occasional consultations to weekly "pain rounds," to the presence of a formal pain consultation team.

Non-pharmacologic techniques in pain management

When HCT patients report pain, analgesic medications are appropriately considered as a first line of treatment. Concurrently, non-pharmacologic methodologies are almost always incorporated into the treatment of the patient, whether or not these interventions are intended [23]. Even with optimal opioid and other analgesic methods, HCT patients with OPM may receive only 50–60% reductions in pain with analgesics [24,25].

Mechanisms of non-pharmacologic techniques

The broad goal of non-pharmacologic methods is to close the "gate" on pain messages, or to impede the transmission of pain messages and facilitate transmission of non-pain messages. In general, this is done by disrupting afferent pain pathways through the addition of competing sensory inputs, or by modifying descending pathways through changes in thoughts and responses to the sensory messages received.

A number of non-pharmacologic methods seem to operate mechanically, modulating transmission of ascending nociceptive messages by sending competing sensory messages from the body surface or limbs. Putting these mechanical methods into

Categories	Scoring		
	0	1	2
Face	No particular expression or smile	Occasional grimace or frown; withdrawn, disinterested	Frequent to constant frown, clenched jaw, quivering chin
Legs	Normal position or relaxed	Uneasy, restless, tense	Kicking or legs drawn up
Activity	Lying quietly, normal position, moves easily	Squirming, shifting back and forth, tense	Arched, rigid, or jerking
Cry	No cry (awake or asleep)	Moans or whimpers, occasional complaint	Crying steadily, screams or sobs; frequent complaints
Consolability	Content, relaxed	Reassured by occasional touching, hugging, or being talked to; distractable	Difficult to console or comfort
Each of the five categories is scored from 0–2, resulting in a total score between 0 and 10.			
The FLACC scale was developed by Sandra Merkel, MS, RN, Terri Voepel-Lewis, MS, RN, and Shobha Malviya, MD, at C.S. Mott Children's Hospital, University of Michigan Health System, Ann Arbor, MI.			

Figure 100.5 Assessment of Behavioral Score on the FLACC. Each category is scored 0–2 for a possible total of 10. 0, Relaxed and comfortable; 1–3, mild discomfort; 4–6, moderate pain; 7–10, severe discomfort/pain. Source: Merkel et al. 1997 [19]. Reproduced with permission of Pediatric Nursing.

operation requires activity. Patients must increase their physical activity, increase thoughts not focused on pain, and achieve an emotional calmness through a sense of control over some aspects of functioning. Lying in bed in a dark, quiet room, without outside stimuli, does not permit the activation of these mechanical methods and can lead patients to focus on the dominant sensory input – pain.

Staff and family members can assist patients by preparing structured activities and statements that facilitate attention to stimuli other than the pain. These need not be complex. Indeed, patients have limited attention span and often have disruptions in fine motor control. Simple conversation, walking outside the room or movement in the room, and simple games all serve to distract the mind from discomfort.

Education of patients and families, except in rare instances, provides reassurance and enhances the ability to cope with unfamiliar and uncomfortable situations. Autonomic nervous system activity decreases as anxieties and fears are allayed. Resultant changes in 5-HT and NE availability help patients as they prepare to guard against worsening symptoms. Adaptive thoughts are those that reassure or calm the patient and relax the autonomic nervous system.

Emotions are closely tied to and influenced by thoughts. Cognitive–behavioral methodologies for treating depression and anxiety successfully have been adapted specifically for cancer and HCT patients [23,25–28]. Patients who are more distressed pretransplant report greater pain during transplant [10,25]. Over time, unrelieved pain can engender feelings of helplessness and hopelessness. Introduction of alternative experiences of joy or self-control counters these feelings. When any of us feels more in control, we are better able to manage difficulties, and we feel less discomfort and less distress [9–11,29].

Strategies used in normal medical or nursing practice influence the thoughts that patients have about their situation. Information, education, and "reframing" techniques can facilitate coping. Other behaviors that are particularly useful in influencing both the ascending and descending modulatory systems include physical methods such as the use of ice or heat, rest when appropriate, transcutaneous nerve stimulation, and physical positioning (see [30] for details on the use of ice, heat, and massage).

Information

Information that can be helpful to patients includes:

1 Time-frame information. When is something likely to happen? How long will it last? It is helpful to break events into the smallest time frames possible.
2 Procedural information. What will be done? What comfort measures will be used?
3 Sensory information to prepare patients for specific discomforts.

Education

Education empowers patients to participate in their medical care and to modify their own behaviors. In addition to facts, patients must have the opportunity for dialogue to fit their own situation. Increasingly, patients and families administer their own pain medication and participate actively in medical treatment. To adhere to treatments, patients must understand them and not be frightened by the potential side effects.

The common barriers to willingness to use available pain treatments are well established [31,32]. Patients, family members, and medical staff frequently fear opioids and are reluctant to identify when treatment is inadequate. Specific areas where HCT patients and their families need education are listed in Table 100.2. Problems with resistance to medications or lack of understanding about pain are routinely prevented by conducting a brief assessment and education session with patients and families prior to the start of treatment. This session serves the additional purpose of identifying those patients who may experience difficulties achieving comfort.

Education alone can significantly reduce pain, reduce length of hospitalization, decrease complications, and improve overall

Table 100.2 Pain-related education need for hematopoietic cell transplantation (HCT) and their families

Question	Usual information offered
Why treat pain?	Pain is harmful to your physical health There is no benefit to proving you can stand pain without treatment, but there are serious negative health effects from suffering unnecessary pain
What treatments are common?	Most HCT patients need strong pain medications (opioids) at some time
When are treatments used:	
How often?	When pain is constant, scheduled doses provide better relief than waiting until pain is severe to take medication
How much at a time?	Your doctor will only prescribe safe levels of medication: let your nurse or doctor know if: You are too sleepy during the day Nausea increases when you take medication You have other symptoms that are new You have a new pain
How do you know when more is needed?	Pain medication should be used to keep pain under control, rather than waiting until pain is intolerable
Is addiction a concern?	HCT patients with no history of overusing drugs or alcohol have no cravings for the medication when the pain stops. Even people who need high doses of medication do not become addicts
Do people become tolerant to the drugs so that they stop working if pain gets really bad?	If you use the medication to keep the pain under control, the medication will not stop working when the pain increases, or if pain continues longer than expected
What if the medication does not work or makes a person sick?	There are many pain treatments. Tell your doctor or nurse if pain is out of control or if a medication makes you feel bad – numerous treatment options are available in these cases

outcomes [33–35]. Information and education do not cause anxiety where none exists, and they are not harmful [36,37].

Other cognitive–behavioral strategies

Reframing, active distraction, and relaxation are all techniques that can be valuable adjuncts to the use of analgesic medications. To the degree possible, speak about events in positive terms, give patients a sense of control (including participation in clinical decisions), involve patients in activities (if only as an observer at times), and provide a calm and caring environment. Research has shown that more specialized techniques, such as imagery and hypnosis, can be helpful but rarely eliminate the need for analgesic medications [25,27,38]. More widely available, and simple, techniques, such as progressive muscle relaxation, deep breathing, and concentrating on pleasant thoughts and places, also are helpful adjuncts to pain relief [39,40].

Pediatric considerations

Most of these methods need little adaptation for use with children. Age-appropriate information is important for children. As with adults, this information should reinforce areas over which the child has some control, such as asking for medication or understanding rewards for self-care activities. Children can plan for their own active distractions and can be engaged in play even when quite ill. Story telling, art projects, and play are effective imagery and distraction methods for children with pain.

It is crucially important to be aware that many children will withdraw from socializing when they are in pain. Their quiet demeanor should not be confused with comfort. Socialization and playfulness are often the best indicators of effective pain relief.

Non-opioid pharmacologic management of pain

NSAIDs and acetaminophen

Many non-opioid analgesics, both over-the-counter and prescription, are available. Two major groups are NSAIDs and phenacetin derivatives, acetaminophen being the only clinically used drug. All of these analgesics exhibit a "ceiling effect." Efficacy will increase with increasing doses up to a particular level; dosing beyond that point will not improve analgesia, but will expose the patient to dose-related side effects.

Other than the distinction that NSAIDs suppress inflammation and acetaminophen has few anti-inflammatory effects, there are few hard data on the comparable efficacy of the various agents as analgesics. Different agents offer varying durations of action, degree of side effects, and individual patient tolerance. These actions tend to be quite idiosyncratic, so it would be unwise to recommend any specific medication regimen. It is essential to tailor therapy on an individual basis.

Non-opioid analgesics are suitable for use in mild-to-moderate nociceptive and inflammatory pain. Alone they provide inadequate analgesia for moderate-to-severe nociceptive pain but, if used in conjunction with opioids, they may improve the quality of analgesia and demonstrate an "opioid-sparing effect," reducing the dose of opioid necessary to produce a given level of analgesia and thus reducing the potential for side effects. Non-opioid analgesics in the management of neuropathic pain currently are under study. Emerging data suggest a limited, but expanding, role for *N*-methyl-D-aspartate (NMDA) antagonists such as subanesthetic ketamine [41].

Pharmacology of NSAIDs and acetaminophen

The pharmacologic effect of NSAIDs is to inhibit the enzyme cyclooxygenase, part of the arachidonic acid cascade, reducing production of prostaglandins, one of the algogenic agents released by injured tissue (others being bradykinin, histamine, and 5-hydroxytryptamine) that stimulate or sensitize nociceptors. Consistent with their anti-inflammatory efficacy, the primary effect of NSAIDs classically has been believed to be peripheral. However, prostaglandins also play a role in the CNS transmission of pain [42–45]. Selective cyclooxygenase-2 (COX-2) inhibitors have limited antiplatelet activity and have less gastric toxicity than other NSAIDs, but are associated with an increased risk of major cardiovascular events [46]. (The only COX-2 inhibitor currently available in the United States is celecoxib.)

Acetaminophen produces similar analgesia to NSAIDs but, unlike NSAIDs, has only minor anti-inflammatory effects. Acetaminophen is as potent as aspirin in inhibiting prostaglandin synthesis in the CNS, but has little effect on prostaglandin metabolism peripherally [47–51]. Analgesic effects of acetaminophen and NSAIDs appear to be additive [52–54].

Side effects of NSAIDs and acetaminophen

Non-opioids have a variety of side effects that are of significance for, and limit their use in, the HCT patient:

1. Interference with platelet function. This lasts for days after stopping the drug in the case of aspirin, or for shorter periods with other NSAIDs. Although COX-2 inhibitors have little, or no, effect on platelet aggregation, they have recently been shown to increase the risk of cardiovascular events [46]. Acetaminophen has virtually no hematologic effects.
2. Gastric irritation and occult upper gastrointestinal bleeding. This is a feature of virtually all NSAIDs, to some extent, even the COX-2 inhibitors. Acetaminophen is benign to the gut.

3 Renal effects. In high dosage, NSAIDs have direct renal toxic effects. The effect of moderate NSAID dose upon already compromised renal function is not clear. Acetaminophen has virtually no renal effects.

4 Hepatic effects. In high dosage, acetaminophen is severely hepatotoxic, and it is generally recommended that patients not ingest more than 4 g daily, although the current thinking suggests that lowering the maximum daily dose to 3 g is safer [55]. The HCT patient may be at higher risk of acetaminophen toxicity due to the high incidence of concurrent or recent hepatotoxic therapy, graft-versus-host disease (GVHD), hepatitis, and poor nutritional status.

Use of NSAIDs and acetaminophen in HCT

The role of NSAIDs for pain management in the acute phase of HCT is limited by the fact that they are available only in oral form (except ketorolac), by their hematologic and gastrointestinal side effects, and by the fact that they may suppress pyrexia. The use of acetaminophen is limited primarily by its potential to suppress pyrexia. Table 100.3 includes dosing data for acetaminophen and the NSAIDs [56].

Table 100.3 Dosing data for acetaminophen and NSAIDs

Drug	Usual dose for adults and children >50 kg body weight	Usual dose for children[a] and adults[b] <50 kg body weight
Acetaminophen[c]	650 mg/4 h 975 mg/6 h	10–15 mg/kg/4 h 15–20 mg/kg/4 h (rectal)
Aspirin[d]	650 mg/4 h 975 mg/4 h	10–15 mg/kg/4 h 15–20 mg/kg/4 h (rectal)
Carboprofen	100 mg TID	
Choline magnesium trisalicylate[e]	1000–1500 mg TID	25 mg/kg TID
Choline salicylate[e]	870 mg/3–4 h	
Diflunisal[f]	500 mg/12 h	
Etodolac	200–400 mg/6–8 h	
Fenoprofen calcium	300–600 mg/6 h	
Ibuprofen	400–600 mg/6 h	10 mg/kg/6–8 h
Ketoprofen	25–60 mg/6–8 h	
Ketorolac tromethamine oral[g]	10 mg/4–6 h (to a maximum of 40 mg/day)	
Ketorolac tromethamine intravenous[g,h]	30 mg initially, then 15 mg/8 h	
Magnesium salicylate	650 mg/4 h	
Meclofenamate[i]	50–100 mg/6 h	
Naproxen	250–275 mg/6–8 h	5 mg/kg/8 h
Naproxen sodium	275 mg/6–8 h	
Sodium salicylate	325–650 mg/3–4	
Celecoxib[j]	200 mg/day	
Rofecoxib[j]	12.5–25 mg/day	

TID, three times daily.

[a] Only drugs that are approved by the US Food and Drug Administration as an analgesic for use in children are included.

[b] Acetaminophen and NSAID dosages for adults weighing less than 50 kg should be adjusted for weight.

[c] No antiplatelet activity.

[d] The standard against which other NSAIDs are compared. May inhibit platelet aggregation for more than 1 week and may cause bleeding. Aspirin is contraindicated in children with fever or other viral disease because of its association with Reye syndrome.

[e] May have minimal antiplatelet activity.

[f] Administration with antacids may decrease absorption.

[g] For short-term use (less than 5 days).

[h] Has the same gastrointestinal toxicities as oral NSAIDs.

[i] Coombs-positive autoimmune hemolytic anemia has been associated with prolonged use.

[j] Cyclooxygenase-2 inhibitors.

Source: adapted from Dahl and Raeder 2000 [52].

Antidepressants

TCAs have established their efficacy over decades and still are in widespread use. They have analgesic effects independent of their antidepressant effect, providing pain relief in the non-depressed patient, or providing pain relief, without affecting mood, in the depressed patient [57–59]. Moreover, the analgesic effects of many TCAs occur at lower dose and blood levels of drug than those which produce antidepressant effects. Newer selective serotonin reuptake inhibitors (SSRIs) are commonly used to treat depression, and they too may have analgesic effects.

TCAs are thought to relieve pain by altering the 5-HT and NE levels in the descending antinociceptive modulating system. Studies suggest that NE and 5-HT specificity are important in producing analgesia. In humans, concomitant 5-HT and NE enhancement is effective [60–62]. More recent work suggests that NE sites are more active; increased 5-HT levels have intrinsically weak analgesic effects but enhance the analgesic effects at NE sites [63–65].

Antidepressants for neuropathic pain

There is a growing literature on the use of antidepressants for neuropathic pain; see the section Neuropathic pain for details.

Antidepressant administration in HCT

There is concern about the use of TCAs in the HCT patient, as many TCAs appear to have marrow-suppressant effects [66]. Clinical experience indicates that up to 25% of HCT patients will have significant marrow suppression in the early engraftment period with TCAs. If TCAs are used, it is desirable to begin with a low dose, for example, amitriptyline 10 mg, and titrate upwards against side effects, which often are the limiting factors. It is helpful to have patients take the drug at bedtime so that sedation and other side effects are less problematic; better sleep may also lead to lower pain levels. All TCAs have long elimination half-lives. The analgesic effects of TCAs can occur within days or weeks, making it necessary to monitor clinical and hematologic function frequently.

Little is known about the clinical use of SSRIs in HCT patients, although this class of drugs is not normally associated with bone marrow suppression. The same can be said for the SNRIs.

Opioids in the management of pain

Drugs that produce analgesia by stimulating morphine-accepting CNS receptors are colloquially referred to as narcotic analgesics. It is more appropriate, however, to refer to these drugs as "opioids" rather than "narcotics." Semantically, the latter term associates the drugs with opium, the source of the prototype drug morphine. "Narcotic" is a legislative, administrative, or legal word, which is applied to a number of psychoactive drugs with abuse potential. In the current social and legislative atmosphere, the word "narcotic" should be avoided in medical settings.

The avoidance of the use of opioids by patients or healthcare professionals, in circumstances where they could be used safely and appropriately, is a grave disservice to those suffering from treatable pain. Opioids are not without liabilities, but the incidence and severity are often overstated. Pain relief is integral to humane treatment and, indeed, has been shown to attenuate disease in animals [67,68].

Opioid pharmacology

Opioids act at a number of different levels of the CNS to produce analgesia:

1 They inhibit the transmission of nociceptive input in the dorsal horn of the spinal cord (Figure 100.1).
2 They activate descending inhibitory systems in the basal ganglia (Figure 100.1) which modulate peripheral nociceptive input at the spinal cord level.
3 They affect the limbic system, altering the emotional response to pain, thereby making it more bearable.

Opioids bind primarily to μ and κ receptors in the CNS. Agonists, such as morphine, activate inhibitory pain pathways in both supraspinal and spinal regions. Antagonists, such as naloxone, competitively inhibit the analgesic effects of opioid agonists. Some opioid drugs exhibit both an antagonistic action (at μ receptors) and an agonist action (at κ receptors); these "mixed agonist–antagonists," such as nalbuphine and butorphanol, have clinical efficacy in HCT patients who experience a wide variety of gastrointestinal syndromes, especially those associated with cramping. Medications with mixed effects can precipitate an abstinence syndrome in people already taking opioid pain relievers, so must be used with vigilance.

Opioid pharmacokinetics

A schema of opioid pharmacokinetics with various modes of administration is given in Figure 100.6. As with many drugs, the blood levels of opioids that result can be measured and interpreted to yield a number of "half-lives." For individual patients, opioid pharmacokinetics and half-lives are fairly consistent. This is true to the extent that the blood levels resulting from a single dose of opioid may be used in sophisticated pharmacokinetic analyses to calculate infusion regimens that hold blood levels of opioid very close to a predetermined level [69]. However, opioid pharmacokinetics are highly variable from patient to patient, with half-lives and rates of elimination varying between two- and fourfold.

Opioid pharmacodynamics

Opioids exhibit dose-dependent effects in many realms of activity. The analgesic dose–response curve of opioids is not a straight line; they have a threshold of efficacy and a narrow "therapeutic window," and among individuals there is a wide range of effective analgesic blood levels of drug.

A useful concept is the minimum effective analgesic concentration (MEAC), the blood level of opioid at which a patient reports satisfactory pain relief (Figure 100.6) [70]. For an individual patient with a specific pain, the MEAC is fairly consistent. However, among individual patients with similar pains MEACs vary over a five- to sixfold range. For example, the mean MEAC of morphine is 16 ng/mL (range 6–33 ng/mL), and the mean MEAC of meperidine 455 ng/mL (range 94–754 ng/mL) [71].

The variability of individual MEACs, in conjunction with the individually variable drug half-lives and speeds of elimination, results in a wide range of opioid doses necessary to maintain analgesic blood levels. The practical manifestation is that opioid doses must be individualized, based on the effects of the drug, which in practice are derived from patient reports of pain relief and occurrence of side effects.

Opioid side effects

Opioids have a wide variety of side effects, many of which will abate or disappear with exposure over a several-day period.

Gastrointestinal effects

Opioids can induce nausea and vomiting in 10–40% of patients through several distinct mechanisms, including direct stimulation of the chemotactic trigger zone (CTZ), sensitization of the vestibular apparatus (patients with a history of motion sickness are especially vulnerable), and delayed gastric emptying [72]. Nausea and vomiting attributable to opioids usually occur at the onset of therapy or with escalation of dose. These symptoms, although common, often subside quickly with continued opioid exposure and may be relieved by changing opioid or using smaller doses of drug at more frequent intervals. The choice of antiemetic for symptom control should be tailored based on the presumed underlying mechanism of opioid induced nausea. Nausea and vomiting due to delayed gut motility (clinically presenting with postprandial nausea and vomiting or early satiety) may improve with promotility agents such as metoclopramide. Nausea and vomiting due to stimulation of the CTZ may be relieved with dopamine or 5-HT antagonists, and symptoms

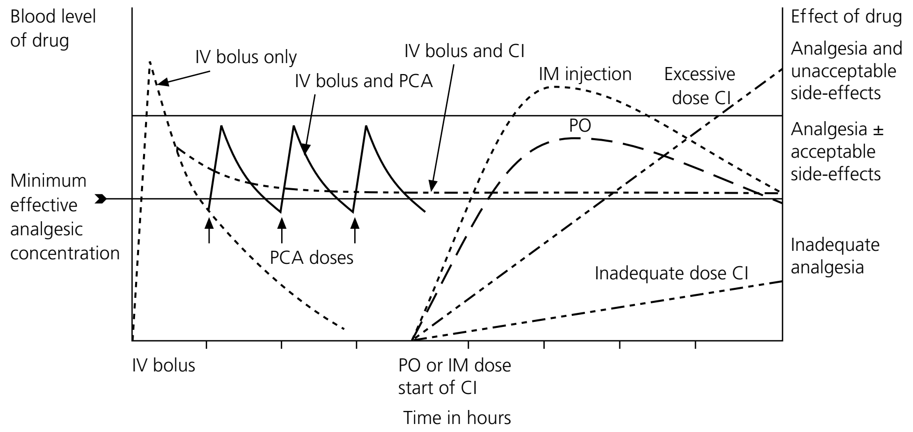

Figure 100.6 Pharmacokinetics of opioids with various routes of administration. The "therapeutic window" of opioids is the range of blood levels of opioid within which they produce satisfactory pain relief without unacceptable side effects. Following administration by various routes, plasma levels of opioids rise, according to the mode of administration, and then fall, depending on the speed of redistribution or elimination. As a general rule, as plasma levels of opioids rise they will reach the minimum effective analgesic concentration and enter the drug's therapeutic window. Rises in plasma level of drug beyond this therapeutic window produce some improvements in analgesia, but a large increase in the incidence and magnitude of opioid side effects. CI, continuous infusion; IM, intramuscular; IV, intravenous; PCA, patient-controlled analgesia; PO, oral.

exacerbated by movement may respond to diphenhydramine (a histamine-1 blocker) or scopolamine (an anticholinergic) [72].

Constipation, although a common side effect of opioids in general, is manifested differentially in the HCT patient due to the predilection for diarrhea from a variety of causes, including mucotoxic conditioning regimens, infections or GVHD. Prophylactic stool softeners and stimulants are therefore not routinely administered, and treatment is symptom based.

Genitourinary effects

Increased tone and contractility of the ureters and increased detrusor tone may lead to urinary retention.

Pruritus

Pruritus is a well documented yet poorly understood occasional side effect of opioid therapy. Antihistamines, rotation of opioids, and opioid dose reduction are common treatment strategies. In addition, mixed opioid agonists–antagonists, e.g. nalbuphine, can reverse itching, but this may also reduce the analgesic effects [72].

Sedation

Sedation is an almost universal side effect of opioids and usually occurs at onset of therapy or with dose escalation. Opioid-induced sedation typically resolves within 72 hours of initiating therapy. It is important to remember that the differential diagnosis of sedation in the HCT patient is broad and includes CNS-active drugs (antiemetics, anxiolytics, antihistamines, and calcineurin inhibitors), metabolic derangements (renal dysfunction and electrolyte disturbances), fatigue (sleep deprivation and disturbed biorhythms), and the prodrome of sepsis. In patients with persistent opioid-induced sedation, small doses of methylphenidate (5–10 mg two or three times per day), donepezil, or modafinil can ameliorate symptoms [73].

Euphoria

Opioid euphoria is rare in the HCT patient.

Dysphoria

If dysphoria occurs, it typically is early during opioid exposure and progressively declines with more prolonged exposure.

Delirium

Patients undergoing HCT can develop delirium (as defined by transiently impaired cognitive, emotional, or behavioral functions) for a variety of reasons. Because of this, a broad investigation into potential metabolic, infectious, and CNS etiologies should be undertaken. Opioids are potential contributors to delirium with the suggestion that opioid naïveté, comorbid illness, higher doses of therapy, and parenteral administration confer greater risk [73,74].

Cases of opioid-induced delirium that do not upset or scare the patient can be treated with reassurance alone. In the event of an agitated delirium, haloperidol or rotation of analgesic therapy to another opioid may be effective [75,76]. Reduction of the opioid dose by 25% can also improve symptoms [68]. However, it is important to note that, in other patient populations, poorly controlled pain confers a greater risk of delirium than the administration of appropriate parenteral opioids [77,78].

Respiratory depression

When monitored by sensitive indices, all opioids blunt the respiratory drive in a dose-dependent fashion. In the dose range that produces analgesia, respiratory depression (defined as an arterial partial pressure of carbon dioxide >5.6 kPa) is uncommon. Patients with pre-existing respiratory disease, those with sleep apnea-like symptoms, and those receiving other CNS-active drugs, especially benzodiazepines, are most at risk of respiratory depression. The degree of respiratory depression parallels the slowing respiratory rate and degree of sedation; these signs can be monitored if respiratory depression is a concern.

Opioid tolerance and dependence

Tolerance

Tolerance is the progressively decreasing effect (analgesic or other effects) of a given dose of drug when that dose is administered repetitively. Tolerance may be seen when a patient who has been achieving satisfactory analgesia with a given dose of drug progressively fails to achieve analgesia without evidence of an increase in nociception. If it occurs in the HCT patient, tolerance may be overcome by increasing the dose of drug or changing to a different opioid. Tolerance is a drug-related effect only and has little or no relation to addiction.

Incomplete cross-tolerance

When changing from one opioid to another, it often takes less than an equianalgesic dose of the second opioid (Table 100.4). This phenomenon is observed frequently, but the mechanisms are not completely understood. If there is a need to switch opioid medications, a good rule of thumb is to give approximately two-thirds of an equianalgesic dose and provide liberal amounts of breakthrough medication until a new equilibrium is established. Although most opioid-related side effects dissipate spontaneously after a few days, a change can provide analgesia at "lower" doses of the second drug, perhaps with fewer side effects.

Physical dependence

Physical dependence is characterized by the occurrence of an abstinence syndrome (yawning, lacrimation, sneezing, agitation, tremors, fever, tachycardia, and other signs of sympathetic arousal) if the opioid abruptly is withdrawn. Like tolerance, dependence is a drug characteristic, not a patient characteristic, and is a separate entity from addiction. Few patients who are opioid dependent are addicts (see below). Abstinence syndromes can be avoided by slow reduction of opioid dose (10–20% every day or every other day) or by using adrenergic α_2-agonists such as clonidine, which mask some of the peripheral adrenergic symptoms [56]. Using the strategies described below in the section on opioid tapering, it is rare to see a true abstinence syndrome when opioids are used with HCT patients.

Psychologic dependence (addiction)

This complication is characterized by an abnormal pattern of drug use; of drug craving for effects other than pain relief; by an overwhelming involvement with procuring and consuming the drug, even when such activity is contrary to the individual's best interests; and with a tendency to relapse into drug use after tapering off the drug. Addiction is the conjunction of genetic, psychologic, social, and cultural factors that affect an individual. Unlike tolerance and physical dependence, psychologic dependence is a patient issue, not a drug issue.

Until recently, practitioners believed that patients with no previous history of substance abuse who receive opioids therapeutically have an extremely low incidence of iatrogenic addiction, of the order of 1 in 2000–4000 [79], so that fear of psychologic dependence

Table 100.4 Dose equivalents and starting doses for opioid analgesics

Drug	Approximate equianalgesic dose (mg)		Usual starting dose			
			>50 kg		<50 kg	
	Oral	Parenteral	Oral	Parenteral	Oral	Parenteral
Morphine	30	10	30 mg/3–4 h	10 mg/3–4 h	0.3 mg/kg/3–4 h	0.1 mg/kg/3–4 h
Hydromorphone	7–15	1.5–3.5	6 mg/3–4 h	1.5 mg/3–4 h	0.06 mg/kg/3–4 h	0.015 mg/kg/3–4 h
Oxycodone	30	N/A	10 mg/3–4 h	N/A	0.2 mg/kg/3–4 h	N/A
Hydrocodone	30	N/A	10 mg/3–4 h	N/A	0.3 mg/kg/3–4 h	N/A
Methadone	10–20	10	10–20 mg/6–8 h	10 mg/6–8 h	0.1–0.2 mg/kg/6–8 h	0.1 mg/kg/6–8 h
Levorphanol	2	1	4 mg/6–8 h	2 mg/6–8 h	0.04 mg/kg/6–8 h	0.02 mg/kg/6–8 h
Meperidine	100	100	N/R	100 mg/2–3 h	N/R	0.75 mg/kg/2–3 h
Codeine	200	130	60 mg/3–4 h	60 mg/2–3 h	0.5–1 mg/kg/6–8 h	N/R
Sufentanil	N/A	0.01	N/A	10 μg/10 min	N/A	0.1 μg/kg/10 min
Fentanyl	0.2(transmucosal)	0.1	200–400 μg/h	50 μg/10 min	2–4 μg/kg/h	1–2 μg/kg/10 min

N/A, not available; N/R, not recommended.
Source: adapted from Dahl and Raeder 2000 [52].

in those patients should not be a concern. That has been called into question in the last several years and, in fact, most pain management practitioners now believe the risk of addiction not only is real, but is fairly high, particularly in patients receiving opioids for chronic pain, even in cancer patients [80].

Opioids commonly used in the HCT patient

Table 100.4 includes a list of opioids, their approximate equianalgesic doses, and dosage intervals for oral or intravenous use. The choice of agent should be limited to opioid agonists. The following are commonly used opioids in the HCT patient and some of their specific complications.

Morphine

The prototypical opioid agonist morphine can be given by any route but has low oral bioavailability (Table 100.4). Oral duration of effect is 3–5 hours, which can be extended to 6–12 hours by the use of sustained-release preparations. Morphine is conjugated in the liver to two major metabolites, 3-glucuronide and 6-glucuronide, which may produce unintended side effects (sedation and confusion). Glucuronidation is a high-capacity conjugative pathway in the liver, so morphine is relatively safe to use in patients with moderate degrees of hepatic failure. Since both glucuronides are excreted by the kidneys, morphine should be used cautiously in patients with any degree of renal impairment. On initial use, morphine has a 15–25% incidence of pruritus, or nausea and vomiting, which often diminish with use over a few days.

Meperidine (Demerol)

This opioid agonist has about one-tenth of the potency of morphine. Meperidine is metabolized to normeperidine, a renally excreted metabolite, which has pronounced excitatory and convulsive effects. In patients with normal renal function, normeperidine levels do not become problematic until relatively large doses of the parent drug have been administered over a prolonged period, for example, 800–900 mg/day over about 5–7 days. However, if used as an analgesic, this meperidine dose could be met or exceeded in the HCT patient. Therefore, patients with normal renal function should be restricted to short-term, low-dose use, for example, for chills and premedication before platelet transfusions. In patients with abnormal renal function, meperidine should be avoided, or its use greatly restricted.

Hydromorphone (Dilaudid)

This agonist has a range of effects similar to that of morphine. Hydromorphone is thought to be five to eight times more potent than morphine, although data from the HCT population support a dose equivalency of about 3 [56,81]. It is generally believed to have fewer side effects than morphine, but this belief is not substantiated by double-blind studies [82]. It is metabolized to a number of renally excreted metabolites, which appear to be pharmacologically inert, at least in clinically relevant doses. Hence hydromorphone is a good choice of opioid in patients with impaired renal function or those with unacceptable side effects with morphine.

Fentanyl

This is a classic agonist with a range of effects similar to that of morphine. It is 100 times more potent than morphine and has a faster onset of effect. In short-term use, it has a relatively short duration of effect, but in longer-term use, its duration of effect is similar to hydromorphone. Fentanyl is metabolized to a number of pharmacologically inert metabolites, and is a good choice of opioid in patients with impaired renal function or those with unacceptable side effects with morphine or hydromorphone. Fentanyl predominantly is administered intravenously. It is also available as an oral transmucosal product that is useful in some HCT patients. Transdermal fentanyl (Duragesic) is a useful alternative to intravenous opioids for patients needing long-term stable dosing, in the absence of fever, and with intact skin. It should be noted that the patch takes 16–24 hours to achieve stable plasma levels of drug, and conversely when the patch is removed, it takes 16–24 hours for plasma levels of drug to fall. Fentanyl patches are not recommended for treatment of acute pain in any patients and, even for chronic use, they may be problematic in HCT patients who may have skin problems or fluctuating body temperatures that make absorption unreliable and potentially dangerous.

Oxycodone

Available only in oral forms, both short- and long-acting, oxycodone has become a favorite (often in fixed combination with acetaminophen) in the treatment of both acute and chronic pain. Its potency is roughly 1.5 times that of oral morphine. It has a well-deserved reputation for being a drug with high abuse potential; chronic use, therefore, should be monitored carefully [83].

Butorphanol

This is a mixed agonist–antagonist with marked κ-agonist effect and moderate μ-antagonist effect. It is a useful drug for HCT patients in treating the pain from GVHD of the gut. Compared with μ-agonists, it is believed to impair visceral nociceptive transmission more and yet causes less gut stasis. Butorphanol may be given intravenously or by nasal inhalation. It is not recommended for moderate-to-severe pain syndromes because it has a relatively low analgesic ceiling and can precipitate abstinence syndrome if the patient is on other opioids [56].

Nalbuphine

This is another mixed κ-agonist–μ-antagonist opioid. It effectively treats opioid-induced pruritus without reversing analgesia. Additionally, clinical experience has demonstrated efficacy in treating pruritus associated with cholestasis [84,85].

Tramadol

This is a centrally acting synthetic analgesic. It has weak opioid activity and inhibits reuptake of both 5-HT and NE. The side-effect profile is similar to that of opioids; respiratory depression may occur with overdose, and an abstinence syndrome can occur with abrupt withdrawal.

Buprenorphine/naloxone (Suboxone)

Buprenorphine alone is a μ-agonist and κ-antagonist that has been used in the treatment of chronic pain because it has a "ceiling" effect, both for analgesia and, more importantly, for respiratory depression. Naloxone, of course, is a powerful μ-antagonist used primarily in the emergency treatment of opioid overdose. The combination of the two drugs, Suboxone, is now a common treatment for chronic pain in patients with a history of opioid abuse. Its use in acute pain management for the opioid-naïve patient is contraindicated and it presents great challenges, due to the μ-antagonist effects, in the management of pain in a patient already on the drug. Discussion of its use in the HCT patient is beyond the scope of this chapter; if a patient who is on Suboxone presents for treatment, it is mandatory to have a pain management specialist involved.

Opioid use in clinical practice

The basic precept in providing analgesia with opioids is to raise and maintain the patient's plasma level of opioid above the patient's MEAC and within the therapeutic window. Figure 100.6 will help with understanding the following paragraphs.

Oral administration

The gut readily absorbs opioids but they undergo extensive first-pass metabolism by the liver, so that to produce a given blood level, the oral dose may be three to six times the parenteral dose (Table 100.4). After oral administration, blood levels rise slowly, reach the effective range in 30–45 minutes, and remain within the therapeutic window for about 3–4 hours; hence 3–4-hourly dosing is necessary, except with methadone, which is effective taken every 6–8 hours. Slow-release preparations of morphine and oxycodone extend this dosing schedule to 8–12 hours.

Rectal administration

This route should be avoided in the HCT patient because of the bacterial shower produced by inserting a suppository.

Intramuscular and subcutaneous opioids

Intramuscular and subcutaneous opioids are rarely used in the HCT population as frequent doses may be necessary, and the mixture of intramuscular/subcutaneous injections and thrombocytopenia results in hematoma formation.

Intravenous bolus

Intravenous bolus dosing rapidly raises the plasma level of a drug. However, the drug is then rapidly redistributed and metabolized, and blood levels fall quickly. Consequently, therapeutic levels, above the MEAC, are maintained for only a short period, less than 30 minutes. Intravenous bolus administration is widely used in the HCT population as most patients have indwelling venous access. The drug reliably and rapidly reaches its targets.

Fixed-rate (continuous) infusion

Infusions of opioid take a long time to raise plasma levels to the effective range, leaving the patient in pain. Moreover, to choose an infusion rate that will maintain an effective plasma level of opioid, the rate at which the individual patient metabolizes and excretes the opioid must be known. Chosen infusion rates can be excessive, leading to plasma levels of drug rising into the toxic range, or inadequate, leading to plasma levels falling into the poor analgesia range (Figure 100.6).

Intravenous bolus followed by a fixed-rate infusion

An intravenous bolus will rapidly raise plasma levels of drug to therapeutic levels that then can be sustained with continuous infusion. Patient report will determine the initial effective bolus (loading) dose. An hourly infusion rate equal to approximately 20% of the effective loading dose usually provides good relief. Intensive nurse monitoring is necessary to guarantee relief and avoid overdose.

Patient-controlled analgesia

In the early 1970s, the revolutionary concept that patients might be capable of acting as their own "sensor" of pain relief and analgesic need was advanced. The concept held that if patients were given as-needed access to opioids, they would titrate their dose to keep plasma concentrations at analgesic levels, close to the MEAC, thereby achieving satisfactory analgesia, and would not over- or underuse the opioid, even when the drug was taken over long periods. This concept reached clinical reality with the advent of computerized pumps that enable patients to self-administer small doses of opioids at frequent intervals, the clinical technique of patient-controlled analgesia (PCA).

The plasma levels of drug, which result from a loading dose plus PCA, are shown in Figure 100.6. By individualizing the dosage of opioid, allowing the patient to titrate to analgesic effect, PCA circumvents the problems posed by other modes of administration, that is, variations in MEAC, pharmacokinetic differences, and changes in degree of nociception. Virtually all adult patients have the cognitive and discriminative ability to use PCA. PCA can be used reliably by children down to at least the age of 12 years [86], and may even be safely and successfully used in some children down to the age of 4 or 5 years [87]. Current clinical practice is to offer PCA to younger children, provided they are able to grasp the concept and use the technique. If a child can play a video game, he or she probably can use PCA.

In HCT patients, the classical instance of PCA use is for mucositis pain. As the mucositis pain becomes worse, patients use more drug, and then, as their mouth and throat heal and the pain lessens, they naturally taper their opioid dose (Figure 100.7). In randomized controlled clinical trials, testing patients with PCA versus continuous infusions of opioid for mucositis pain, patients using PCA tend to have lower pain scores and use less drug over shorter periods, and hence a tendency to have fewer side effects [88]. PCA pumps have various parameters that must be defined for safe and effective use. When used effectively, PCA can decrease nursing time, provide more timely pain relief, and involve the patient importantly in symptom relief.

PCA bolus dose

Initial recommended bolus sizes are morphine 0.5–2 mg (10–30 μg/kg) in adults and 10–20 μg/kg in children, or equivalent doses of other drugs. As nociception and pain worsen, bolus size should be increased; if nociception and pain become less, dose size should be decreased. Experience has shown that the optimal bolus size is one that permits the patient's 24-hour drug need, obtained from the nursing records or the PCA pump memory, delivered in 25–40 doses.

PCA lock-out interval

After a bolus of opioid has been delivered, the pump will not deliver another dose for this set interval, usually 6–10 minutes. This is a safety feature as it prevents the administration of a further dose of drug until after the patient has had the opportunity to experience the effects (analgesia and sedation) of the previous dose.

PCA plus fixed infusion

PCA alone has some disadvantages. If a patient sleeps or is disconnected from the intravenous infusion and does not self-administer opioid, blood levels of the drug will fall. The patient may then have difficulty in self-administering sufficient drug to raise the blood level of opioid back into the analgesic range. To circumvent this problem, PCA pumps have a constant infusion option. This option should not be used until a patient has established their individual dose requirement, that is, by opioid use via PCA alone. For example, a patient may have used 84 mg of morphine in the previous 24 hours. This averages 3.5 mg of morphine per hour. It is advised that the infusion be set at not greater than 30–50% of the expected opioid need with some day–night variability as needed – in this case 1.0–1.8 mg/hour. In addition to helping with sleep, the addition of an infusion means that the blood levels of opioid fall more slowly after each PCA dose, giving the patient a longer period between the need for repeat PCA doses.

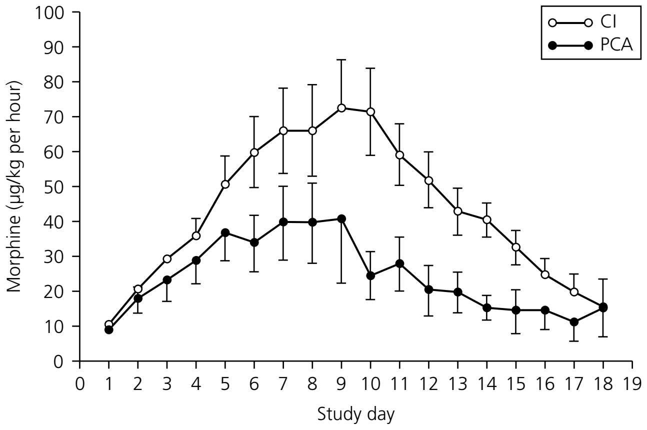

Figure 100.7 Daily mean morphine dose for adolescents with mucositis pain during hematopoietic cell transplantation. Morphine was delivered by physician-prescribed continuous infusion (CI) or patient-controlled analgesia (PCA). As the pain worsened, the PCA patients delivered more drug, and as the mucositis healed, they automatically tapered their dosage. Note also the lesser amount of drug used by the PCA patients, even though the pain scores were very similar in the two groups. Results were the same in adults [22]. Source: Mackie et al. 1991 [86]. Reproduced with permission of Elsevier.

Studies in postoperative patients using PCA for 24–48 hours have not shown a marked improvement in analgesia or patient satisfaction by the addition of an infusion to PCA [89,90]. Nonetheless, there is a strong body of opinion that the addition of an infusion to a PCA improves the quality of analgesia in HCT patients when pain persists over days, and can help with sleep.

Opioid tapering

Some HCT patients have pain problems of extended duration, which necessitate opioid therapy for long periods. Usually, as the pain problem diminishes, a patient's PCA or oral self-administered opioid dose is gradually reduced and ultimately eliminated. However, with relatively long-term (10–14 days) use, a patient may be physically dependent on the opioid; that is, abrupt cessation of opioid intake may precipitate an abstinence syndrome. In the HCT patient, these symptoms can be confused with stress reactions or can be attributed to infections or symptoms of GVHD.

It is important that both physician and patient understand that these symptoms are entirely unrelated to addiction (see the sections on physical dependence and psychologic dependence) [56], and that even if an abstinence syndrome does occur, it is rarely physically dangerous and does not have sinister long-term implications, although it is very unpleasant. The onset of these symptoms can occur within 6–12 hours after an opioid dose reduction, and peak at 24–72 hours. For both humanitarian reasons and treatment success, any tapering process should be designed to minimize the possibility of an abstinence syndrome; and if an abstinence syndrome occurs, it should be treated by using small doses of opioid until the symptoms dissipate.

Practical realities of tapering

All patients should be watched closely for abstinence symptoms since they may not be aware that their experiences are related to changes in opioid dose. If an abstinence syndrome does occur, an intravenous infusion or PCA dosing schedule that eliminates symptoms is the first intervention. This dose is then reduced by about 10–20% per day, assuming that the pain or abstinence syndrome does not reappear [91].

For ambulatory patients, continuous monitoring for symptoms by medical staff is not possible, and therefore a slower taper is advised. One simple method is the "pill count." The patient is prescribed the usual opioid in a tablet preparation, and is instructed to take it on a time-contingent basis (consistent with the duration of effect of the opioid) rather than as needed. Reductions of 10–20% of the dose are successively made until the patient is taking no drug. Immediate-release opioid preparations usually provide greater dosing flexibility than do sustained-release preparations which cannot be divided. Peripheral adrenergic symptoms during the taper can be avoided or masked by using adrenergic α_2-agonists such as clonidine [56].

For more complex patients, such as those who are overly focused on the dose or number of tablets, an alternative to the pill count method is to use "pain cocktail" [91,92]. In this strategy, the patient's mean daily intake of opioid is combined into a fixed volume of a taste-masked liquid vehicle, for example, cherry syrup, and the volume is split into regularly scheduled doses appropriate to the expected duration of action of the drug. When reducing the opioid content, the total daily volume stays stable, but the milligram opioid content is reduced by 10–20%.

Clinical pain management in the HCT patient

Pre transplant

Approaching and entering a transplantation program is an exceedingly stressful experience for patients, and may result in more vulnerability than usual to a variety of stresses, including pain. This is also a time of peak motivation to understand and participate actively in anything that will improve treatment comfort or success. Patients and families should be assured that symptom management, including pain, will be treated effectively, safely, and in a timely manner. In particular, education about the appropriate and safe use of opioids in HCT is important so that fears of addiction may be dispelled prior to the need for these medications. The pre-transplant period is the ideal time to learn, practice, and perfect non-pharmacologic pain-relieving skills.

As the time of the transplant comes closer, analgesic drugs that may be contraindicated during the transplant, for example TCAs, SSRIs, and NSAIDs, should be stopped, and safer substitutes, for example, opioids or shorter-acting anxiolytics such as clonazepam, begun.

In-hospital, peri-transplant period

This is the most vulnerable time for the patient. Pain management measures used are those least likely to impede engraftment or to interfere with the patient's ability to function. Analgesic drugs are usually given intravenously, as patients are frequently unable to take oral medication, and intramuscular injections are contraindicated.

It is more difficult to evaluate and treat patients who use non-prescription psychoactive drugs. In these individuals, who may likely be opioid tolerant, analgesic use is usually greater than normal, and the clinician evaluating the success of therapy may have to rely on external observable criteria rather than the usual patient report criteria. Contracts may need to be set to outline explicit behaviors that must be adhered to by the patient, to ensure that drug delivery is maintained or increased. Expected behaviors can include all the self-care activities necessary, along with appropriate interactions with staff in relation to anger control, manipulations, inappropriate drug-seeking activities, and number of requests for medications. When the nociceptive phase of the transplant is over, it may be necessary to impose an externally derived, relatively rigid opioid tapering schedule, rather than allowing the patient to self-taper as is normally done.

Pseudoaddiction

Care must be taken not to underdose patients who have previous, licit or illicit, experience with opioids, or who simply have high opioid requirements due to idiosyncrasies associated with the drugs. Persistent underdosing of patients leaves them in pain, and often sets up an undesirable cycle in which the patient continually asks for medication and the staff, frustrated, begin to deny medication or label the patient as a "drug-seeker." These behaviors can lead to accusations of addiction when, in fact, it is the failure to provide adequate pain relief that is at fault.

Patients with prior history of substance abuse

Patients who have a history of substance abuse (tobacco, alcohol, illicit drugs, opioids, or sedatives) may present management problems in the peri-transplant period, especially with opioid tapering. It is essential to identify these at-risk patients prior to transplant, provide adequate analgesia (see pseudoaddiction above), set clear guidelines for acceptable behavior, and be cognizant of increased opioid requirements. Opioid withdrawal may be made easier by providing time-contingent medications, rather than "as-needed" dosing, removing a layer of control from the patient.

The following are the most common pain problems and their management approaches.

Oropharyngeal mucositis

The conditioning regimens and GVHD prophylaxis used in HCT can result in OPM requiring systemic opioid analgesia in 60–100% of patients [10,93,94]. Onset of oropharyngeal discomfort typically occurs 5–7 days after the initiation of chemoradiotherapy, and peaks at day 9–13 of HCT. Resolution of symptoms usually occurs 3–4 weeks after transplantation and parallels neutrophil recovery. The duration of analgesic need may range from a few days to several weeks. Biologic predictors of mucositis-related pain correlate with the degree of tissue damage and include type and intensity of chemoradiotherapy, the use of methotrexate for GVHD prophylaxis, age, clinical grade of mucositis, and source of stem cells. Psychosocial predictors of mucositis pain have also been identified and include pre-transplant HCT-related distress, type of pain-coping strategy employed, depression, and solitary living status [10,95].

Recent approaches to combat mucositis have focused on prevention. There is a growing experience with reduced-intensity or alternative conditioning regimens that are less mucotoxic. In addition, alternatives to standard methotrexate for GVHD prophylaxis are under investigation. A systematic review found convincing evidence that ice chips, amifostine, hydrolytic enzymes, antibiotic paste, calcium phosphate rinses, and good oral care protocols are effective in reducing the severity of mucositis [96]. Keratinocyte growth factors have been shown to be safe and to decrease the duration of mucositis pain in the autologous transplant setting [97]. Studies are ongoing to assess the safety and efficacy of this class of agents in the allogeneic setting.

Once OPM pain has been established, topical analgesics such as viscous lidocaine and benzydamine can offer effective pain control [96,98]. These agents should be swished and expectorated several times daily, and are often used in conjunction with mucosal coating agents such as diphenhydramine. As the severity of OPM increases, these topical agents become inadequate, and systemic opioids are often required.

Oral opioids are of limited use owing to the high frequency of concurrent odynophagia, nausea/vomiting, and poor gastrointestinal absorption. The systemic management of mucositis pain is therefore usually accomplished with parenteral opioids. When frequent intravenous doses are required, the use of PCA is appropriate in adults and children who can manage it. In patients who cannot manage PCA, continuous infusion plus bolus dosing when needed is often required. Specifics on PCA use for mucositis pain are described above in the sections Opioid use in clinical practice and Opioid tapering.

Despite current preventive strategies and systemic opioid therapy, many patients still report ongoing mucositis pain [95]. Psychologic techniques (such as relaxation and cognitive–behavioral training) may be helpful in conjunction with systemic opioids [10]. Further investigations into preventive and non-opioid alternative pain management strategies are needed.

Conditioning regimen-related cutaneous skin toxicity

In HCT, this complication occurs predominantly in the hands, feet, groin, axillae, and genitalia. The magnitude of skin disruption varies from mild erythema to frank lesions similar to second-degree burns which usually heal 6–10 days postengraftment. The skin lesions may require treatment with silver-containing creams or biologic dressings such as pig skin. For mild toxicity, cold packs often supply adequate pain control. For more severe pain, opioids, as used for OPM, are appropriate.

Hepatic capsule distention

Sinusoidal obstructive syndrome (SOS) of the liver may cause liver engorgement with capsular distention. Intravenous or PCA opioids as for OPM are appropriate. Patients with SOS will tolerate and metabolize opioids normally until either the SOS becomes severe (bilirubin ≥20 mg/dL), hepatic failure ensues, or renal dysfunction occurs. It is essential to remember that the high-capacity hepatic degradation pathways may overwhelm a mildly dysfunctional kidney leading to accumulation of degradation products, particularly important with meperidine and morphine.

Pain associated with hematopoietic growth factors

The recombinant granulocyte colony-stimulating factor filgrastim is commonly used to hasten neutrophil recovery and to mobilize donor stem cells. Headaches and bone pain (usually in the pelvic and long bones) are frequently reported and are dose dependent. Bone pain can occur within hours of filgrastim administration and usually resolves within a few days of drug cessation. Pain is typically well controlled with acetaminophen or NSAIDs, although low-dose oral opioids are occasionally required. In published series, between 60 and 100% of donors undergoing stem-cell mobilization with filgrastim report bone pain severe enough to require some type of analgesia. Although rare, bone pain can occasionally be severe enough that hospitalization is required [99]. A growing experience with the longer-acting pegfilgrastim shows comparable incidences of bone pain [100,101]. Outpatients receiving filgrastim or pegfilgrastim should have access to ample “as-needed” doses of a rapid-onset oral opioid such as morphine, hydromorphone, oxycodone, or hydrocodone, often in combination with acetaminophen. Of interest, loratidine is being explored as a safe alternative to treat pegfilgrastim induced pain [102].

Postengraftment

Graft-versus-host-disease

Patients who receive allogeneic transplants are at risk for GVHD, despite prophylaxis with immunosuppressive drugs and steroids. The following are commonly occurring sites of GVHD that tend to have accompanying pain:

1. *Oral.* Oral GVHD can overlap with the signs and symptoms of OPM. Symptom-based treatment includes good oral hygiene, topical analgesics, and oral or systemic opioids titrated to effect.
2. *Skin.* The pain of skin GVHD responds to opioids. Skin GVHD also associated with pruritis can be effectively treated with oral hydroxyzine or nalbuphine, as pruritus may be mediated through systems that are opioid sensitive [84,103]. The TCA doxepin can be fairly effective in treating itch from any cause.
3. *Gastrointestinal tract.* Gut GVHD may cause pain for a variety of reasons. Bowel wall inflammation may cause pain at rest and with movement. Vigorous peristalsis of inflamed gut may cause episodic cramping pain that is not responsive to opioids. Indeed, opioids may make the pain worse by contributing to bowel dysmotility. Clinical experience strongly suggests that κ-agonist opioids such as butorphanol produce better pain relief and less gut atony than μ-agonist opioids. There may be a place for the use of invasive analgesic techniques such as epidural analgesia, which provide potent analgesia with low-dose drug use, in this circumstance. Chronic GVHD is a difficult pain management problem, and long-term opioids or one of the invasive analgesic techniques should be considered.

In circumstances in which opioids are not very helpful, and pain is unpredictable and brief, as in chronic GVHD gut cramping, patients can use an image that counteracts the physical sensation, combined with deep breathing. For example, a patient with a cramping pain might imagine a tight fist that progressively relaxes as they blow out each slow, deep breath. Or, in the case of burning pain, the patient might imagine breathing arctic air through the hot body part, and watching as the red turns to orange and cools to green or blue. Both adults and children have used this method to shorten the duration of pain.

Pain associated with steroid tapering

This usually occurs at the time when steroids are being tapered slowly. Typically, the pain is severe, of rapid onset and short duration, and usually occurs in the distal femur and proximal tibia in the early morning hours. Usually this pain is responsive to opioids – but doses need to be large enough to abort the pain episode.

Pain due to herpes zoster

The acute episode is painful in about 75% of cases. Pain may persist beyond the acute episode. Pain that persists beyond 1 month may be due to peripheral nerve damage and is therefore termed postherpetic neuralgia (PHN). In the acute episode, non-opioid agents and opioid analgesics are appropriate and effective. For PHN, non-opioid and opioid analgesics are relatively ineffectual. With PHN, three types of pain, which respond to differing therapeutic modalities, occur:

1. Severe skin sensitivity with pain to the slightest touch (allodynia). Eutectic Mixture of Local Anesthetics (EMLA cream, a 2.4% lidocaine–2.5% prilocaine mixture applied topically) or more recent preparations such as lidocaine patches might be considered [104]. Effective systemic drugs include anticonvulsants and antidepressants (see the section Neuropathic pain below).
2. Episodic shooting pain best thought of as an epileptiform discharge from damaged dorsal horn neurons (see the section Neuropathic pain below).
3. A background burning pain, which is the most common form of PHN. This often responds well to TCAs [61,62] and occasionally to opioids.

Prevention of PHN has been the subject of various studies. The early suggestion that corticosteroids might prevent PHN [105] has not been confirmed [106]. More recent studies strongly suggest that early use of antiviral medications may reduce the incidence and duration of PHN [107,108]. Even more exciting is gathering evidence that early sympathetic blockade (e.g. by using epidural analgesia) shortens the acute phase and reduces the incidence, duration, and severity of PHN [109,110]; combined with antiviral therapy, this would now appear to be the intervention of choice, especially in patients over 50 years old.

Neuropathic pain

Damage to nerves may cause neuropathic pain. Such pain typically is out of proportion to the stimulus intensity; it can involve myelinated Aδ nerve fibers or smaller, unmyelinated C fibers. Damage to the latter in particular may cause "sympathetically mediated" pain syndromes. These syndromes may be amenable to neural blockade, whereas non-sympathetically mediated pain syndromes usually are not.

Neuropathic pain often is associated with sensory or autonomic nervous system dysfunction. Patients tend to use words such as "burning," "raw," "gnawing," "aching," or "tightness" to describe the discomfort of neuropathic conditions. Shooting and shock-like pain syndromes also can occur. Common causes include exposure to chemotherapeutic agents (particularly vinca alkaloids), calcineurin inhibitors, damage to large neural plexi by radiation or surgery, spinal cord compression, or nerve root infiltration in conditions such as herpes zoster. The incidence of neuropathic pain in HCT patients is unknown. Mechanisms of the various syndromes are still poorly understood; consequently, there are many treatment options, and it is difficult to predict efficacy. In the last few years, however, coherent approaches to the management of neuropathic pain have emerged, based on our current understanding of NE and 5-HT neurochemistry [63,65,111]. See the following two sections.

Antidepressants for neuropathic pain

Many HCT patients suffer from neuropathic pain. Even though there have been no clinical trials of TCA or SSRI analgesic efficacy in HCT patients, it probably is reasonable to extrapolate to the HCT population data from trials in other conditions, usually diabetic neuropathy or PHN models [112].

TCAs have been shown to be variably effective in some patients. Compared with placebo, amitriptyline, imipramine, and desipramine produce benefits in neuropathic pain when administered in either low or high dose [58,60,62,113,114]. Benefits appear after about 2 weeks; a frank correlation between blood level of drug and analgesic efficacy has not been established. Amitriptyline is probably the drug of choice, but desipramine is a good alternative with similar efficacy and possibly fewer side effects [61,62].

Although it has been difficult to show that SSRIs have clear analgesic efficacy in neuropathic pain syndromes, there is emerging evidence that they may be beneficial, in particular that SSRIs may enhance analgesia from TCAs [63,64]. An even newer antidepressant, duloxetine, which is a 5-HT and NE reuptake inhibitor, is less well studied but also shows promise in refractory neuropathic pain syndromes [115,116]. There is a growing consensus that SNRIs are effective in treating neuropathic pain in non-HCT patients [117] and these strategies may legitimately be extrapolated to HCT patients.

Anticonvulsants for neuropathic pain

A wide variety of anticonvulsants, including carbamazepine, phenytoin, valproic acid, and clonazepam, have been used to treat neuropathic pain, with some success [112,118]. The more recently developed anticonvulsants gabapentin and pregabalin, however, appear to be more effective, safer, and better tolerated by cancer patients, especially in those patients at risk for developing PHN [117,119–124].

Topical preparations for neuropathic pain

Topical application of lidocaine patches [125,126] and capsaicin [127,128] has been used successfully to control peripheral neuropathic pain. Topical capsaicin has been used to treat mucocutaneous neuropathic pain, such as oral mucositis [129], although it is recommended only for early or mild cases because capsaicin can cause severe pain by releasing substance P.

Opioid treatment of neuropathic pain

The historical dogma that opioids are ineffective in treatment of neuropathic pain has been shown to be false [130–132]. Neuropathic pain does, indeed, respond to opioid treatment, but may require higher doses than in the treatment of nociceptive pain. Further, adjunctive treatments may enhance and complement analgesia provided by opioids in the setting of neuropathic cancer pain [133,134]. It is often helpful to treat neuropathic pain with opioids until the effect plateaus or unacceptable side effects emerge. As noted above in the section on addiction, long-term opioid treatment may incur risks of psychologic dependence and abuse.

Pain due to diagnostic or therapeutic maneuvers

Postlumbar puncture headache

Postlumbar puncture headache (PLPHA) is believed to be caused by a continuing leak of cerebrospinal fluid through the hole in the dura created by the lumbar puncture. This leak may cause a low-pressure headache or traction on the base of brain structures as they migrate distally through the foramen magnum. Additionally, there likely is a reflex vasodilation is response to the abnormal hydrostatic pressure gradient across the brain [135]. Lumbar punctures should be performed with the smallest needle possible, certainly no larger than 22 G, and preferably 25 G. With a 22 G needle, the incidence of PLPHA is 20–30% in 20–30-year-olds but only 2–3% in those over 60 years old.

A classic PLPHA is an occiput to frontal headache that occurs after a lumbar puncture and that is exquisitely sensitive to positional changes. It may be associated with photophobia, nausea, vomiting, tinnitus, diplopia, and changes in auditory and visual acuity. Not all headaches postlumbar puncture are PLPHAs. Patients with a history of headaches are more likely to get one after lumbar puncture, but whether these are PLPHAs is often debatable.

Prophylactic measures that minimize the incidence of PLPHA include:

- Using small needles, preferably those with non-cutting "pencil point" tips, such as Whitacre or Sprotte needles.
- Single punctures.
- Lying flat after the lumbar puncture, presumably allowing the dural hole to heal. A 1-hour initial period followed by as much time as possible recumbent for 24 hours is advised. This has never been shown to be true evidentially but is, nevertheless, advised by experts.

Once a headache has occurred, a variety of treatments can be used:

- Bed rest, mild analgesics, and copious fluids.
- Caffeine infusion, 500 mg in 500–1000 mL over 1–2 hours repeated twice, if needed, barring side effects such as tachycardia or uncontrolled anxiety.
- Wearing an abdominal binder while out of bed. This increases intra-abdominal pressure, increases epidural blood flow, and reduces transdural pressure gradients, thus reducing cerebrospinal fluid loss.
- Epidural blood patch. This is performed by doing an epidural puncture at the site of the lumbar puncture, and then instilling 10–15 mL of the patient's own blood into the epidural space at the

site of the previous puncture. The blood either decreases the transdural pressure gradient, allowing the hole to heal, or acts as "physiologic glue," sealing the hole. Epidural blood patches should be used with caution in the HCT patient because of the risk of creating an epidural hematoma in the coagulation-impaired patient, and of introducing a perfect bacterial culture medium close to the neuraxis in an immunocompromised patient.

- Use of triptans. Anecdotal evidence and clinical experience indicate that the migraine-aborting medication sumatriptan, available orally and as a nasal spray, and also applied by the intramuscular route, is useful in the treatment of PLPHAs and may obviate the need for blood patching.

Surgical pain

HCT patients often undergo a number of procedures, ranging from the simple, with little postoperative pain, for example indwelling central line placement, to the complex and painful, for example lung biopsy. The management of postoperative pain in HCT patients should be aggressive. Invasive techniques, such as epidural catheters for administration of spinal opioids and local anesthetics, should be weighed carefully. After thoracic and abdominal surgery, epidural analgesia can aid pulmonary function and overall activity; there is a small risk of infection at the catheter site [136,137]. A complicating feature of postoperative pain management in HCT is that many patients will be opioid tolerant. In such cases, it is recommended to replace presurgical opioids completely with a basal infusion of drug and provide liberal breakthrough dosing either by PCA or frequent nurse-delivered boluses, depending upon the cognitive function of the patient. Even though opioids do not have true logarithmic dose–response curves, they behave as if they do in patients who are tolerant. Hence it is not surprising that many patients will require, at least, double their previous daily opioid requirements in the acute postoperative phase. Self-titration via PCA allows the patient to escalate and subsequently wean medication to acceptable levels of comfort with minimal risk of respiratory depression.

Analgesia/sedation for painful and unpleasant procedures

HCT patients have to undergo a number of different painful, unpleasant, and anxiety-provoking procedures. In small children, the only way in which these can be accomplished is under deep sedation or general anesthesia. Preprocedural fears and intraprocedural discomforts can be managed in a variety of ways, as described below.

Non-pharmacologic management of procedures

Cognitive–behavioral and physical methods can be effective for both preprocedural anxiety and active distraction during procedures [138]. These methods require patients to be alert; even moderate sedation with benzodiazepines disrupts the effectiveness of these methods. Relaxation and imagery can effect cognitive distraction and reduce physical stress.

Pharmacologic management of procedures

Pharmacologic management can take a variety of forms, depending on the procedure, the patient, and local resources. Adults often will tolerate procedures with local anesthetic and a minimum of CNS-active drug. Children are likely to need more CNS-active drugs. In increasing order of complexity and invasiveness, the following techniques can be used.

Cutaneous local anesthetic

A 1-hour cutaneous application of EMLA will produce dense skin anesthesia. Infiltration of skin and deep structures with percutaneous local anesthetic is highly efficacious. Local anesthetics do not work instantaneously, and it is good practice to leave the local in place for about 5 minutes before attempting any potentially painful maneuver. The addition of 2–3 mL of standard sterile sodium bicarbonate solution to a 30 mL vial of 1% lidocaine will reduce or eliminate the stinging pain of injection.

Premedication

Small doses of an anxiolytic (e.g. midazolam) and a short-acting opioid (e.g. fentanyl) prior to the procedure will make injection of local anesthetic more pleasant for patients. Patients often will have no recollection of the procedure, but will recall the experience less unpleasantly even when there were difficulties or the patient expressed discomfort.

Conscious sedation

Conscious sedation is achieved with opioids and sedatives, given in doses higher than those used simply for premedication, such that patients remain responsive to verbal stimuli and are able to protect their airway and breathe spontaneously. Only qualified nurses and physicians should provide conscious sedation due to the potential need, albeit small, for airway support.

One approach is the use of lorazepam plus an opioid such as morphine or hydromorphone, medications that patients often are on already. The strategy is to "top off" the existing sedative and analgesic for the procedure with the knowledge that respiratory compromise would be extraordinarily rare because patients will previously have developed respiratory tolerance to those drugs. Another common combination of drugs is midazolam plus an opioid; this is less safe since the respiratory-depressant effects of midazolam are more pronounced and unpredictable than with lorazepam, and because patients rarely are on midazolam chronically so will not have built up a respiratory tolerance to the medication. In either case, it is important to weigh the risks of aspiration against the benefits of providing deeper sedation than would be achieved with premedication alone, if, for example, the patient has severe mucositis.

Deep sedation and general anesthesia

The continuum from conscious sedation to deep sedation, when the patient is virtually unarousable, to general anesthesia, when the patient is rendered unconscious, is indistinct. Whereas well-trained nurses can administer conscious sedation safely, only those providers who are highly skilled in airway management and interventions for cardiovascular instability should provide deep sedation and general anesthesia. Such regimens typically consist of a short-acting, potent opioid plus an intravenous anesthetic; fentanyl (short-acting due to rapid redistribution) and remifentanil (which degrades spontaneously in the plasma) plus propofol are two commonly used methods. The authors discourage the use of ketamine, another alternative, because it is longer acting, creates copious secretions, and often is associated with postadministration delirium. The American Society of Pediatrics has provided practice guidelines for such situations [139].

Invasive analgesic techniques in the HCT patient

In the non-HCT population, patients whose longer-term nociceptive pain is poorly managed by systemic analgesics are candidates for one of a number of potent, but invasive, analgesic techniques. In HCT patients, these techniques have been used infrequently owing to the risks of hematoma and infection. Post-HCT, there are patients with severe pain problems that are not responsive to systemic analgesics, for example, chronic gut GVHD or pathologic fractures, who should be considered for these potent techniques. Examples of the techniques, their potential indications in the HCT patient, and potential complications are as follows:

1. Peripheral nerve blocks with local anesthetic for pain in well-circumscribed anatomic distributions. For example, intercostal blocks may be appropriate for fractured ribs, chest tube placement, or following thoracotomy.
2. Sympathetic nerve blocks with local anesthetic in conditions that have a sympathetic nervous system-mediated component. For example, stellate ganglion blocks may be appropriate for head and neck herpes zoster.
3. Neurodestructive sympathetic nerve blocks for pain that is likely to be longstanding and resistant to other analgesics. For example, neurodestructive celiac plexus block may sometimes be considered for pain associated with intra-abdominal candidiasis.
4. Neuraxial (epidural or subarachnoid) blocks with either opioids or local anesthetic. The techniques may be used on a short-term basis, for example, for postoperative pain after thoracic or abdominal surgery, via percutaneously inserted catheters. For pain of likely longer duration, such as gut GVHD, more complex and expensive implanted systems may be considered.
5. Spinal cord stimulation is a highly specialized technique that utilizes electricity to modulate efferent and afferent pain signals in the central nervous system. Clinical data have demonstrated varying degrees of efficacy and, in all cases, careful patient selection is paramount.

Analgesia and sedation in dying patients

In providing pharmacologic relief of discomfort in dying patients, the choice of the most effective and least burdensome route of drug delivery is often a problem. Choosing the route of drug administration in the HCT patient is simplified by the fact that most people have indwelling central venous catheters. The fundamental drug in providing comfort care is an opioid. The choice of opioid may be suggested by what has been used effectively earlier in the patient's course.

Both continuous infusion of opioids and PCA have a role to play, although, as the patient's condition deteriorates, they will less likely be able to use PCA effectively. If patients are not receiving any opioid, one should start with either PCA or continuous infusion as for mucositis, and adjust doses upwards based on response. If patients are receiving opioids, doses should be adjusted upwards to achieve comfort. It is often necessary to use high doses of opioid to obtain comfort, and the usual dose increases may be inadequate. In these circumstances, a bolus dose of 1 hour's worth of opioid, followed by an increase of 25–50%, may be necessary. The occurrence of unwanted side effects, such as nausea, delirium, agitation, tremulousness, or other CNS phenomena, can be treated by changing to an equianalgesic dose of another opioid or by the addition of further CNS-active drugs, including low-dose (0.5–1.0 mg/kg) ketamine once or twice per day [140].

In the event that opioids do not provide adequate comfort, especially if there is poor control of anxiety or tremulousness, sedative drugs such as benzodiazepines or barbiturates should be considered. Initial benzodiazepine regimes would be midazolam 1–2 mg/hour and lorazepam 1–2 mg/hour; dose increases of 25–50% are appropriate. As with opioids, substantial doses may be needed. Another useful category of drug is the butyrophenones, such as haloperidol and droperidol. These can be used to treat delirium and confusion, and, in association with opioids, they may produce a calm, neuroleptic-like state. Sample regimens would be haloperidol 0.25–1.0 mg/kg or droperidol 0.1–0.2 mg, intravenously every 4–6 hours.

In the terminal stages, agitation may require the use of potent CNS depressants such as barbiturates (pentobarbital 1–2 mg/kg bolus, and continuous infusion 1–5 mg/kg/hour or phenobarbital 200 mg IV/SC bolus, then CII/CSI at 600 mg/day; usual maintenance dose, 600–1600 mg/day) or propofol (50–200 µg/kg/min) to provide "palliative sedation" [141,142]. In some instances, comfort measures can shorten a patient's life. The Rule of Double Effect (also called the Doctrine of Double Effect), which invokes the axiom that intervening on the patient's behalf may incur risks, including the possibility of death, is a well-accepted ethical principle; it protects the practitioner legally and permits extreme comfort measures in dying patients. The Rule of Double Effect derives from Catholic moral theology and has roots as far back as Thomas Aquinas [143]. A full discussion of end-of-life ethics is beyond the scope of this chapter; ethical principles are fundamental but often do not account for the complex sociology surrounding death and dying [144].

Conclusion

This chapter has outlined the neurophysiology and pharmacology of pain, and has described the common sources of discomfort in patients undergoing HCT. It provides practical guidelines for the evaluation and pharmacologic treatment of pain in HCT. Most HCT patients can be managed effectively by the oncology team. In situations where continued inadequate or ineffective analgesia prevails, it is strongly recommended that a multidisciplinary expert pain relief team be consulted and involved.

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CHAPTER 101

Oral Complications of Hematopoietic Cell Transplantation

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Introduction

Oral complications can present a wide range of lesions and changes for hematopoietic cell transplantation (HCT) patients ranging from mild transient complications to chronic disabling lesions to life-threatening disease. Lesions can arise from pre-existing dental or other oral diseases or can arise from HCT treatment specifics (e.g. conditioning regimens) or HCT-associated complications [e.g. graft-versus-host disease (GVHD)]. The importance and impact of comprehensive supportive oral care for HCT patients are recognized as an integral component of HCT patient management. Comprehensive HCT oral supportive care can reduce patient suffering, the risk for medical complications, and healthcare resource utilization and also improve the long-term outcome of transplantation. A multidisciplinary team approach to incorporating HCT oral care protocols can result in optimal oral and systemic management. Evidenced-based management guidelines continue to be developed and can be utilized to provide direct benefits to the patient's overall clinical course.

Correlations with the phases of transplantation

Oral complications occur in all phases of transplantation (Table 101.1) and generally reflect the patient's oral health status as influenced by the patient's overall status, including pre-existing dental health problems, HCT-associated adverse sequelae, and their associated management.

Risk factors for oral complications include, but are not limited to, underlying systemic and oral disease, patient age, type of donor, HCT conditioning regimen, post-HCT immune status, and availability of supportive care. For example, the risk for chronic oral complications (e.g., GVHD, salivary hypofunction) is considerably elevated in allogeneic recipients compared with autologous HCT recipients [1]. The ability to recognize patterns for oral complications can help with the diagnosis and management of oral complications since many of the varied oral lesions and infections can have a non-specific presentation.

Pre-transplantation oral evaluation and stabilization

The primary goal of pre-HCT oral evaluation and care is to identify and eliminate pre-existing oral and dental disease that could contribute significantly to oral complications post-HCT [2–5]. All oral lesions should be assessed and diagnosed, including those that involve the dentition, periodontium, maxilla and mandible, mucosa, and salivary glands. Causes of these lesions could include infection, drug reactions (including bone-modifying agents), or neoplasia. Clinical examinations should be augmented by radiographic imaging and appropriate tests. Microbiology and virology testing for lesions and/or special tissue tests including tissue biopsy may be indicated.

Patients should be informed about potential oral complications of HCT and should be educated about standard oral care protocols. Transplant candidates should be advised to seek complete oral and dental evaluations and treatment as early possible prior to presenting for transplant. The dental team needs to be involved with the pre-HCT oral evaluation whenever possible; it is imperative, however, that the dental care providers clearly understand the goals and objectives of pre-HCT stabilization and provide appropriate and realistic care. To maximize ideal oral management, the oncology team should advise the dentist and hygienist as to the patient's medical status, timing of treatment, and expected course post-HCT recovery. Dental care should be targeted at eliminating or stabilizing oral and dental diseases that could complicate HCT recovery. It should be recognized that routine dental care will need to be postponed until immunologic recovery has occurred post-HCT – this can vary from 6 months post- HCT for autologous patients to as long as several years for allogeneic HCT patients with chronic GVHD (cGVHD). For patients whose medical condition prohibits dental treatment, medically necessary non-invasive oral care should be provided until routine dental care can be performed.

Dental treatment

Any medically necessitated pre-HCT dental treatment ideally should not interfere with the planned start of transplant conditioning or induce situations that could increase the risk of complications (e.g. non-healed dental extraction sites). The level and complexity of care and the time required to complete pre-HCT need to be balanced by the medical, financial, and emotional considerations relative to the patient's situation.

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Table 101.1 Phases of hematopoietic cell transplantation in relation to oral complications and management

Transplant phase	Time interval	Oral complications	Highlights of oral management
Phase I	Prior to conditioning	Pre-existing oral/dental disease – Dental caries – Endodontic infections – Periodontal disease: gingivitis, periodontitis – Oral mucosal infections: viral, fungal, bacterial Oral complications of recent cancer therapy Oral manifestations of malignancy – Gingival leukemic infiltrates – Oral lymphoma TMD	• Pre-HCT dental examination and radiographs • Elimination of dental, periodontal, endodontic, and oral mucosal lesions/infections • Patient education relative to oral complications and their management
Phase II	Initiation of conditioning therapy through trilineage engraftment	Acute conditioning regimen toxicities – Oropharyngeal mucositis – Oral infections – Xerostomia – Taste Dysfunction Mucosal infections: viral, fungal, bacterial Periodontal/dental infections Oral hemorrhage Acute GVHD (allogeneic transplants) GVHD prophylaxis toxicity (methotrexate)	• Maintain routine oral hygiene measures • Mucositis prevention and symptom control protocols • Infection surveillance and treatment as indicated – Viral (HSV, CMV) – Fungal (*Candida*, molds) – Bacterial • Local hemorrhage control • Management of xerostomia symptoms • Topical/symptomatic therapy for oral aGVHD • sTMD management
Phase III	Trilineage marrow engraftment to normal circulating counts	Persistent conditioning regimen toxicities – Xerostomia – Taste dysfunction Oral mucosal infections – Fungal (*Candida*, molds) – Viral (HSV, CMV) – Bacterial[a] Oral aGVHD (allogeneic transplants) – Mucosal lesions – Xerostomia Neurotoxicity (tremors, numbness, pain, taste dysfunction) Dental hypersensitivity TMD	• Maintain basic oral hygiene • Symptomatic management of xerostomia, including dental caries prevention • Infection surveillance and treatment as indicated • Surveillance for/diagnosis of oral aGVHD
Phase IV	Normal hematologic counts to immunologic recovery	Chronic toxicities of conditioning regimens – Xerostomia – Taste dysfunction Oral manifestations of GVHD – Resolving acute GVHD – Emerging chronic GVHD Oral mucosal infections – Oral candidiasis – Viral infections (VZV, HPV, HSV) – Bacterial infections[b]	• Maintain basic oral hygiene • Management of xerostomia including dental caries prevention • Surveillance for/diagnosis of oral GVHD with treatment as indicated • Infection surveillance/ treatment of infections • Resume routine dental examinations (4–6 months post-HCT) – Avoid elective and less than urgent treatments – Emergency dental care with medical support/ avoid elective dental treatment
Phase V	Long-term survival: full functional immunologic recovery	Chronic toxicities of conditioning regimens – Xerostomia – Taste dysfunction Oral manifestations of cGVHD Oral mucosal infections – Oral candidiasis – Viral infections (HPV, VZV, HSV) – Bacterial infections[b] Second malignancies post-transplant – Lymphoproliferative disorders – Squamous cell carcinoma – Salivary gland tumors Craniofacial growth and development abnormalities in children	• Maintain basic oral hygiene • Management of xerostomia, including dental caries prevention • Surveillance for/diagnosis of oral GVHD with treatment as indicated • Infection surveillance and treatment • Surveillance for potential second malignancies post-transplant

CMV, cytomegalovirus; GVHD, graft-versus-host disease; HCT, hematopoietic cell transplantation; HPV, human papillomavirus; HSV, herpes simplex virus; TMD, temporomandibular dysfunction; VZV, varicella-zoster virus.

[a] Mucosal bacterial infections during this phase become increasingly uncommon unless engraftment is slow or if the patient has aGVHD or is on GVHD therapy.

[b] Mucosal bacterial infections are rare unless the patient has severe cGVHD, especially when on intensive therapy for GVHD.

Dental caries and endodontic infections

Dental caries and endodontic infections are common dental problems requiring stabilization prior to transplantation [5,6]. Dental caries treatment may need to be prioritized: more extensive caries is at higher risk for causing systemic infectious complications post-HCT, and therefore a high priority for pre-HCT stabilization. Less urgent decay can be managed conservatively or even deferred until it is medically appropriate to resume routine dental care post-HCT.

If a pulpal infection occurs post-HCT, the resulting endodontic infection can be difficult to manage and can even be life threatening. Therefore, endodontically infected teeth should be treated prior to HCT with either endodontic therapy or extraction.

Periapical radiolucencies associated with endodontically treated teeth should be carefully evaluated. Radiographic changes associated with endodontically treated teeth can result from a number of factors, including infection, inflammatory reactions, apical fibrous scars, cysts, and even malignant lesions [2]. In cases where periapical radiolucency is associated with an endodontically treated tooth and there are no signs or symptoms of infection, retreatment or extraction is not typically needed since the radiolucency is most likely due to an apical scar [2]. However, if risk exists for infection or treatment failure after transplantation for these teeth, retreatment or extraction should be performed.

Periodontal disease stabilization

Infectious periodontal diseases (gingivitis and periodontitis) pose the most frequent and significant infectious disease risks for patients post-HCT [7]. Periodontal disease in patients pre-transplantation can range from mild marginal gingivitis to severe chronic periodontitis with deep periodontal pocketing, bone loss, and chronic abscesses. In diseased sites, the tissues lining the periodontal pockets are infected and ulcerated and at risk for causing local, regional, and systemic infection, with also an increased risk for bleeding. Gingival bleeding predominantly results from pre-existing periodontal disease and is not due solely to low platelet counts. Healthy gingival tissue does not typically bleed unless traumatized. Patients should not be instructed to stop routine brushing and flossing "because their platelets are low" – rather, it is important to continue with appropriately administered oral hygiene as described below, in order to prevent infection and reduce the risk for gingival bleeding.

The single most important consideration for controlling periodontal infections is effective and consistent tooth brushing and flossing – effective dental bacterial plaque removal can render mild-to-moderate gingival/periodontal infections at "low risk" for spread of infection. More severe disease often requires additional antimicrobial therapy (topical, systemic, or both) and subgingival periodontal treatment (root planing and curettage). Periodontal disease-stabilizing procedures need to be completed prior to myeloablation; patients then need to maintain excellent oral hygiene [1]. However, teeth with severe periodontal involvement (i.e. significant bone loss, often accompanied by mobility) should be extracted. In situations where extractions are not feasible owing to medical status, subgingival time-release minocycline can be placed in diseased periodontal pockets around day 0; this strategy can reduce bacterial colonization for 3–4 weeks and thus reduce the risk for periodontally related infection risks during the period of most profound neutropenia.

Debate exists as to the management of unerupted or partially erupted third molars ("wisdom teeth") prior to transplantation. Some authors recommend extraction of all fully impacted or partially erupted third molars prior to HCT. However, many authors favor a more conservative approach that endorses extraction of third molars only if the teeth have a history of acute periodontal infections (pericoronitis) during the previous 12–18 months and/or if gingival health cannot be maintained with routine oral hygiene measures.

Dental extractions

When dental extractions are indicated, they should be performed as atraumatically as possible, with primary closure if feasible. It is difficult to define a minimal time for healing of extraction sites before initiating conditioning, but the more time that can be afforded for healing after surgery before myelosuppression post-HCT, the better. Epithelialization of the extraction sites can reduce the risk for local infection, bacteremia, distant infection, and bleeding [8]. The amount of time necessary for initial healing generally increases when infection is present (periodontal and/or endodontic infections), or with surgical extractions. For example, a simple extraction with primary closure of a non-infected tooth will generally initially epithelialize in 14 days. In contrast, extraction of multiple adjacent teeth or an impacted mandibular third molar can require as long as 21–28 days to heal adequately. The healing of extraction sites for patients previously treated with bisphosphonates is often delayed and can take upwards of 4–6 weeks (see below).

Dentures and orthodontic appliances

Removable prostheses (e.g., full or partial dentures, occlusal guards, etc.) should be examined thoroughly and adjusted as needed prior to HCT. Poorly fitting appliances can traumatize oral mucosa compromised by cytotoxic chemotherapy or radiation and can increase the risk for mucosal ulceration, thus increasing the risk for pain and infection. During HCT conditioning and for the first several weeks post-HCT, dentures should be worn minimally (e.g. only during eating) to reduce the risk of mucosal injury and secondary infection. Antimicrobial soaking solutions are strongly recommended when dentures are being stored, and soaking solutions should be changed daily [1].

Orthodontic bands and fixed appliances, with the exception of fixed lingual retainers, should be removed prior to conditioning to reduce the risk for mucosal trauma. Additionally, metal dental restorative materials and orthodontic appliances produce radiation backscatter with total body irradiation (TBI) that can increase radiation damage for tissues overlying the metal surfaces [9]. Large metallic dental restorations or metal orthodontic appliances can be covered with 3–4 mm thick vinyl mouth guards during radiation treatment to hold tissues away from the metal surfaces and reduce radiation damage. The use of removable orthodontic retainers can be discontinued for the first 3–4 weeks post-HCT and then use can be resumed. If orthodontic bands and brackets cannot be removed, custom vinyl mouth guards should be utilized to help reduce mucosal trauma.

Temporomandibular dysfunction

It is important to identify patients with a history of temporomandibular dysfunction (TMD), especially those presenting with a myofascial pain component. Parafunctional habits (cheek or lip chewing, clenching, and/or bruxism) should be identified, and efforts should be considered to reduce these behaviors. Sleep dysfunction and emotional stress can acutely increase parafunctional clenching/bruxing habits. Physical therapy modalities (moist heat/cold packs, massage and muscle stretching for muscles, and cold packs for painful joints) can help control symptoms. Muscle relaxants can be useful for managing acute muscle-based signs and symptoms of TMD. Efforts to improve sleep and reduce stress will often help to resolve myalgia and headache pain.

Prophylactic antibiotics

The need for prophylactic antibiotics prior to any dental procedures escalates as the risk for bacteremia and infection increases for immunosuppressed patients. However, there are no evidence-based guidelines for the use of prophylactic antibiotics for dental treatment prior to and following HCT. Prudent clinical practice suggests that prophylactic antimicrobials may well be of benefit for patients with low neutrophil counts ($<1000/mm^3$) or who are otherwise immunocompromised. Some authors have recommended antibiotic prophylaxis prior to dental procedures to reduce bacteremia and potential colonization of central venous access catheters by oral bacteria. This recommendation is empiric and based on anecdotal experience but not evidence-based analysis [10,11]. The policy endorsed by the authors established in collaboration with the input from infectious disease consultants recommends the following:

1 Patients at increased risk of infection due to poor immune status (e.g. neutrophils $<1000/\text{mm}^3$) should receive appropriate antibiotics prior to dental treatment and, as the risk of infection increases, the intensity of antibiotic supportive therapy needs to be increased.
2 Until further evidence is available, patients who have central venous access catheters in place should receive antibiotics to prevent colonization of the catheter following dental treatment-induced bacteremia, using the American Heart Association's prophylactic antibiotics guidelines for patients at high risk for infectious endocarditis. Standard prophylactic antibiotics for specific medical situations (e.g. endocarditis risk, dialysis catheters) should be used.

Post-HCT oral complications

Post-HCT, oral complications generally have distinct patterns of occurrence and reflect parallel systemic HCT toxicities and pathologies (Table 101.1). Early post-HCT oral complications primarily arise from the direct toxicities of conditioning regimens or indirectly from systemic toxicities and immune status. These complications can involve only the oral cavity or may represent oral manifestations of systemic involvement. Specific oral complications can occur acutely (days 0–100 post-HCT), possibly becoming chronic, or initially arise later post-HCT. Distinct patterns of oral complications are recognized with specifics of HCT (e.g. autologous/syngeneic versus allogeneic HCTs or myeloablative versus reduced-intensity conditioning regimens). The recognition of the risk factors for oral complications and their clinical patterns can significantly improve the diagnosis and management of HCT oral complications.

Acute direct conditioning regimen toxicities are dominated by oropharyngeal mucositis, salivary gland dysfunction, and taste dysfunction. Acute indirect toxicities are generally related to infectious complications, thrombocytopenia, or acute GVHD (aGVHD). Chronic oral complications are generally related to chronic toxicities of conditioning regimens and cGVHD and include infections, xerostomia, oral cGVHD lesions, second malignancies post-HCT, and compromised dental and craniofacial growth in children.

Oral care protocols

Standard HCT oral care protocols are designed to maintain oral health and prevent infectious and bleeding complications. These protocols can be subclassified into (1) oral hygiene protocols to prevent infection due to dental/periodontal disease and (2) strategies to maintain mucosal stability and oral comfort [5] (Table 101.2).

Oral hygiene

There can be significant benefits of maintaining oral health, especially periodontal health. Bacterial dental plaque accumulation causes infection and can progress to regional infection and bacteremia [12,13]. Maintaining gingival health by reducing oral bacterial plaque accumulation can reduce the risk of bacteremia and gingival bleeding. Basic components of oral hygiene protocols are tooth brushing and flossing. Tooth brushing and flossing represent the most appropriate and cost-effective approach to dental plaque control relative to the prevention of gingivitis and periodontal disease. Oral care protocols that mandate that patients discontinue brushing/flossing when blood counts (notably platelets) decrease below defined thresholds are mistakenly based on the concept that these oral hygiene activities cause undue risk of bleeding. Consistent plaque control promotes gingival health, decreases exacerbation of oral mucositis due to oral microbial colonization, and reduces risk of bacteremia and bleeding in myelosuppressed transplant patients [3].

Table 101.2 Routine oral hygiene and mucosal care

Tooth brushing
Soft/ultrasoft nylon-bristle brush
Electric/ultrasonic toothbrushes with soft bristles are acceptable
Brush twice per day with the Bass sulcular scrub method
Rinse frequently with water or normal saline
– Toothpaste as tolerated – discontinue use if causes pain (consider using non-mint flavors)
– Frequent rinsing with 0.9% saline solution or water if not using toothpaste
Brush with 1.1% sodium fluoride when there is increased risk of dental caries (salivary gland dysfunction, inadequate oral hygiene)
Foam tooth brushes: not recommended
– Use *only* when impossible to use a regular soft nylon toothbrush
– Use *with* antimicrobial rinses (e.g. chlorhexidine) when possible
– Resume use of a nylon-bristled toothbrush when possible
Flossing
Floss once daily with an atraumatic technique
Interproximal brushes: use once daily as instructed
Bland oral rinses
Types of rinse
– 0.9% saline solution (three-quarters of a teaspoon of NaCl in 32 oz of water)
– Sodium bicarbonate solution (2 tablespoons of sodium bicarbonate in 32 oz of water)
– 0.9% saline/sodium bicarbonate solution (three-quarters of a teaspoon of NaCl + 2 tablespoons of sodium bicarbonate in 32 oz of water)
Total volume of 12–16 oz rinsed, held mouthful by mouthful, and expectorated
Repeat every 1–4 hours or as required for pain/irritation
Topical fluorides
1.1% neutral sodium fluoride gel (preferred)
0.4% stannous fluoride gel
Brush on for 3–5 minutes, expectorated, and rinse as directed. Apply once per day
Topical antimicrobial rinses
0.12–0.20% chlorhexidine oral rinse
Minocycline or doxycycline rinse (100 mg/4 oz water)
Rinse, hold for 1–2 minutes and expectorated. Repeat 2–4 times per day

Tooth brushing

Daily removal of bacterial dental plaque from teeth and gingival tissues is critical for maintaining oral health [3,5]. Patients should be given instructions and trained in proper brushing technique prior to HCT; their ability to comply with correct brushing technique should be reviewed on a regular basis throughout HCT recovery. Patients should brush with a soft or extra-soft nylon-bristled toothbrush twice per day using a standard sulcular brushing technique. If toothpaste causes burning or stinging, it can be discontinued and water or saline solution rinsing substituted until mucositis resolves. Vigorous oral rinsing with water or saline solution while brushing will aid in removal of bacteria and debris dislodged by the brushing. Brushes should be air dried between uses. Electric/ultrasonic toothbrushes can be used if patients are properly trained in their use. If it becomes necessary to suspend the use of standard tooth brushing, it should be resumed as soon as possible after oral mucosal lesions improve.

Foam toothbrushes are not effective in removing dental plaque and do not promote gingival health. Furthermore, foam brushes can abrade mucosal surfaces and can potentially promote mucosal breakdown when patients are at risk for mucositis. The use of foam brushes in conjunction with a topical antimicrobial solution has been reported to be more effective than the use of only foam brushes but still is not equal to standard brushing.

Dental flossing

The removal of bacterial plaque interdentally is critical for maintaining gingival health. If patients demonstrate correct dental flossing techniques, they should continue flossing throughout HCT recovery. Patients with fixed lingual retainers have to floss involved teeth; if they are unable to or unwilling to commit to the effort required to floss these teeth, the retainer should be removed and can be replaced once the patient has been able to resume routine dental care. Although interproximal brushes can help with removing debris from inter proximal areas, they are not a substitute for flossing.

Antimicrobial rinses

Topical antimicrobial agents can aid in the control of bacterial colonization of gingival tissues. Chlorhexidine oral rinses are the most commonly available product for this purpose. Other potentially antimicrobial rinses include tetracycline, minocycline, doxycycline, and povidone iodine [3]. Antimicrobial rinses should be utilized when patients are unable to perform standard brushing and flossing.

Topical fluorides and remineralizing products

Patients with active dental decay or at increased risk for dental caries, especially those patients with xerostomia, should use topical daily fluoride therapy and/or remineralizing therapy. Neutral 1.1% sodium fluoride gel can be applied with a brush-on technique (Table 101.2).

Oral mucosal care

Oral mucosal care protocols should be utilized on a daily basis [1]. Bland oral rinses not only promote patient comfort, but also help maintain hydration of the oral mucosa and thus reduce risk of secondary infection.

Oral mucosal protection protocols are based on non-medicated care strategies that emphasize frequent rinsing with bland rinses (e.g. saline solution, sodium bicarbonate or saline–sodium bicarbonate solutions) (Table 101.2). These approaches are directed at keeping mucosal surfaces moist, neutralizing acidity, eliminating thick mucus secretions, and soothing mild-to-moderate mucosal discomfort. Some patients prefer to use "iced" bland rinses where ice chips are added to the basic solutions immediately prior to use. These solutions do not typically need to be sterile and can be prepared as needed.

Hydrogen peroxide rinses should not be used as a routine mouth rinse because of their tendency to dry tissues and interfere with wound healing. However, a mixture of 3% hydrogen peroxide and 0.9% saline solution (1:1 to 1:3 by volume) can aid in removal of blood clots or mucous accumulations. Lemon glycerin swabs also should not be used as glycerin can cause mucosal dryness. Commercial mouthwashes are not generally recommended because they can cause stinging/burning and increase mucosal dryness.

HCT patients should be encouraged to minimize potential trauma and irritation to oral mucosa. Avoiding rough textured foods/vigorous chewing, stopping parafunctional habits (e.g. lip–cheek chewing habits), and careful oral hygiene can reduce the risk of mucosal injury and breakdown.

Medicated care strategies are utilized to manage specific oral lesions. Examples include the use of topical antibiotics for "neutropenic ulcerations," treatment of candidiasis with antifungal agents, and topical steroids for aphthous ulcerations. Diagnosis of oral lesions is critical in order to deliver appropriately targeted interventions.

Patient education

Patient education and compliance with care protocols are an important element of the oral care plan. In addition to oral hygiene care instructions, patients must be educated as to potential oral complications of HCT, such as mucositis, oral bleeding, oral infections, GVHD, and pain. Patient motivation is key to acceptance of responsibility for their self-care protocols, and they should understand that their efforts can have a positive impact on their clinical course. Individual patient needs must be addressed in developing oral care regimens [14].

Oropharyngeal mucositis (OM)

Oral mucositis is a frequent, serious toxicity of phase II of HCT therapy [14,15]. Approximately 80% of patients who receive high-dose conditioning regimens prior to HCT develop clinically significant OM [1]. For patients receiving myeloablative conditioning regimens, OM has been reported to be the single most debilitating complication of a transplant, causing severe pain and affecting nutritional intake, mouth care, oral function, and quality of life. OM increases the risk for bleeding and systemic infection and, when severe, can compromise the upper airway, requiring endotracheal intubation.

The current pathobiologic model of oral mucositis incorporates five stages [15] (Figure 101.1). Evidence supports the impact of both direct cellular damage from cancer chemotherapy and radiation, and derangements in proinflammatory cytokines [e.g. tumor necrosis factor-alpha (TNF-α), interleukin-1] and altered nuclear factor-kappa B (NFκB) transcription as possible key contributors to the development of OM. Further research addressed at understanding these mechanisms and related processes may lead to novel, molecularly targeted preventive or therapeutic strategies in the future.

Clinically apparent oral mucosal changes typically emerge 5–10 days after initiation of high-dose conditioning regimens. Initially, mucosal atrophy and erythema occur, notably on non-keratinized oral mucosa. Ulceration typically becomes most severe approximately 6–11 days after-HCT (Figures 26.6, 26.7, 101.2). The mucosa then gradually heals over the next 1–2 weeks. Oral mucosal sites most commonly affected include the lateral and ventral tongue, and buccal and labial mucosa. Severity of OM is primarily related to the type of conditioning regimen utilized. The degree of genetic match between the donor and patient (e.g. syngeneic, autologous, allogeneic, and unrelated donors) can affect the clinical expression of mucositis, since oral aGVHD can significantly confound the clinical presentation of conditioning regimen-related OM.

Mucosal injury can be accentuated by a number of factors including, but not limited to, salivary gland dysfunction, mucosal trauma (including trauma resulting from normal oral function), and infection or irritation caused by indigenous oral flora, acquired pathogens, and reactivation of latent organisms including herpes group viruses [e.g. herpes simplex virus (HSV)] [1]. Oral aGVHD should be included in the differential diagnosis for allogeneic HCT patients whose oral mucositis fails to demonstrate a clear trend for healing by 21 days post-HCT.

Oral mucositis management

Oral mucositis management can be challenging for the clinician and patient. Currently, management strategies are primarily directed towards symptom management [1]. The interventions are

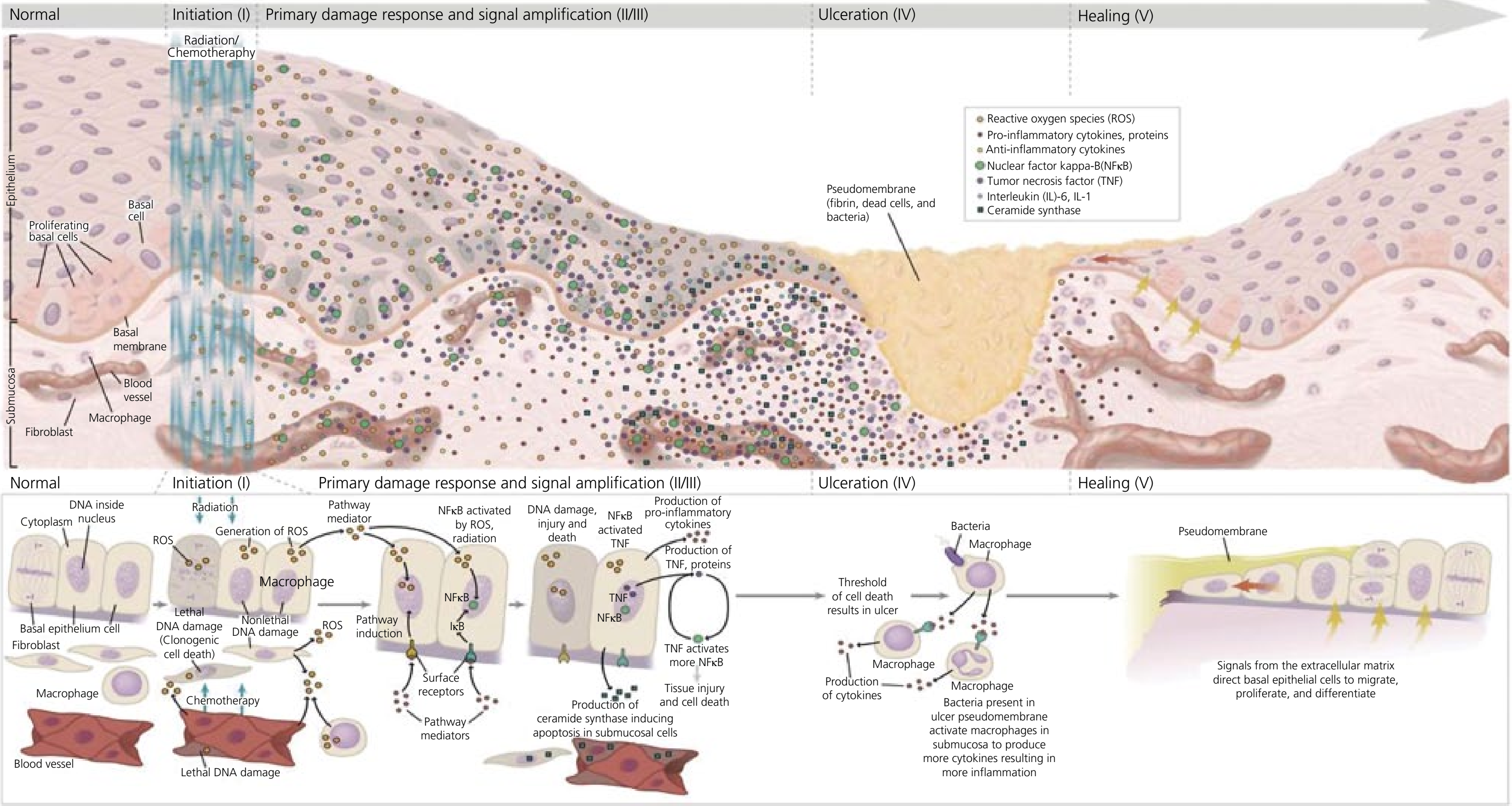

Normal
Initiation (I)
Primary damage response and signal amplification (II/III)
Ulceration (IV)
Healing (V)
Radiation/
Chemotheraphy
Epithelium
Submucosa
Proliferating basal cells
Basal cell
Basal membrane
Blood vessel
Macrophage
Fibroblast
Pseudomembrane (fibrin, dead cells, and bacteria)
● Reactive oxygen species (ROS)
● Pro-inflammatory cytokines, proteins
● Anti-inflammatory cytokines
● Nuclear factor kappa-B(NFκB)
● Tumor necrosis factor (TNF)
● Interleukin (IL)-6, IL-1
■ Ceramide synthase
Normal
Initiation (I)
Primary damage response and signal amplification (II/III)
Ulceration (IV)
Healing (V)
Cytoplasm
DNA inside nucleus
Basal epithelium cell
Fibroblast
Macrophage
Blood vessel
Radiation
ROS
Generation of ROS
Macrophage
Lethal DNA damage (Clonogenic cell death)
Nonlethal DNA damage
ROS
Chemotherapy
Lethal DNA damage
Pathway mediator
NFκB activated by ROS, radiation
NFκB
IκB
Pathway induction
Surface receptors
Pathway mediators
DNA damage, injury and death
NFκB activated TNF
Production of pro-inflammatory cytokines
Production of TNF, proteins
TNF
NFκB
TNF activates more NFκB
Tissue injury and cell death
Production of ceramide synthase inducing apoptosis in submucosal cells
Threshold of cell death results in ulcer
Bacteria
Macrophage
Macrophage
Production of cytokines
Macrophage
Bacteria present in ulcer pseudomembrane activate macrophages in submucosa to produce more cytokines resulting in more inflammation
Pseudomembrane
Signals from the extracellular matrix direct basal epithelial cells to migrate, proliferate, and differentiate

Figure 101.1 The phases of mucositis following exposure of mucosa to radiation or chemotherapy can be arbitrarily arranged into five phases that in actuality can occur simultaneously.

Phase I: Initiation. Chemotherapy and radiation produce varying degrees of direct damage to DNA, usually through the production of reactive oxygen species. Additionally, there is simultaneous activation of other pathways involved with subsequent steps.

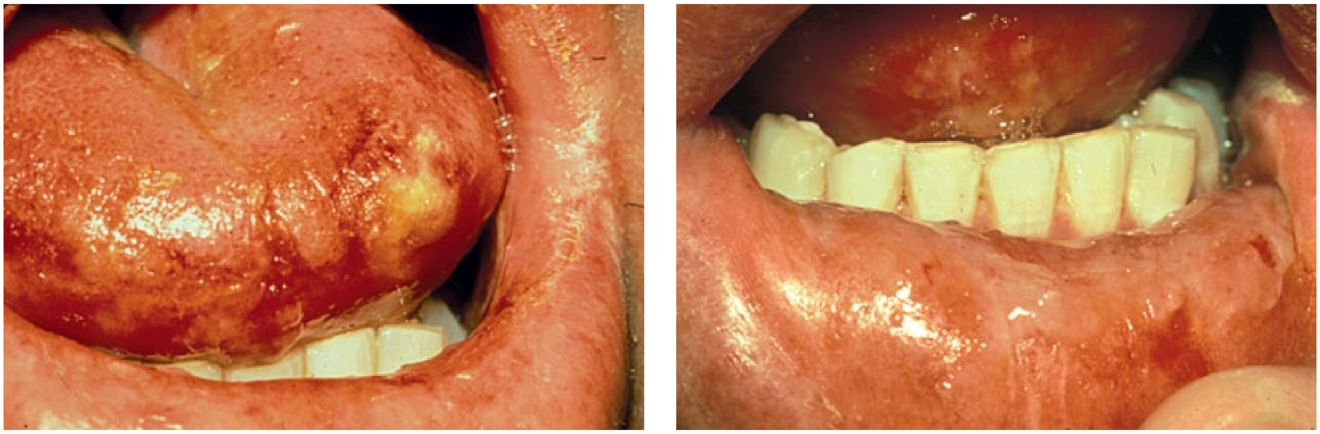
Phase II: Upregulation and message generation. Transcription factors such as nuclear factor-kappa B (NFκB) are activated to upregulate many genes in the endothelium, epithelium, fibroblasts, and macrophages, which results in the production of messaging and effector proteins including proinflammatory cytokines and enzymes. This results in apoptosis and widespread tissue damage.

Phase III: Amplification and signaling. Inflammatory cytokines can directly feed back to reupregulate transcription factors, such as NFκB, to set off a new wave of factors that can induce further damage throughout the epithelium and submucosa. Trauma and further cytokine release increase damage to epithelial surface.

Phase IV: Ulcerative/bacterial phase. This phase will usually peak between 6 and 12 days after transplant. With atrophy and ulceration of the mucosal surfaces, fibrin–exudate pseudomembranes form. These can become colonized by oral bacteria, which unfortunately is usually during the period of most profound neutropenia. Included in invading organisms are Gram-negative bacteria that can release endotoxins (lipopolysaccharide), which causes further release of cytokines from surrounding tissues and macrophages.

Phase V: Healing phase. Through signaling from the mesenchyme, signaling from the extracellular matrix promotes the recovery of the basal layer mucosal epithelial cells. The mucosal surface is renewed and ulcerations are healed. The return of circulating white blood cells (especially neutrophils) aids in clearing bacteria from tissue.

Source: adapted from Sonis et al. 2004 [57]. Reproduced with permission of John Wiley & Sons.



(a)

(b)

Figure 101.2 Severe oral mucositis occurring 8 days after hematopoietic cell transplantation following conditioning with VP-16, cyclophosphamide, and total body irradiation (World Health Organization mucositis score of 4). Bleeding ulcerations are noted on the anterior ventral area of the tongue (a) in this patient, with simultaneous pseudomembranous fibrin exudate-covered ulcers evident on the lower labial mucosa (b). (For a color version of this figure, please see the color plate section.)

Table 101.3 Mucositis management

Prevention of oral mucositis
- *Oral cryotherapy (ice chips)*: start holding ice chips in the mouth prior to administration of melphalan and continue for up to 6 hours
- *Radiation backscatter protection*: use vinyl mouth guards, cotton rolls, etc., to hold soft tissues away from metal restorations during delivery of radiation
- Epithelial growth factor (palifermin): for patients with hematologic malignancies scheduled to receive conditioning with protocols expected to have a high risk of grade III/IV

Symptomatic management of oral mucositis

Step 1
- Bland rinses (room temperature or chilled):
  - 0.9% saline solution
  - Sodium bicarbonate solution
  - 0.9% saline solution/sodium bicarbonate solution

Step 2
- Topical anesthetics
  - Lidocaine: viscous, ointment, sprays
  - Diphenhydramine solution
  - Other: doxepin solution, other antihistamines, benzydamine[a]
  - Mucosal protectant: hydroxypropylcellulose film-forming agents (Zilactin)

Step 3
- Analgesics
- Topical agents: benzydamine hydrochloride[a], doxepin rinse
- Opioid drugs: morphine, fentanyl, hydromorphone, others
  - Oral (regular release, time release)
  - Intravenous (bolus, continuous infusion, patient-controlled analgesia)
  - Transdermal patches
  - Oral transmucosal products

[a] Benzydamine is not licensed in the United States but is available in Canada and Europe.

based on the severity of tissue damage and symptoms, infection prevention and therapy, nutritional support, and use of standardized pain management decision matrices (pain management ladders) to determine the need for analgesics and narcotics (Table 101.3) [58]. An important advance in mucositis management was achieved with the US Food and Drug Administration (FDA) approval of keratinocyte growth factor-1 (palifermin) [16]. Clinical studies have shown that palifermin produces a significant reduction in several key adverse outcomes in patients undergoing autologous HCT who received palifermin prior to and immediately after conditioning with an aggressive ££TBI-based preparative regimen. The outcomes included a greater than 40% reduction in the incidence of severe OM (World Health Organization, grade 4).

Oral cryotherapy (ice chip therapy) is a technique that has been shown to be effective for reducing mucositis caused by single chemotherapeutic agents with relatively short half-lives delivered as bolus doses, such as melphalan [17]. Patients are instructed to start holding ice chips in their mouths 10 minutes prior to administration of the chemotherapy and then continue to hold ice in their mouth for the next 6 hours to induce local vasoconstriction and apparently reduce drug delivery to oral mucosal tissues. Oral laser therapy has also shown promise relative to efficacy and safety, but further research is needed [18,19].

Oral pain management

Since palifermin is the only agent currently approved for preventing mucositis, pain management strategies remain the primary means of managing OM symptoms until healing can occur. Symptom management and the prevention/management of factors that aggravate OM represent the primary objectives for HCT mucositis management. It is recommended that a “stepped” or “ladder” approach to pain management be utilized, such that a systematic stepwise protocol is used with treatments and agents being added to provide increasingly effective symptom relief for patients with mucositis [1,20]. Even though systemic treatments are usually required to manage severe mucositis, as each “step up” is added, it is important that patients continue to utilize the previous steps as part of the overall management strategy until mucositis has resolved.

Initially, the frequency of bland rinse use (saline and/or sodium bicarbonate) is increased to hydrate oral tissues, rinse away debris, and provide symptomatic relief for mild mucositis. Mucosal coating agents, such as antacid and kaolin solutions, can be added as discomfort increases. As mucosal breakdown and pain increase, the next step adds topical anesthetics. A wide variety of topical anesthetic agents is available, ranging from antihistamines (e.g. diphenhydramine) to topical local anesthetics (e.g. lidocaine, benzocaine) and analgesics (e.g. benzydamine, doxepin). One of the most common complications associated with topical anesthetic use is inadvertent mucosal damage due to trauma and irritation when trying to eat or carry out oral hygiene when the mouth is temporarily anesthetized. Generalized oral rinsing/gargling with anesthetics also carries the risk of impairing the gag reflex due to oropharyngeal anesthesia, which can result in aspiration and pneumonia. Therefore, local application to areas of ulceration may be more appropriate than extensive oral rinsing.

Although chlorhexidine can reduce bacterial colonization and help with gingivitis, there are conflicting reports relative to its ability to prevent mucositis. Current guidelines do not support the use of chlorhexidine to prevent oral mucositis [14].

Systemic pain medications, especially opioids, represent the last step of mucositis symptom management. These products are indicated when bland rinses and topical anesthetic fail to produce sufficient pain relief (see Chapter 100). OM typically produces gradually increasing pain that peaks between 6 and 11 days post-HCT. Morphine has generally been the most frequently utilized agent and has been found to be extremely effective when administered with patient-controlled analgesia. Opiates including hydromorphone, meperidine, and time-release oral or intravenous morphine and fentanyl can also be used.

Oral infections

The patterns and types of oral infection observed in HCT patients are, in general, similar to systemic infection risk patterns for HCT patients. However, advances in the prevention and management of infectious complications in HCT patients have dramatically reduced the incidence and impact of oral infections – antifungal prophylactic protocols have reduced the frequency of oral candidiasis, and prophylaxis for HSV and protocols to prevent cytomegalovirus (CMV) infections have significantly reduced the occurrence of oral HSV and CMV infections (see Chapters 86 and 87). Maintaining consistent and effective oral hygiene and the use of systemic prophylactic antibacterial protocols can dramatically reduce the risk for oral bacterial infections. HCT patients are at risk for oral infections until there is full immune reconstitution. The types and severity of these infections are related to a number of factors, including pre-transplant oral infections, the use of prophylactic infectious disease protocols, the degree and duration of immunosuppression, the degree and duration of mucosal damage (e.g. mucositis or GVHD), and salivary gland dysfunction. Phase II is the period of highest risk (early oral infections), although oral infections can be problematic long-term post-HCT (late oral infections).

Early oral infections

Myeloablation and immunosuppressive therapy directly increases the risk for both acute oral and systemic infections of oral origin [1]. The types of organism causing acute oral infections are primarily related to the colonizing oral microflora, acquired oral flora, and risk for reactivation of latent viruses. The risk of oral bacterial infection has been reduced with the implementation of standard oral hygiene protocols and improved bacterial prophylaxis and treatment. Opportunistic Gram-negative and Gram-positive pathogens and also Gram-positive streptococci and staphylococci, however, remain a substantial concern (see Chapter 84). Key risk factors for early oral infection include the degree and duration of immunosuppression, the severity and duration of damage to oral mucosa (OM and GVHD), salivary gland dysfunction, and patient adherence to oral hygiene protocols [1].

Fungal infections

Candidiasis is the most frequent oral infection occurring in HCT patients (see Chapter 85). The risk for oral *Candida* infections increases as the severity and duration of immunosuppression increases. However, with improved systemic antifungal prophylaxis protocols, the incidence and extent of oral *Candida* infections have decreased over the last decade. *Candida albicans* is considered to be the most pathogenic and most frequently isolated yeast species associated with oral infection, but other *Candida* species are increasingly being isolated. However, since *C. albicans* is a component of the normal oral flora, identification of *C. albicans* in culture specimens from oropharyngeal mucosa may only indicate colonization, and not infection, unless recovered in high numbers. It is important to correlate clinical findings of mucosal atrophy, erythema, and/or pseudomembranous plaques with the results of laboratory tests to identify yeasts both to determine the need to treat these infections and to judge infection response to therapy.

Pseudomembranous candidiasis is the most common clinical presentation for oral candidiasis and is usually described as white surface "debris-like" lesions that can be wiped off, most often involving the tongue, the buccal and labial mucosa, the tonsillar pillars, and the soft palate. *Candida* involvement of the lip commissures is noted as inflammation, chapping, and fissuring. The risk of oral candidiasis is highest during phase II and the first half of phase III. The most prominent risk factors for this infection are neutropenia, GVHD, GVHD therapy, salivary gland dysfunction, and use of broad-spectrum antibiotics. Food debris, pseudomembranous fibrin exudates, mucous secretions, and hyperkeratotic lichenoid changes related to GVHD may cause white "changes" that can be misinterpreted as pseudomembranous candidiasis.

A variety of topical antifungal strategies are available when localized oral candidiasis is present. Clotrimazole (troches or cream) or nystatin (oral solutions, pastilles, powder) can be used to control acute oral candidiasis. Systemic antifungal agents have demonstrated effectiveness against oropharyngeal candidiasis.

HCT patients are also at risk for oral infection caused by invasive fungal organisms, including aspergillosis and mucormycosis [21,22]. Systemic antifungal prophylaxis has significantly reduced the incidence of these infections. The tendency for these infections to result in aggressive local oral invasion and systemic spread, often with lethal outcome, is a major concern. Diagnosis of these infections often requires histopathologic examinations and culture of tissue specimens. Therapy requires the selection of appropriate systemic agents, often in conjunction with local surgical revision.

Viral infections

HCT patients are at high risk for acute oropharyngeal viral infections (see Chapters 86–89) [1]. During phase II and early phase III disease, oral viral infections are predominately caused by HSV. CMV infections can occur but are less common. Infections caused by varicella zoster virus (VZV), Epstein–Barr virus (EBV), and Coxsackie virus are extremely uncommon. Oral human papillomavirus (HPV) infections can be noted late post-HCT.

HSV reactivation early post-HCT can exacerbate mucositis and result in hemorrhagic, severe ulcerative lesions [23]. Clinically, HSV stomatitis can be difficult to recognize and is often confused with severe OM. As immune responsiveness recovers, HSV lesions appear as more classic focal ulcerations, often involving the attached gingiva and hard palate. Extraoral HSV lesions usually present with patterns similar to herpes labialis lesions.

The prophylactic use of acyclovir for HSV-seropositive patients has dramatically reduced the incidence of HSV reactivation. For patients not receiving prophylactic or therapeutic doses of acyclovir, or in instances where either oral acyclovir may not be absorbed adequately or resistant virus may be present, early diagnosis of HSV infection is important [24]. If not treated promptly, infection can cause significant oral pain and tissue damage, and it may even disseminate, with serious systemic sequelae. Diagnosis utilizes viral culture (including shell vial testing) or direct immunofluorescent staining of scrapings for ulcers (see Chapter 87).

CMV prophylaxis has dramatically reduced the incidence of oral lesions (see Chapter 86). CMV can cause oral ulcerative lesions during phases II and III [25]. CMV lesions have a non-specific clinical appearance, with a tendency for shallow-to-moderate-depth, irregular ulcerations covered with pseudomembranous fibrin exudate. In severe cases, lesions can be extensive and involve large areas of mucosa. Surface swab cultures often yield inconsistent results. Shell vial cultures can improve the possibility of detecting CMV, but histological examination using immunohistochemical tissue stains specific for CMV and biopsy materials is the most reliable technique to diagnose this disease. Ganciclovir and valganciclovir are the drugs of choice for treatment [26].

Bacterial infections

Bacterial infections in the HCT patient can be caused by “normal oral flora” or by nosocomial pathogens (see Chapter 84). Organisms typically classified as being of low virulence in immunocompetent patients can produce both local and systemic infections in HCT patients. Examples include Gram-positive organisms such as *Streptococcus viridans* and *Streptococcus mutans* [27].

Antibiotic prophylaxis in neutropenic HCT patients reduces mortality, episodes of fever, and the number of documented bacterial infections. The spectrum of infections tends to shift as new prophylactic antibiotic protocols evolve. However, the risk of viridans group streptococcal bacteremia and infection in patients with severe OM remains problematic, especially in those individuals receiving oral, non-absorbable quinolone antibiotics [12]. In addition, a number of pathogens, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*, can be acquired. These highly pathogenic organisms can cause serious morbidity and can be fatal. Infection management strategies should involve preventive or therapeutic regimens that target these microbes. Microbiologic documentation of causative organisms is essential given the non-specific presentation of bacterial infections. It is important to recognize that secondary bacterial infection of all types of oral ulcerative lesions in immunosuppressed HCT patients can occur and complicate diagnosis and management.

Immunocompromised HCT patients with periodontal disease may develop acute periodontal infections with associated systemic sequelae. Although not frequent in HCT patients, these lesions can occur in subtle fashion, thus necessitating careful patient examination.

Late oral infections

Although the risk of infection decreases with immune recovery post-HCT, the oral cavity can remain at risk for a variety of infections as immunologic recovery progresses through late phase III and IV. Viral and fungal organisms cause most significant oral infections during these phases. Serious oral bacterial infections are less common, primarily developing secondary to periodontal or dental infections.

The most common oral fungal infection during these later phases is candidiasis, commonly presenting with pseudomembranous white plaques and striae; however, atrophic oral candidiasis can also occur. Since the appearance of oral GVHD and oral mucosal debris can be similar to that of candidiasis (Figures 84.13, 101.3, and 101.4), it is important that the specimen recovered from lesions be subjected to the appropriate laboratory identification of responsible organisms.

In phase III, oral HSV infections can range from herpetic stomatitis in severely immunosuppressed patients with widespread oral ulcerations to relatively minor herpes labialis lesions in patients with recovering immune function. CMV lesions are associated with a more compromised immune status and can be difficult to diagnose. Biopsy with immunofluorescence is usually needed to detect CMV associated with chronic painful oral ulcerations, especially in patients who have CMV reactivation.

VZV reactivation in HCT patients usually occurs after day 100 post-HCT, with the peak incidence between 3 and 12 months post-HCT. Orofacial lesions present with herpes zoster trigeminal dermatomal distribution patterns. Infection tends to occur during mid- to late-phase III and phase IV. Extended antiviral prophylaxis protocols have dramatically reduced the incidence of this infection.

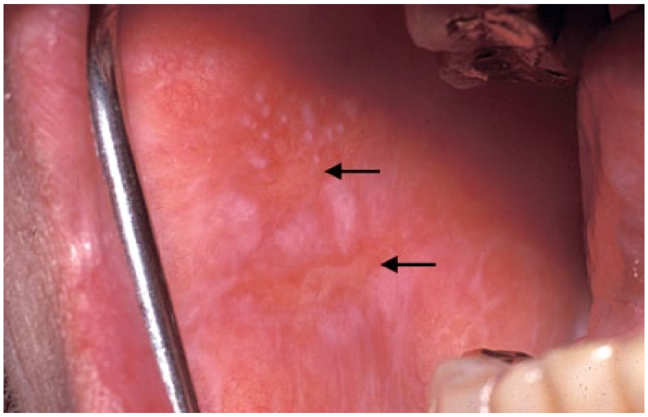


Figure 101.3 Oral chronic graft-versus-host disease in a patient 1 year post-HCT, with lichenoid hyperkeratotic changes (stria-, papule-, and plaque-like changes) associated with generalized atrophic and erythematous mucosal changes involving the right buccal mucosa. Small initial pseudomembranous fibrin exudate-covered ulcers are indicated by arrows. (For a color version of this figure, please see the color plate section.)

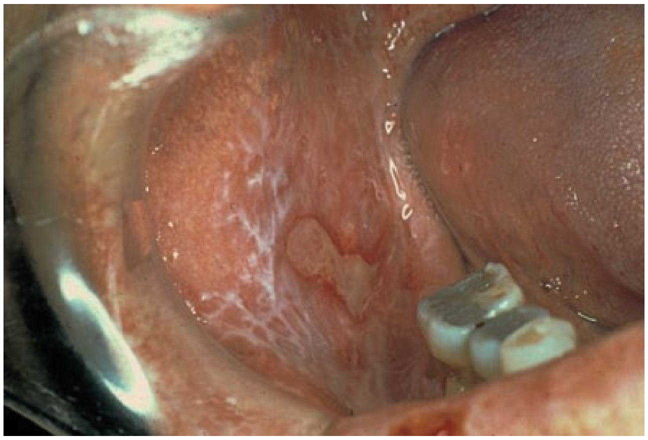


Figure 101.4 Severe oral chronic graft-versus-host disease showing distinct hyperkeratotic lichenoid striae and a pseudomembranous fibrin exudate-covered ulceration with intense surrounding inflammation in a patient approximately 18 months post-HCT. (For a color version of this figure, please see the color plate section.)

Chronically immunocompromised patients post-HCT, especially those receiving high-dose GVHD immunosuppressive therapy, are at increased risk for oral HPV infections [28]. Most cases appear to represent reactivation of latent virus, with patients reporting skin lesions prior to transplant. Oral HPV lesions can vary from exophytic oral verrucous vulgaris-like masses to a more flat condyloma acuminata-like appearance.

Oral hemorrhage

Oral hemorrhage in HCT patients is less common now as effective platelet support protocols and anti HSV prophylaxis have been developed. Risk factors for oral bleeding include thrombocytopenia, conditioning regime-associated vascular damage, use of anticoagulant therapy, presence of liver disease, and infections [29,30]. Local risk factors for bleeding include mucositis, infections, and trauma. Mucosal petechiae can be observed when the platelet count falls below 20,000/mL. Spontaneous gingival bleeding primarily occurs when there is active periodontal disease, especially when oral hygiene is inadequate.

Oral hemorrhage associated with thrombocytopenia is rarely a serious complication, although it can be both frustrating and alarming. Local measures are directed at decreasing blood flow along with promoting adequate clot formation, followed by clot protection until healing has occurred. Topically, treatment includes direct pressure applied via gauze soaked in vasoconstrictors (e.g. epinephrine) to reduce blood flow. Topical application of antifibrinolytic agents (ε-aminocaproic acid or tranexamic acid) with drug-soaked gauze can further help with clot stabilization. Clot-forming agents, especially hemostatic collagen products (e.g. microfibrillar collagen), fibrin glue, and chitosan can be used to organize and stabilize clots [31].

Salivary gland hypofunction

HCT conditioning regimens, especially those including TBI, anticholinergic medications, and the GVHD involvement of salivary glands, are major risk factors for salivary gland dysfunction post-HCT [32]. Hypofunction can be due to destruction of the secretory units of salivary glands leading to permanent reduction or alteration in saliva production [33,34].

The clinical signs of the salivary gland hypofunction can vary. Early post-HCT, patients may seem to have excessive saliva secretion that actually represents inadequate saliva clearance due to dysphagia and/or inadequate swallowing of secretions due to painful OM, pharyngitis, or nausea and vomiting triggered by swallowing oral secretions. More commonly, hyposalivation is observed to occur early post-transplant as a consequence of conditioning regime salivary gland damage; additionally, numerous drugs can suppress salivary gland secretions [35]. Saliva will often be more viscous due to decreased serous saliva secretion.

Xerostomia can significantly compromise the quality of life of patients, increasing the risk of oral lesions caused by trauma or the colonizing microflora. Strategies to promote mucosal moisturization and lubrication include frequent sipping of fluids, rinsing with bland oral rinses (0.9% saline or sodium bicarbonate rinses), and the use of artificial saliva and oral moisturizers. Sugar-free candies drops, mints, and gums can transiently increase salivary flow rates due to stimulation of glands through taste stimulation. Sialagogues (pilocarpine, cevimeline, and bethanechol) can be used to stimulate the salivary glands and improve oral moisturization [36,37] (Table 101.4).

Infection of the major salivary glands can occur when major salivary gland flow rates decrease and there is a retrograde spread of oral bacteria up ductal structures to involve salivary gland parenchyma. Treatment includes antibiotics along with stimulation of glands (e.g. lemon drops or sialogogues) and application of heat over the glands [38].

Table 101.4 Management of post-transplantation salivary gland dysfunction

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Oral moisturization
- Frequent sipping of fluids
- Artificial saliva and oral lubricating agents

Stimulation of salivary glands
- Sugar-free candy, mints, and gum
- Sialagogues
  - – Pilocarpine (5–10 mg 3–4 times per day, maximum of 30 mg/day)
  - – Cevimeline (30 mg 3 times per day)
  - – Bethanechol (25–50 mg 3–4 times per day to a maximum of 150 mg/day)

Xerostomia decay prevention
- Plaque removal: tooth brushing, flossing, other oral hygiene aids
- Remineralization
  - – Topical prescription-strength fluorides[a]
    - 1.1% neutral sodium fluoride gel (preferred)
    - 0.4% stannous fluoride gel
  - – Brush on for 3–5 minutes, spit out, avoid rinsing for 20 minutes. Apply once per day
  - – Calcium/phosphate remineralizing solutions
- Topical antimicrobials
  - – 0.12–0.20% chlorhexidine oral rinse (use alcohol-free preparations whenever possible): rinse, hold in the mouth for 1–2 minutes and spit out
  - – Tetracycline (250 mg/4 oz water), minocycline (100 mg/4 oz water), or doxycycline (100 mg/4 oz water): rinse/hold 1 oz for 1 minute and then spit out; repeat 2–4 times per day
- Diet modification: reduce exposure to refined carbohydrates, sugars, etc.

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[a] Non-prescription fluoride preparations are inadequate for moderate-to-high dental caries risk.

GVHD-induced salivary gland dysfunction results from ductal and acinar GVHD-induced tissue damage. Clinical manifestation of salivary gland GVHD involvement can additionally include mucoceles that can occur, with the labial and soft palate mucosa being the most frequently involved sites (Figure 84.15).

Conditioning regime-related salivary gland dysfunction will generally improve over the first 3–6 months after transplantation. Timely GVHD treatment appears to allow for some salivary gland recovery, although in some instances damage to the glands may be permanent [33,34]. Patients with chronic xerostomia are at increased risk of dental caries and should be placed on appropriate regimens to minimize dental plaque and provide for enamel remineralization via topical fluorides and/or remineralizing solutions [35] (Tables 101.2 and 101.4).

Oral GVHD

The oral cavity is frequently affected by GVHD [35]. Clinically, oral GVHD mimics a number of naturally occurring autoimmune disorders, including lichen planus, lupus erythematosus, and systemic sclerosis [35]. As noted above, GVHD involvement of the salivary glands can result in salivary gland dysfunction and xerostomia. General oral mucosal sensitivity and discomfort to normally tolerated agents (e.g. spices, mint flavoring agents, acidic foods) can be associated with oral GVHD. While the clinical presentation of oral GVHD is similar in both acute and cGVHD (mucosal erythema, atrophy, and white lichenoid hyperkeratotic striae, plaques and papules), patterns of changes are usually more distinct with cGVHD (Figures 84.13, 101.3, and 101.4). In more severe cases, pseudomembranous ulcerations can be noted. The posterior buccal and lower labial mucosa and lateral/ventral tongue are the most commonly involved sites.

In allogeneic HCT, early oral aGVHD is usually not discernible from OM until after 21–24 days post-HCT; early (prior to day 21) especially hyperacute, oral aGVHD will generally be attributed to severe OM that is slow to resolve. Oral aGVHD occurring between 28 and 120 days post-HCT will present with clinical changes with varying degrees of atrophy, erythema, and lichenoid hyperkeratotic lesions. Oral cGVHD mucosal changes are more distinct and pronounced. Oral GVHD can cause significant pain and produce an increased risk of oral infection. With extensive cGVHD, the extent and severity of oral lesions can become disabling. Chronic dental and periodontal infections have been noted to exacerbate oral GVHD, and elimination of these infections can often dramatically improve patients' oral status. Sclerodermatous changes can result in perioral fibrosis that decreases oral opening and interferes with oral function; in more severe cases, submucosal buccal fibrosis can further restrict oral opening. Oral secondary infections caused by HSV, CMV, and *Candida* species, or trauma, can exacerbate oral GVHD lesions and confound the diagnosis [35].

In 2006, the National Institutes of Health (NIH) published the Consensus Criteria for cGVHD [39]. The goal of this document was to standardize criteria for the assessment of cGVHD and to propose tools for scoring cGVHD organ involvement [39] (see Chapter 83). This scale has utility for determining oral cGVHD severity and then being used over time to judge the response to cGVHD therapy [40].

Symptomatic oral GVHD will often respond to topical immunosuppressive therapy, although more severe or recalcitrant cases require systemic immunosuppressive therapy. Topical management of oral GVHD can include topical application of steroids (rinses, creams, or gels), tacrolimus, and treatment with psoralen–ultraviolet A irradiation (PUVA) therapy (Table 101.5); while the effectiveness of topical cyclosporine and azathioprine have been reported with small case series, their effectiveness has not been generally appreciated. Topical therapy can reduce the symptoms from oral lesions and help resolve pseudomembranous ulcerations, but topical therapy will generally not produce complete resolution of oral involvement [41]. Extracorporeal photopheresis has been shown to reduce the severity of oral cGVHD [42].

As noted above, GVHD-induced salivary hypofunction is often noted, and although usually associated with oral mucosal lesions, it can occur independently of mucosal involvement. Salivary hypofunction interferes with oral function and can lead to symptoms of burning and mucosal irritation. Salivary hypofunction can increase the risk for dental decay due to the loss of specific salivary proteins that help control oral flora (which can result in a significant shift in oral flora to a predominance of dental decay-causing bacteria) and a reduction in salivary calcium and phosphates, which are important for remineralizing enamel and dentine. Patients need to be able to perform extremely efficient oral hygiene (brushing and flossing) along with using daily higher strengths of topical fluorides, calcium and phosphate remineralizing dental products, and possibly antibacterial mouth rinses. Attempts to improve salivary flow rates with sialagogues can increase resting salivary gland flow rates and improve patient comfort, but effects on oral health and caries reduction have not been demonstrated to date [35]. Permanent damage to salivary glands from GVHD will result in chronic xerostomia and decreased salivary flow rates.

Taste dysfunction

Taste dysfunction post-HCT is a neurosensory toxicity associated with conditioning regimens, medications (e.g. cyclosporine), and GVHD [43]. Decreased taste sensitivity is not only distressing for patients but also can affect their quality of life and can lead to food aversion, resulting in weight loss and nutritional decline.

Ageusia (loss of taste function) post-HCT results from widespread damage to taste receptor cells that results in insufficient detection of taste stimuli. HCT patients often describe symptoms consistent with dysgeusia (disordered or persistent unpleasant taste) due to lesser degree of taste receptor damage. Chronic GVHD flares have been associated with taste dysfunction; effective management of GVHD appears to support the recovery of taste acuity in these instances.

Normal taste function typically returns between 60 and 120 days following HCT. However, taste dysfunction can be persistent up to several years after HCT or even permanent [44]. Taste dysfunction in HCT patients is not preventable; management primarily consists of supportive care focusing on diet adjustment (food choices, preparation, etc.).

Neurotoxicity, neuropathies, and orofacial pain

Increased tooth hypersensitivity to thermal stimuli is frequently reported between 2 and 4 months after HCT. The mechanism is not understood, but possible etiologies may include gingival recession (dentine exposure), conditioning regimen toxicity, or cyclosporine toxicity. Symptoms usually resolve spontaneously within a few months. Topical brush-on fluorides, desensitizing toothpaste, and fluoride varnishes will generally reduce or eliminate symptoms.

Neuropathies involving the face and jaws can be noted pre-HCT due to tumor involvement of cranial nerves (e.g. leukemic infiltrates, multiple myeloma, lymphomas) and also neuropathies secondary to medications (e.g. lenolinomide, bortizomib, vinca alkaloids). It is important to obtain careful histories to identify potential risk factors. Reversibility of these neuropathies is unpredictable and management suggestions for painful neuropathies, especially medication-related neuropathies, will center around the use of agents such as carbamazepine, gabapentin, or pregabalin.

Oral neuromuscular complications resulting from treatment-induced neurotoxicity have been associated with a number of medications, including cyclophosphamide, steroids, cyclosporine, and tacrolimus (FK506) (see Chapter 105). Tongue and jaw muscle tremors, hot/cold tooth sensitivity, and taste dysfunction have been specifically associated with high-dose cyclosporine therapy. Reducing or discontinuing cyclosporine usually promotes resolution. Similar neurotoxicity problems have been associated with thalidomide.

TMD may present as facial pain, headache, muscle spasms or joint dysfunction, with headache and possibly referral of pain to the ear or throat. In most instances patients will report clenching or bruxing as a result of stress, sleep dysfunction, or, occasionally, central nervous system toxicity from selected medications. Masticatory muscle palpation tenderness, temporomandibular joint noises, and pain radiating to the ear are hallmark findings. The short-term use of muscle relaxants or anxiety-reducing agents plus physical therapy (moist heat applications, massage, and gentle stretching) will often resolve these symptoms.

Reduced-intensity conditioning regimen for HCT transplantation

Experience with reduced-intensity conditioning regimens (RICRs) for HCT continues to grow (see Chapter 20). Since the "intensity" of RICRs can vary significantly, the range and severity of oral complications can vary. For protocols utilizing fludarabine (150 mg/m^2) plus TBI (200 cGy), there is often no OM but salivary hypofunction and taste dysfunction are noted. With protocols of increasing intensity (fludarabine/cyclophosphamide/TBI and protocols

Table 101.5 Topical management of oral chronic graft-versus-host disease

(a)

Topical oral therapy	**Considerations for use**
Pre-use oral care	1. Rinse mouth with plain water; avoid normal saline 2. Try not to eat, drink or brush teeth with toothpaste for 15–20 minutes before use of topical agent
Rinses	1. Use adequate volume to easily coat oral tissues (5–10 mL) 2. Hold in mouth for 4–6 minutes, spit out. Rinses can be held longer if (a) oral lesions are more severe and/or (b) the patient is not able to use rinse the prescribed number of times per day 3. Avoid eating or drinking for 20–30 minutes after use
Gels	1. Dry lesion before application as this will improve adhesion to the lesion 2. Hold gel on lesions for 30–40 seconds: Gently apply gel to the lesion with a cotton swab or a clean fingertip. Apply like "icing a cake," do not "rub" into the tissue 3. Avoid eating or drinking for 20–30 minutes after use when possible Alternatively: apply ointment to a 2 × 2 sterile gauze and place on top of the lesion, keeping in place for 10–15[a] minutes. Remove gauze and avoid eating or drinking for 20–30 minutes after use when possible
Creams	1. Dry the lesion before applying 2. Warm a metal spoon in hot water, dry and place medication in the spoon to semi-liquefy 3. Apply softened/liquefied cream to the lesion 4. Avoid eating or drinking for 20–30 minutes after use when possible[a]
Ointments	1. Dry the lesion before application as this will improve adhesion to the lesion 2. Ointments can be warmed slightly as noted above for creams 3. Gently apply warmed ointment to the lesion with a cotton swab or a clean fingertip. The key is to apply like "icing a cake," not by trying to rub into the tissue 4. Avoid eating or drinking for 20–30 minutes after use when possible[a] Alternatively: apply ointment to a 2 × 2 sterile gauze and place on top of the lesion, keeping in place for 10–15[a] minutes. Remove gauze; avoid eating or drinking for 20–30 minutes
Inhalers	1. Dry oral tissues slightly 2. Use one or two "puffs" **without** inhaling – can be directed specifically towards lesions 3. For general oral GVHD, gently coat the mouth with the medication 4. If problems occur with oral candidiasis, in addition to prescribing antifungals, consider having the patient rinse the mouth 10–15 minutes after use

[a] Patients should avoid swallowing topical drugs after rinsing/applying.

Notes: (a) systemic uptake can occur from absorption through tissues or swallowing – patients may require monitoring of absorbed drug; (b) monitor mouth oral candidiasis; *Candida* cultures may be indicated.

(b)

	Agent	**Instructions**
Corticosteroids[a]		
Rinses	Dexamethasone	0.1–0.4 mg /mL rinse. 5–10 mL swish/held for 3–5 minutes. Spit out. Repeat 3–6 times per day
	Budesonide	0.3–0.6 mg/mL rinse. 10 mL swished/held for 15 minutes. Spit out. Repeat 2–4 times per day
	Betamethasone	0.5 mg tablet dissolved in 10 mL, held for 3 minutes, spit out. Repeat 4 times per day
Sprays/inhalers	Beclomethasone	1–2 puffs in mouth 2–4 times per day
	Fluticasone	1–2 puffs in mouth 2–4 times per day
	Betamethasone	1–2 puffs in mouth 2–4 times per day
	Triamcinolone	1–2 puffs in mouth 2–4 times per day
Gels, creams, and ointments	0.05% Clobetasol cream, ointment, gel, solution	Apply to lesions 2 times per day
	0.05% Halobetasol cream, ointment	Apply to lesions 2 times per day
	0.05% Fluocinonide cream, ointment, gel	Apply to lesions 2 times per day
	0.1–0.5% Triamcinolone cream	Apply to lesions 2 times per day
	0.05–0.1% Betamethasone cream, ointment	Apply to lesions 2 times per day
Non-steroidal immunosuppressives		
Rinses	Cyclosporine[b]	Rinse: 100 mg/mL, 5 mL swished/held for several minutes, spit out, repeat 3 times per day 1.5% gel applied to lesions 2 times per day
	Azathioprine	5–10 mg/mL, 5–10 mL swished and held for 3–5 minutes, spit out. Repeat 2–6 times per day
Gels/other	Cyclosporine[b]	0.5 mg/dL in bioadherent base
	Azathioprine	5 mg/mL in 3% methylcellulose gel, 1–2 mL applied to lesion 3–4 times per day
	0.1% Tacrolimus	Apply 0.5 inch to gauze, hold over lesion for 15–20 minutes 2 times per day. Monitor plasma levels
Phototherapy	PUVA	0.3 mg/kg psoralen followed by 0.5–6 J/cm^2 UVA radiation (dose increased as tolerated), 3–4 times per week
	UVB	0.02 mJ/cm^2 with escalating 0.02 mJ/cm^2 every 4th treatment
	Extracorporeal photopheresis (systemic/extracorporeal treatment)	
Dental infections	Aggressive prevention/treatment/elimination of all dental and periodontal infections; extract non-restorable involved teeth	

PUVA, psoralen–ultraviolet A irradiation; UVB, ultraviolet B irradiation.

[a] Anti-*Candida* prophylaxis may be needed, especially with inhalers.

[b] Cyclosporine is expensive and has met with mixed effectiveness.

Source: adapted from Schubert and Correa 2008 [35].

utilizing ≥300 cGy TBI), there is increasing frequency and severity of oral complications [45]. The severity and duration of neutropenia can vary with RICRs, hence the risk of oral infections can vary. Bacterial dental and periodontal infections remain a concern, but prophylactic antifungal, antiviral, and antibacterial protocols will generally reduce the risk for oral infections. Oral aGVHD can emerge post-HCT and oral cGVHD changes can be noted at the time of day 100 examinations, but oral cGVHD changes often will not become evident until immunosuppression is significantly reduced or withdrawn 4–6 months post-HCT with the clinical presentation similar to that seen in standard allogeneic transplant patients.

Evaluations for cGVHD: oral assessments

Standardized evaluation of the oral cavity of allogeneic HCT patients is recommended every 3 months after transplantation (see Chapter 83). Oral examinations obtained at regular intervals can provide important information relative to overall GVHD status. Clinical evaluations should be directed to determining the presence and severity of oral GVHD changes and also assessing overall oral health.

Initial examination

Initial cGVHD oral examinations should be obtained between 80 and 100 days post-HCT and should attempt to detect oral cGVHD changes and to assess the patient's general oral and dental health. Detection of oral GVHD changes at this time can aid in overall GVHD diagnosis and staging. Oral mucosal changes including erythema, atrophy, lichenoid hyperkeratotic striae, and pseudomembranous ulcerations are associated with oral GVHD [35] (Figures 101.3 and 101.4). Additionally, dental examinations should aim to detect the presence of or risk for dental, endodontic, and periodontal infections. It has been recognized that oral infections, including gingivitis, periodontitis, and dental abscesses, can aggravate GVHD. Patients should be encouraged to continue adequate oral hygiene. Patients with active decay or at risk for caries should be managed with a comprehensive program that can include topical fluorides, tooth remineralizing products, and antibacterial products [35]. Restorative and periodontal treatment should be prioritized and, if deemed immediately necessary, patients will need to receive prophylactic antibiotics along with strategies to reduce bacteremia and risk for aspiration pneumonia.

Generally, careful oral mucosal examinations are sensitive enough to detect the presence of oral GVHD. However, when clinical examinations are not conclusive, oral labial mucosal with minor salivary gland biopsies can provide data to support the diagnosis of GVHD [46]. Patients with clearly clinically evident oral GVHD mucosal changes will generally not require biopsy to confirm oral mucosal and minor salivary gland involvement by GVHD [40].

Long-term follow-up oral examinations

Follow-up oral examinations can provide important information relative to ongoing oral cGVHD activity and help judge response to systemic therapy. Oral pain/sensitivity and dryness related to oral GVHD, if identified, should be managed appropriately. The goal of topical oral GVHD therapy is to ameliorate symptoms and improve oral function and health [35] (Table 101.5). Assessment for chronic dental and periodontal infections needs to be made, and appropriate dental treatment to eliminate them needs to be carried out.

Antiosteoclastic therapy-associated osteonecrosis of the jaws

Antiosteoclastic therapy-associated osteonecrosis of the jaws (AOTONJ) is a recognized oral complication of patients receiving antiosteoclastic therapy with bisphosphonates and denosumab and is currently estimated to occur in 2–4% of patients receiving intravenous bisphosphonates and subcutaneous denosumab [47–49]. In the transplant setting, AOTONJ is most frequently seen in patients with multiple myeloma or patients who have been heavily treated previously for osteoporosis. AOTONJ is defined as the unexpected occurrence of non-healing exposed bone in the maxillofacial region that persists for over 8 weeks in patients who have received bisphosphonate or denosumab treatment without a history of radiation treatment to the involved bone. Although intravenous bisphosphonates has been most frequently associated with AOTONJ, oral bisphosphonates and subcutaneous denosumab for osteoporosis has also been associated with AOTONJ (Figures 101.5 and 101.6). Dental extractions, periodontal surgery, and denture trauma are the most commonly identified causes of AOTONJ, but spontaneous lesions can also occur [47,48]. Although the pathobiology of AOTONJ has not been fully established, it appears clear that osteoclast inactivation and secondary local infection are primary factors. A careful pre-HCT oral examination of patients receiving AOTONJ needs to be carried out [50]. Treatment of established AOTONJ involves the administration of topical and systemic antibiotics and pain medications. Advances in the surgical revision of AOTONJ lesions have been reported more recently [50]. It is important to note that the authors have not noted a worsening of AOTONJ lesions in patients following HCT, with most of these observations having been made in autologous HCT patients treated for multiple myeloma.

mTOR inhibitor oral ulcerations

Other than the use of sirolimus for treatment of cGVHD, tyrosine kinase inhibitors and mammalian target of rapamycin (mTOR) inhibitors are not commonly utilized with HCT protocols. However, these agents have clearly been associated with oral mucosal lesions that mimic recurrent aphthous ulcerations [51].

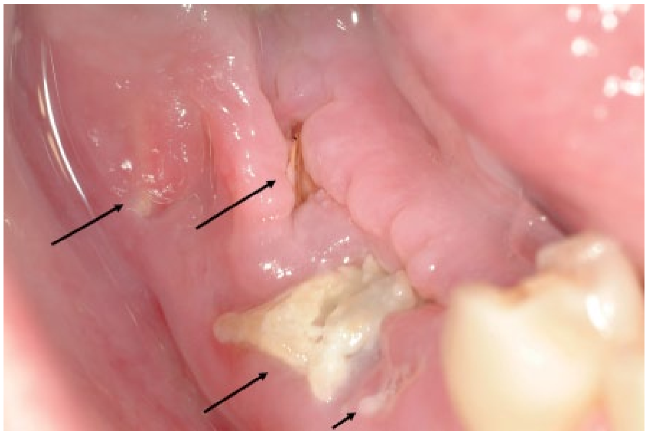

Figure 101.5 A 45-year-old male with a history of multiple myeloma who had been treated with intravenous zoledronate for 2 years prior to having two lower right molars extracted. He presented with three areas of bisphosphonate-associated osteonecrosis of the lower right mandible 6 months following the extractions. Lesions were non-painful and stable. Arrows indicate areas of bone exposure. (For a color version of this figure, please see the color plate section.)

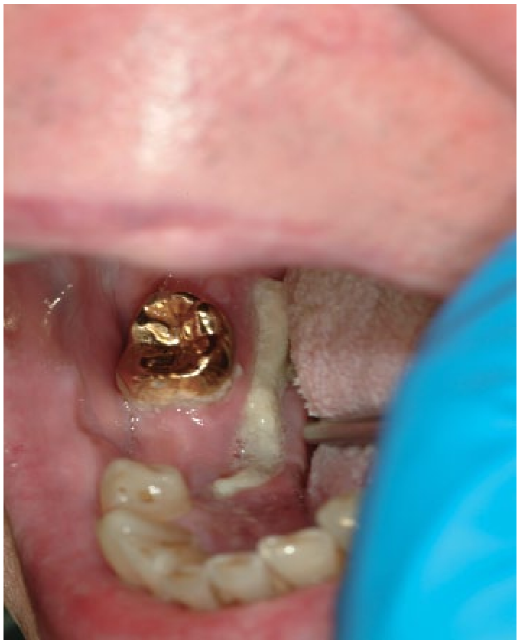

Figure 101.6 Bisphosphonate-associated osteonecrosis of the jaw involving the lower right lingual ridge in a patient with multiple myeloma treated with intravenous zoledronate monthly for 20 months. The patient's second bicuspid and first molar had been extracted 3 months earlier due to what was thought to be severe peridontal disease; the primary extraction site healed, but an extensive area of osteonecrosis developed along the mandibular lingual torus that was present lingual to the original extraction site. (For a color version of this figure, please see the color plate section.)

These lesions can be extremely painful and can be a dose-limiting toxicity. Early clinical reports suggest that these ulcerations can be responsive to topical, intralesional, and/or systemic steroids. Sirolimus-associated oral ulcers have not been reported, but could be responsible for additional mucosal damage in cGVHD patients being treated with sirolimus.

Routine dental care guidelines post-HCT

Complete dental examinations can be resumed generally between 120 and 180 days post-HCT and should include examination of teeth and periodontal tissues and radiographic assessments as indicated. However, resumption of routine dental treatment, including routine dental cleanings and restorations, is dependent upon the immune reconstitution and overall medical stabilization of the patient. Premature resumption of dental treatment can put the patient at risk for local infection, bacteremia, and aspiration pneumonia. In general, patients who have undergone autologous transplants can resume routine care approximately 5–6 months post-HCT with recovery of immune function; however, transplants utilizing CD34-depleted grafts or conditioning with fludarabine can result in slower immune recovery. Allogeneic HCT patients without cGVHD and who are no longer on immunosuppressive agents will generally require 9–12 months to recover adequate immune function before being candidates for routine dental care (immunosuppressive therapy should have been discontinued for approximately 6 weeks before resuming routine dental treatment). As long as the patient has active GVHD and is receiving immunosuppressive therapy, routine dental treatment should not be resumed, and if dental treatment is necessitated, infection prevention strategies should be used (see below).

If dental treatment is imperative for patients without full immune or hematologic reconstitution, it is important that the dentist and physician determine what supportive medical care is necessary, including prophylactic antibiotics, possibly immunoglobulin G administration, adjustment of steroid doses, and possibly platelet transfusions. Rinsing with chlorhexidine immediately before treatment is recommended. Prophylactic antibiotic regimens (American Heart Association endocarditis prevention protocols) appear to be efficacious, with regimens being extended if there is ongoing dental infection or if there is concern for delayed healing. Dentists should also utilize techniques to reduce the risk of aspiration (e.g. the use of rubber dams and high-volume suction), in addition to reducing the complexity of treatments and shortening treatment times.

Edentulous patients can generally receive prosthetic care once engraftment has occurred and their medical condition has stabilized. Either transitional or permanent dentures are typically feasible in these circumstances.

Orofacial/dental growth and development

Children younger than 16 years who undergo HCT are at risk for the development of abnormalities in their developing dental and/or skeletal structures [52] (see Chapter 102). Conditioning regimens, in addition to cancer therapy prior to transplantation, can damage tooth buds and result in a number of developmental abnormalities including enamel hypoplasia, root-growth alterations (root tapering and blunting, agenesis with premature apical closure), or complete agenesis. TBI conditioning regimens seem to be associated with increased risk of dental malformation compared with chemotherapy-only protocols. The younger a patient is at the time of transplant, the higher is the risk and extent of dental abnormalities (Figure 101.7).

Altered craniofacial skeletal development has also been noted following HCT and with altered orthognathic and facial development. The reduction in lower facial height reported in pediatric HCT patients could be due to the impaired dental development and the fact that young age is a risk factor for more severe dental disturbances [53], and also changes to facial and jaw skeletal development. It is useful to note that these skeletal changes appear to occur in a symmetric manner such that the child does not appear to have obviously hypoplastic jaws with corresponding esthetic changes; essentially, the face appears smaller but is relatively in proportion to the child's overall stature due to general retardation in growth.

Second malignancy post-HCT

The occurrence of new or second malignancies and lymphoproliferative disorders following HCT has long been recognized as a significant complication of HCT [48,50] (see Chapter 104). Oral cancers, especially squamous cell carcinomas and salivary gland tumors, are among the most common types of oral solid oral malignancies in both adult and pediatric long-term survivors of HCT [52,54]. Studies have shown that age at the time of HCT, cGVHD, and patients who had women as graft donors have an increased risk for development of second solid tumors. However, the presence of cGVHD was been reported as a risk factor for oral squamous cell carcinomas [55]. HPV infections may contribute to oral squamous cell carcinomas for HCT survivors, but the frequency and significance have not been studied or reported.

Unfortunately, oral dysplasia and malignancies can often be obscured by oral cGVHD or even mimic cGVHD, and only biopsy can discriminate between the two. Head and neck lymphoproliferative disorders have been noted, possibly with enlarged salivary

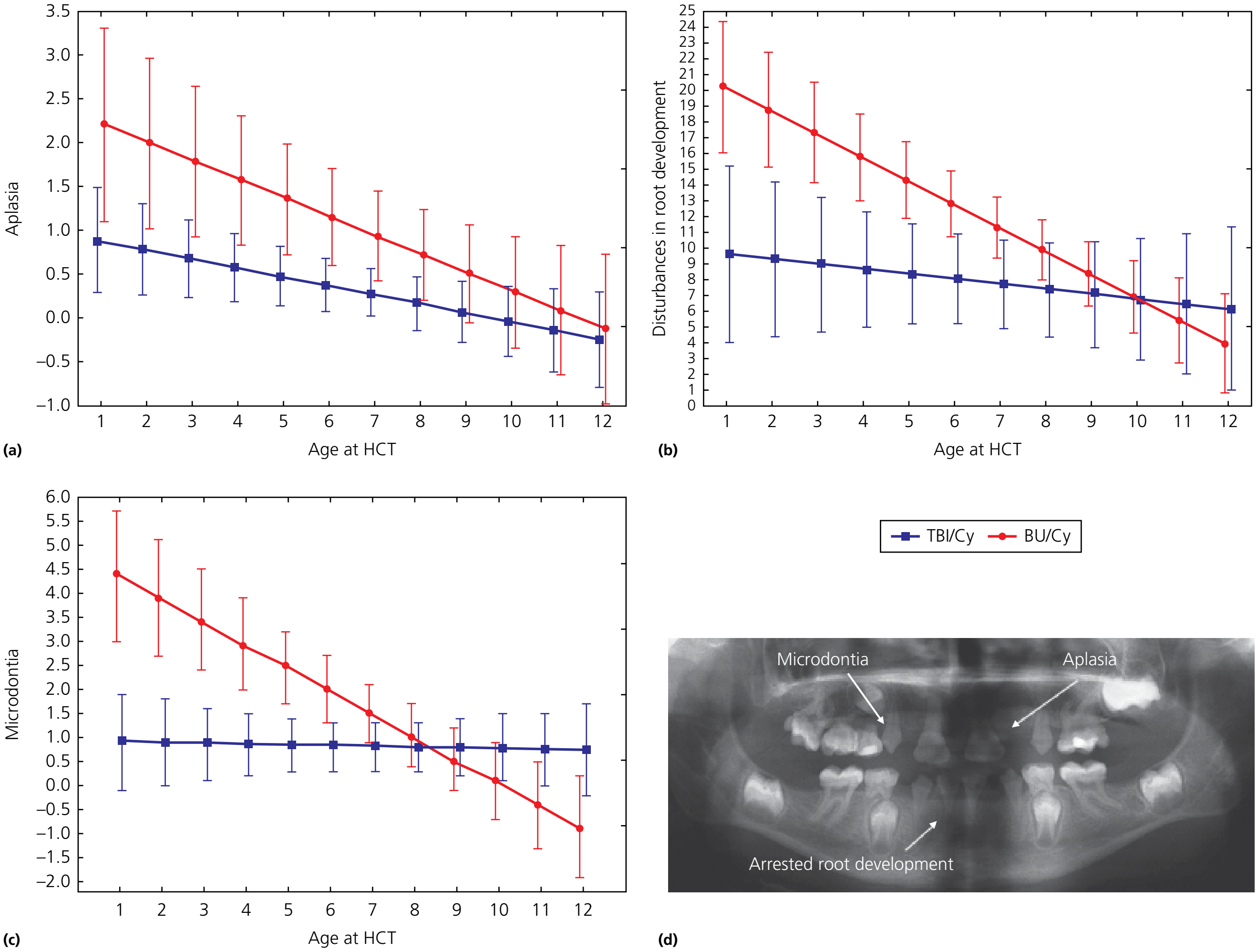

Figure 101.7 Disturbances in dental growth and development in long-term survivors of HCT. Conditioning regimes for HCT can cause severe disturbances in dental growth and development for pediatric HCT patients with TBI-containing regimes causing more problems than chemotherapy-only regimes. The younger the age at the time of transplant, the more significant the disturbance in dental developmental. (a) Number of missing teeth in children subjected to HCT by age. (b) Number of teeth with disturbances in root development by age. (c) Number of teeth with microdontia by age. (d) Panoramic radiograph showing arrested root development, microdontia, and aplasia of teeth in a pediatric long-term survivor post-HCT. Source: reproduced with permission of G. Dahllöf, K. Garming Legert, M. Remberger, and O. Ringdén Support Care Cancer (2012) 20 (Suppl 1):S226; 945. (For a color version of this figure, please see the color plate section.)

glands and cervical lymphadenopathy. Early detection of solid tumors has a crucial role in improving overall survival in this group of patients. Consistent and comprehensive oral soft tissue and head and neck examinations become a critical component of patient follow-up for all post-transplant survivors, with specific attention paid to early detection of potentially dysplastic and malignant lesions. Long-term survivors of HCT should be instructed to monitor oral lesions and changes appropriately: lesions not healing within 2–3 weeks should be carefully assessed and subjected to appropriate diagnostic testing. Clearly, biopsy is the only definitive way to differentiate between GVHD and malignancy in this setting [56].

Conclusion

The development of new technologies and strategies to prevent and manage HCT-related oral complications will have a significant impact on post-HCT patient recovery. Oral mucositis is one of the most significant and feared complications affecting early HCT recovery. However, preventing mucositis could reduce the risk for systemic infection, pain, and the number of hospital days, with a resulting improvement in the quality of life of patients, reducing hospital costs, and possibly reducing day-100 mortality. Additionally, improvements in management protocols for salivary gland dysfunction and taste dysfunction will also have a positive impact on the early phases of transplant recovery.

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CHAPTER 102

Growth and Development After Hematopoietic Cell Transplantation

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Introduction

Hematopoietic cell transplantation (HCT) is a curative therapeutic modality for an ever-increasing number of children and young adults with a variety of malignant and non-malignant disorders. However, an understanding of the late effects resulting from agents used in preparative regimens for HCT (chemotherapy and/or radiation therapy), along with knowledge of pre-HCT exposures, is necessary to appreciate and anticipate the effects on growth and development after transplantation.

Because the preparative regimens are designed not only to suppress the patient's immune system, but also to eradicate malignant cells, the doses of the agents administered are not limited by bone marrow toxicity. Historically, the most common regimens have utilized high-dose cyclophosphamide (CY) given alone or in combination with busulfan (BU), or other chemotherapy agents, and given with or without total body irradiation (TBI). Other chemotherapy agents used in preparative regimens include high-dose carmustine [1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU)], melphalan, etoposide (VP-16), fludarabine, and *N*-triethylenethiophosphoramide (thioTEPA). In addition to TBI, other radiation regimens include thoracoabdominal radiation (TAI) and total lymphoid radiation (TLI). The myeloablative doses of TBI have typically been 8–14.0 Gy, whereas the doses of TBI used for non-myeloablative transplant preparative regimens range from 2 to 6 Gy. Details of various myeloablative and non-myeloablative preparative regimens are discussed elsewhere in this book. It should be noted, however, that our current understanding of the impact on growth and development after non-myeloablative or reduced intensity HCT is basically unknown, although is anticipated to be significantly less than after myeloablative HCT.

Both high-dose chemotherapy and irradiation are known to affect the function of the neuroendocrine system, and therefore growth and development. The risks for the development of endocrine dysfunction depend on a variety of factors, including age at HCT, the type of conditioning regimen utilized, and the gender of the patient.

Normal growth and development require balanced endocrine gland function. Growth- and maturation-promoting hormones include thyroid hormone, growth hormone (GH), androgens, and estrogens. This chapter reviews the late effects that have been observed related to agents used primarily in myeloablative HCT preparative regimens on the endocrine function and the subsequent effects on growth and development of children and young adults.

Thyroid function

Normal thyroid hormone production is necessary for normal linear growth in young children. Consequently, suboptimal thyroid hormone production can contribute to decreased growth. Thyroid function is usually not impaired following conventional chemotherapy, but radiation to the thyroid gland has been associated with the development of compensated hypothyroidism, overt hypothyroidism, thyroiditis, and thyroid neoplasms [1]. Radiation of the thyroid may cause subsequent hyperplasia and induction of nodules and malignancies. The onset of thyroid dysfunction after irradiation usually begins as asymptomatic compensated hypothyroidism with elevated thyroid-stimulating hormone (TSH) and normal thyroid hormone production within the first year, and may progress to overt hypothyroidism with elevated TSH and subnormal thyroid hormone production over the next several decades.

Physical examination is often unreliable in detecting thyroid nodules. Fluctuations in serum thyroxine or triiodothyronine levels do not distinguish benign from malignant disease [2]. Ultrasonography detected thyroid abnormalities in 42 of 96 (44%) survivors of childhood cancer who received head and neck irradiation, compared with 15% with palpable lesions [2]. Thyroid nodules were detected by ultrasonography in 22 of the 96 (23%) patients and were significantly more frequent in patients whose thyroid exposure was more than 31 Gy.

The development of thyroid malignancies is another significant concern after HCT. While some report thyroid malignancy occurring between 1.5 and 6.0 years after radiotherapy exposure, other reports suggest a latency period of up to 40 years with a peak incidence occurring 15–25 years after irradiation exposure of 2–5 Gy [3,4]. The prevalence of thyroid malignancy in irradiated survivors of pediatric cancer is unknown. A 1991 epidemiologic investigation of 9170 patients who survived childhood cancer for at least 2 years reported a 53-fold increased risk of thyroid neoplasia [5]. Doses less than 2 Gy were associated with a 13-fold increased risk. Other studies suggest a radiation dose–response relationship and also a relationship to patient age at time of exposure, with younger age patients at highest risk [4,6].

The susceptibility of the thyroid to carcinogenetic effects of radiation is particularly apparent in younger individuals, and suggests

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that children who have received TBI, TAI, or TLI in their HCT preparative regimens are at risk for development of thyroid abnormalities. Since no single historical factor, physical finding, or clinical laboratory result is pathognomonic of thyroid neoplasia, the use of ultrasound to detect subtle architectural changes in the thyroid gland may be useful as a screening tool to detect asymptomatic nodules.

Preparative regimens consisting of chemotherapy only

After HCT, thyroid function has usually been evaluated with TSH and free T4 plasma levels. Abnormal values often prompt further study, for example, thyroid autoantibodies. The majority of results from reported studies show that chemotherapy regimens including CY, BU plus CY, and other chemotherapy agents plus CY have not resulted in thyroid function abnormalities in the majority of patients, although these have all been limited by relatively small sample size of subjects [7–12]. Children given BU–CY preparative regimens and marrow transplantation for acute myeloid leukemia (AML) have shown a 3% incidence of hypothyroidism, suggesting that patients who received BU–CY are at low risk for development of hypothyroidism [9]. However, in a larger study, the Seattle group published data on 791 children who underwent HCT and survived for more than 1 year and found that by 10 years post-HCT 30% of patients developed hypothyroidism [13]. In multivariate analysis examining the impact of the preparative regimen (Figure 102.1a), there was no difference in the risk of thyroid dysfunction between patients who had received TBI compared with a BU-based regimen [hazard ratio (HR) 1.0 vs. 0.84, $p = 0.48$]. The highest risk of thyroid dysfunction was in the group who had received a preparative regimen that contained both TBI and BU (HR 1.7, $p = 0.009$), and the risk was reduced in those patients who received CY only in their conditioning regimen (HR 0.32, $p = 0.03$). Other significant risk factors from this analysis included local radiotherapy (spinal, mantle, and total lymphoid, HR 1.82, $p = 0.05$), age less than 10 years (HR 1.7, $p = 0.001$, Figure 102.1b), and malignant diagnosis (compared with non-malignant diseases) where the HR ranged from approximately 3.0 for myeloid or lymphoid malignancies to 15 for Hodgkin's disease (overall $p < 0.001$). Of note, however, is that in this analysis, the only patients to develop thyroid malignancy were those who had received TBI. In a larger analysis of over 4000 patients who underwent HCT for either AML or chronic myelogenous leukemia (CML) with a BU-based preparative regimen (no patient had received radiation therapy as treatment for disease before HCT or in preparation for HCT), no increased risk of developing thyroid cancer was identified [14].

Preparative regimens containing irradiation

Detailed evaluations of the hypothalamic–pituitary–thyroid axis have demonstrated that the major effect of irradiation is at the level of the thyroid gland and not at the level of the hypothalamus or pituitary gland [15]. Compensated hypothyroidism and overt hypothyroidism are both frequent events following TBI- or TLI-based preparative regimens [10–12,16–19]. In an analysis of thyroid function in 153 children with more than 5 years of follow-up, 16 (9%) developed overt hypothyroidism at a median of 2.9 years (range 1–10 years) and 31.4% developed compensated hypothyroidism at a median of 2.7 years (range 0.8–11 years) after transplant [11]. Thyroid dysfunction-free survival was 73.2% after 5 years and 59.2% after 10 years. Factors associated with the development of hypothyroidism were patient age less than 9 years, single-dose TBI, and HCT in second complete remission. Thyroid nodules not associated with malignancy developed in 10 of 35 patients evaluated at a median of 7.8 years. Other studies have also confirmed the fact that age younger than 9–10 years [13,20,21] at HCT is a risk factor for developing thyroid dysfunction after HCT, although as noted previously, TBI has not always been identified as an independent risk factor [13].

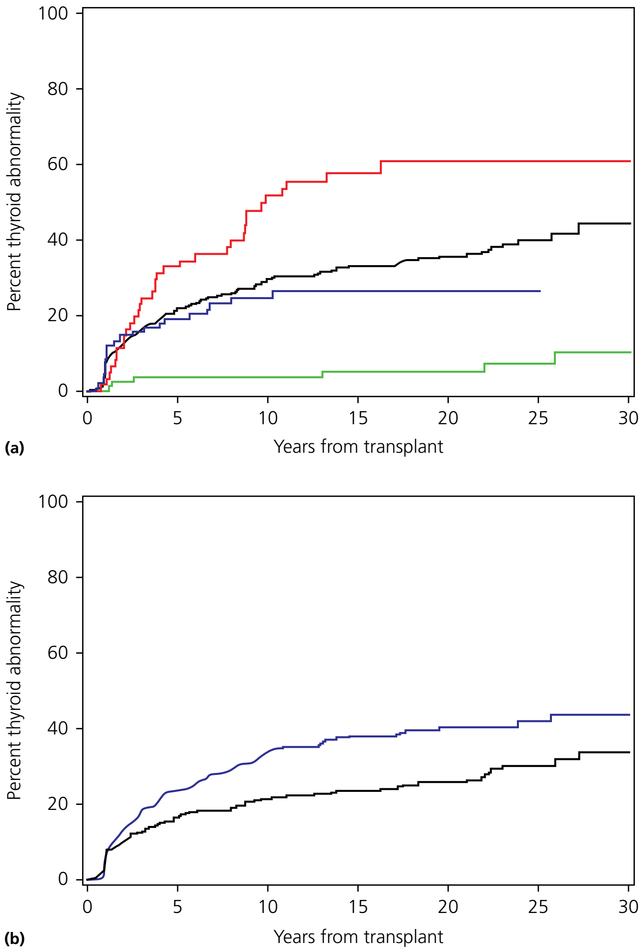

Figure 102.1 (a) Cumulative incidence of developing thyroid dysfunction after HCT according to type of preparative regimen received: from top to bottom, BU + TBI, TBI, BU, or CY only. (b) Cumulative incidence of developing thyroid dysfunction after HCT according to age at time of HCT. The bottom curve represents patients who received HCT between 10 and 17 years of age and the top curve patients between 0 and 9 years of age.

It appears that one of the strongest factors related to the risk of thyroid dysfunction after TBI exposure depends upon how the TBI is delivered and that the impact on thyroid function is much more significant in patients who received single-fraction TLI or TBI. Among children given 7.5 Gy single-exposure TLI or 7.8–10.0 Gy single-exposure TBI, compensated hypothyroidism or overt hypothyroidism developed in approximately 40–48% of patients, in contrast to an incidence of compensated hypothyroidism or overt hypothyroidism of approximately 15% observed among children after 12.0–15.74 Gy fractionated TBI [10,11,16–19]. The patients in these studies where single-exposure TBI was administered were followed on average for more than 8 years after radiation exposure, whereas those given fractionated exposure TBI were followed for a median of 4–6 years after radiation exposure. Although fractionated

TBI appears to result in a lower incidence of thyroid abnormalities, data from patients who received radiation but not HCT have shown that a risk for development of thyroid dysfunction and thyroid malignancies may be delayed several decades. The development of thyroid dysfunction has been associated with age at transplant, with age 0–9 years having higher risk than age greater than 10 years (HR 1.7, $p = 0.001$) [13]. Other factors such as gender or acute or chronic graft-versus-host disease (GVHD) have not been shown to have an impact on this risk.

Treatment

All patients in whom overt hypothyroidism develops should receive treatment with thyroxine, but the benefit of thyroid replacement in patients with compensated hypothyroidism is controversial. Although the carcinogenic potential of thyroid irradiation has been well documented, the ability of thyroid replacement to reduce the incidence of radiation-associated thyroid carcinoma remains unproven [1,22]. Recommendations from pediatric endocrinologists vary with respect to treatment of compensated hypothyroidism after irradiation therapy. Among patients in whom benign thyroid nodules develop after conventional irradiation therapy, treatment with thyroxine decreases the risk of recurrence of these nodules but does not decrease the risk of thyroid carcinoma.

Papillary carcinoma, toxic goiter, and an adenoma were observed between 4 and 14 years in six children after 10.0 Gy single-exposure TBI and in at least five after exposure to 12–15.75 Gy fractionated TBI [15]. All of these children had abnormal thyroid function, and none had received thyroid hormone therapy prior to discovery of the thyroid mass. The adenoma was found at autopsy, but the other patients were treated successfully with thyroidectomy or radioactive iodine thyroid ablation. Hence all patients who have received TBI, TLI, or TAI should be examined annually with physical examination, tests of thyroid function, and perhaps ultrasound.

The euthyroid sick syndrome should also be taken into consideration when interpreting the results of thyroid tests. In this condition, changes in thyroid hormone metabolism are associated with a severe, non-thyroidal illness, including in some cases HCT. The level of TSH decreases rapidly during the first few months after HCT but gradually recovers to normal by 6 months. Acute changes in thyroid hormone levels also gradually recover to pre-HCT levels by 6 months. Increased levels of cytokines, including interleukin-6 and tumor necrosis factor-alpha, post-HCT were found to correlate inversely with the levels of thyroid hormone. The euthyroid sick syndrome does not require specific thyroid hormone therapy [23].

Growth

Linear growth is a continuous and finely regulated phenomenon that is the result of the interaction of genetic make-up, nutritional factors, hormones (GH, thyroid hormone, sex steroids), and metabolism. The importance of each major factor varies with different periods of growth. Growth in infancy is largely determined by nutrition and metabolic factors. Growth in childhood is largely influenced by GH and in puberty by the synergistic action of GH and sex steroids. Impaired linear growth after HCT is multifactorial in origin and can be due to GH deficiency, hypothyroidism, hypogonadism, corticosteroid treatment, and poor nutritional status. The impact of GH deficiency on growth in children who received HCT is frequently difficult to determine since GH studies have not been performed consistently. Data are difficult to interpret as to whether intensive chemotherapy regimens with or without central nervous system (CNS) radiation are associated with persistent growth impairment, since often only growth rates are reported [24–28]. Some studies suggest that the intensity and duration of combination antineoplastic therapy in addition to CNS radiation and age at diagnosis influence patterns of growth [29–31]. The younger the child is at the time of CNS radiation, the greater is the loss of growth during puberty.

Several studies have compared growth outcome for children treated with conventional chemotherapy with or without cranial radiation and HCT. One study [32] of children with AML concluded that patterns of growth were similar between those who were treated with chemotherapy and those treated with HCT. Follow-up for the 26 patients in the chemotherapy group was 7.4 years (range 1.8–15.5 years) and that for the 26 patients in the HCT group was 5.6 years (range 2.0–15.4 years). None of the patients had GH testing performed, 11 chemotherapy patients had received 18–24 Gy cranial radiation, and nine HCT patients had received TBI. The change in height standard deviation (SD) from time of diagnosis to last follow-up was −0.43 for chemotherapy patients and −0.48 for HCT patients. A second comparison study [33] included 10 patients given chemotherapy, 18 patients given chemotherapy plus 18.0–24.0 Gy cranial radiation, and 15 patients given chemotherapy plus 12–15 Gy TBI and HCT. The change in height SD was −0.21 for the chemotherapy group,−1.26 for the chemotherapy plus cranial radiation group ($p = 0.0001$), and −1.33 for the HCT group ($p = 0.0008$). All patients had GH testing performed, and the only patients with GH deficiency were in the HCT group ($p = 0.003$). The third study evaluated infants diagnosed at less than 1 year of age with AML and acute lymphoblastic leukemia (ALL) [34]. Ten were treated with chemotherapy only, 17 received chemotherapy plus cranial radiation, and seven received HCT (six with TBI). The median change in height SD at 5 years after diagnosis was −0.9 for the chemotherapy group, −1.0 for the chemotherapy plus cranial radiation group ($p = 0.07$), and −2.3 for the HCT group ($p = 0.01$). Further follow-up for a small number suggested that height continued to decline with increasing time after diagnosis. Based on these and other studies, patients experience an average loss of 0.9–2.1 SD in height from the time of HCT to final height [35–39].

Recipients of chemotherapy only without cranial radiation have height losses that are substantially less than those observed after HCT using TBI-containing regimens [36,37,39–46]. CNS radiation has been associated with the development of GH deficiency, which appears to be related to the child's age at time of radiation, the radiation dose received, and the length of time elapsed after completion of radiation [47–49]. Radiation-induced GH deficiency is thought to be due mainly to hypothalamic damage, although the pituitary gland also shows sensitivity to radiation [47]. Some children, especially those with ALL, have received 18–24 Gy CNS radiation as part of their initial treatment prior to referral for HCT. When TBI is included in their preparative regimen, their total CNS radiation dose will usually exceed 30 Gy, the estimated threshold for development of GH deficiency [50], although even doses as low as 9–10 Gy may result in GH deficiency [51]. GH deficiency may be expected to develop 2–3 years after radiation in the majority of patients who received a higher total dose of CNS radiation, whereas GH deficiency may not develop in patients who received lower doses of CNS radiation for up to 5–10 years after radiation. Hence it may be anticipated that nearly all children who have received CNS radiation in addition to TBI are likely to develop GH deficiency, and that some children who received only TBI (particularly a single dose of TBI) just prior to or during their growth period may develop GH deficiency [42,43,52,53]. The latter group may not manifest a decline in the rate of growth until the third year after HCT. Fractionated TBI and TLI can also reduce growth velocity and final height, although the effect is less pronounced than after single-dose TBI [9,36,54,55]. Growth failure associated with TBI may also be due to direct radiation damage to the growth plates [56–58].

Because GH secretion is episodic, determination of GH levels involves the use of a stimulus to enhance pituitary GH secretion followed by multiple venous blood samplings. There is no consensus in the literature regarding a definitive GH stimulation test [59,60]. Although the 24-hour spontaneous pulsatile GH secretion test is considered by many to be the most physiologic assessment of GH secretion, it is impractical owing to the large volumes of blood required and the need for frequent sampling [61,62]. The 12-hour overnight sampling schedule has been shown to be well tolerated, reliable, and reproducible, but is less frequently used owing to the inconvenience of overnight hospitalization, extensive blood sampling, and lack of clear advantage over stimulated GH response [63,64]. Commonly used stimuli include exercise or pharmacologic agents such as clonidine, levodopa, arginine, growth hormone-releasing hormone (GHRH), and insulin [59,60,65–67]. The clinical diagnosis of GH deficiency is based on a combination of GH stimulation test, auxological criteria, biochemical tests, and radiologic evaluation [68]. Failure to attain a normal peak GH level (usually after two different stimuli), accompanied by a low insulin-like growth factor-1 (IGF-1) and IGF-binding protein-3 (IGFBP-3) levels, a height more than 2 SDs below the mean, decreased growth rate and height SD score, and a delayed bone age are typical features of classical GH deficiency, although not all of these criteria have to be met to diagnose GH deficiency [66,68]. For example, GH deficiency may be present in children whose height is above –2 SD, but significantly below the mid-parental height, especially if growth velocity is reduced. Also, IGF-1 and IGFBP-3 may not be reliable indicators of GH status after cranial radiation [69,70]. Furthermore, GH deficiency may develop over time, so GH provocative testing may have to be repeated even if the initial test was normal. Children who have received cranial radiation may have variable responses to the provocative stimuli, which may be due to the use of different pharmacologic agents, variations in the interval between radiation and testing, or neurosecretory defects [47,71,72].

Preparative regimens containing chemotherapy

Following preparative regimens with high-dose CY only, normal height SDs are usually observed, although pubertal growth spurt may be blunted [73]. When tested, normal levels of GH were detected. Some children with chronic GVHD treated with corticosteroids had decreased growth velocity during the time chronic GVHD was active and while being treated with corticosteroid therapy. Once chronic GVHD was controlled and corticosteroid therapy discontinued, catch-up growth occurred prior to growth rates returning to normal for age. Final height achieved for 26 girls and 25 boys was at a median of the 25th percentile (range 5–90th) for girls and the 50th percentile (range 5–95th) for boys. The European Group for Blood and Marrow Transplantation reported that mean final height SD among 26 patients prepared with CY only was –0.15 ± 1.68 [36].

BU, an alkylating agent frequently combined with CY in preparative regimens, is an agent that affects both dividing ands non-dividing cells, and crosses the blood–brain barrier [74]. The effect of BU on growth has been reported for children prepared with 14 mg/kg BU and 200 mg/kg CY prior to bone marrow transplantation (BMT) for thalassemia major [8,75]. Both studies found an effect of patient age at the time of HCT on subsequent growth. Among 47 patients evaluable for final adult height, the 26 patients who were aged 7 years or less at HCT had a final adult height consistent with the mid-parental height, whereas the 21 patients who were over 7 years of age at HCT failed to achieve their full genetic height potential. It is not possible from these data to determine if this effect was secondary to BU exposure or whether the correction of thalassemia at an earlier age had a positive impact on growth.

Among children prepared with 16 mg/kg BU and 120 mg/kg or 200 mg/kg CY prior to BMT for AML, similar data have emerged. Data among the AML children who had not received prior CNS radiation have generally demonstrated normal growth velocity [9,15,36,43,46,76,77]. Studies have shown that height SD improves following HCT. One study of 23 children found no significant difference between height SD at HCT and yearly height SD from 1 to 5 years post-HCT, with a mean height at HCT of –0.38 SD to a mean height at 5 years of –0.11 [76]. The European Group for Blood and Marrow Transplantation reported final adult height to be –0.38 ± 1.16 SD for 10 children [36]. We have observed the final adult height *z*-score to be –0.3 to –0.4 (±1.46) SD or height at a mean of the 30th percentile for both boys and girls. Height SD and height growth velocity are shown in Figures 102.2 and 102.3 for 47 boys and 39 girls following 16 mg/kg BU and 120 or 200 mg/kg CY. Both girls and boys had blunted pubertal growth spurt. The impact of chronic illness on height has been demonstrated in one report of 30 children evaluated at 3 years after HCT. The 12 children with no post-HCT complications had a change in height from HCT to 3 years of –0.2 SD, whereas the 18 children with complications (mainly chronic GVHD) had a change in height of –1.7 SD ($p = 0.001$), demonstrating that factors other than the preparative regimen have significant impact on the child's growth [77].

Thus, the impact of chemotherapy-only preparative regimens on growth is less clear. Chemotherapy-only regimens usually do not adversely affect height, although there are rare reports of growth impairment and GH deficiency after BU–CY [8,43,46]. Although BU crosses the blood–brain barrier and can produce dose-related neurotoxicity, a direct causality between the dose of BU and impaired GH secretion remains to be proven [78,79].

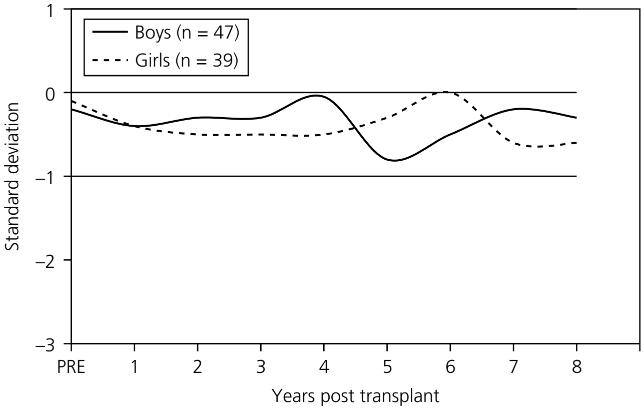

Figure 102.2 Height standard deviation curves for 39 girls and 47 boys transplanted with a preparative regimen of 16 mg/kg busulfan (BU) and 120–200 mg/kg cyclophosphamide (CY). Source: Sanders et al. 1988 [73]. Reproduced with permission of S. Karger AG.

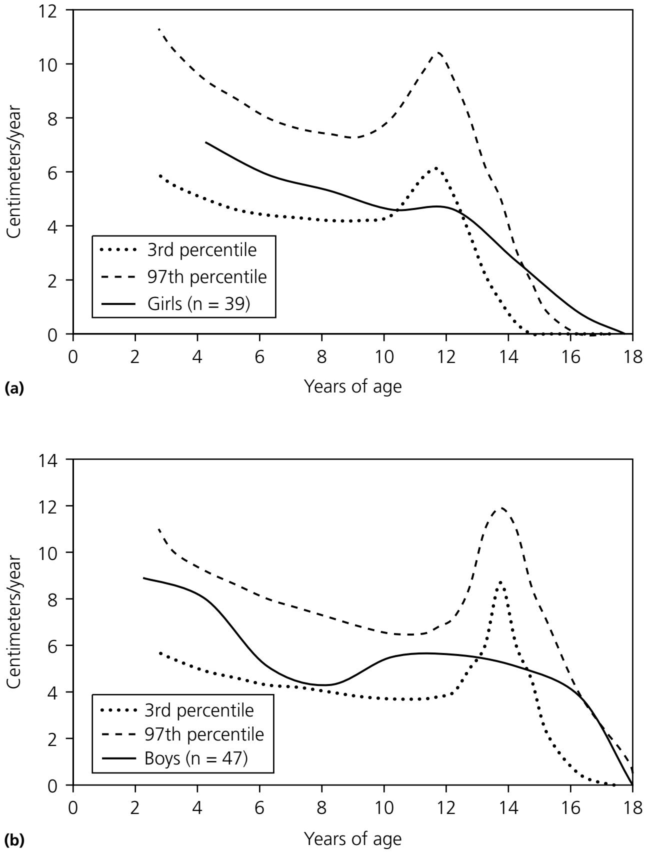

Figure 102.3 (a) Height velocity curve for 39 girls transplanted with a busulfan (BU) and cyclophosphamide (CY) preparative regimen. Note the blunted pubertal growth spurt. (b) Height velocity curve for 47 boys transplanted with a preparative regimen of BU and CY. Again, note the blunted pubertal growth spurt. Source: Sanders et al. 1988 [73]. Reproduced with permission of S. Karger AG.

Radiation-containing preparative regimens

Growth rates

Growth impairment after TBI has been well documented [9,10,15,35–37,40–46,55,57,69,73,80]. Children given single-exposure TBI usually have been the group with the greatest incidence of growth rate impairment, which often has also been associated with decreased GH production. Fractionated TBI was initially thought to have a growth-"sparing" effect, but these children have demonstrated decreased growth rates and decreased height SD scores [39,55,81,82]. One study comparing height SD scores for 26 patients given 9–10 Gy single-exposure TBI with those for 23 patients given 12–14.40 Gy fractionated-exposure TBI found that all had decreased height SD scores. The patients with single-fraction TBI had a change in height SD scores from −0.29 to −0.90 ($p = 0.0001$) between 1 and 3 years after HCT, whereas the height SD scores for patients who received fractionated TBI changed from −0.09 to −0.22 ($p = 0.02$) between 1 and 3 years after HCT [55]. A second study reported the mean cumulative height change during the first 3 years after TBI among prepubertal children not given cranial radiation. These height changes were significantly worse for the 11 patients given 10 Gy single-exposure TBI ($p = 0.001$) (mean height change −1.4 SD) compared with seven children given 12 Gy fractionated TBI (mean cumulative height change −0.4 SD, p = NS) [81]. A third study reported no differences in height SD at 8 years after single-exposure TBI for 81 children. The mean height changes for 237 children who had not received cranial radiation prior to 12.0–15.75 Gy fractionated TBI were −1.0 SD at 3 years and −1.3 SD at 6 years, and among the 79 who received cranial radiation and fractionated TBI the mean height changes were −1.4 SD at 3 years and −1.7 SD at 6 years. Prior cranial radiation and years post-transplant were the only factors found to influence subsequent height SD scores significantly.

Patient age at transplant has been found to be a significant factor in predicting final adult height [36,37,39,40]. Studies have shown that children less than 10 years of age at time of transplant have the greatest risk for growth failure and significantly decreased final adult height. Patient's gender has also been found to have a significant influence on final adult height, with boys being at a greater risk of growth failure than girls [37,39,40]. Other reports have confirmed the negative effect of young patient age and male gender on final height [39,83]. Boys had a significant decrease in sitting height and leg length, whereas girls had a decrease in sitting height SD score that was not accompanied by a reduction in leg length [83]. The decreased sitting height in both boys and girls is most likely related to the effect of radiotherapy on the spinal growth, which is greater than the impact on the leg growth. These investigators found that the mean height SD loss between TBI and final height was −1.1 SD for girls and −1.5 SD for boys.

Growth hormone

The total height gained from GH therapy is inversely related to patient age at the start of treatment and positively correlated with the duration of therapy. GH has additional beneficial effects for the growing child in addition to improving linear growth. Children with GH deficiency are at risk for decreased bone mineral density due to reduced osteoblastic activity, leading to decreased bone mineral accrual [84–86]. Studies in children with GH deficiency have shown that bone loss is reversed with GH therapy [87]. GH also affects body composition and lipid profile. Those who are GH deficient have reduced lean body mass, increased visceral obesity, dyslipidemia, and an increase in cardiovascular risk factors and cardiovascular mortality [88–94]. Treatment with GH has resulted in improved lean body mass and improved cardiovascular risk profile among children not undergoing HCT [95–98].

The incidence of GH deficiency after TBI and HCT varies from 20 to 85% depending upon differences in time of testing after HCT, preparative regimens received, inclusion of patients with and without cranial radiation, and use of different methods of GH testing [18,44,53,57,66,69,99,100]. Even though GH deficiency has been observed, fewer than half have received GH therapy. Retrospective studies have shown that height SD is significantly lower than predicted based on height at HCT and mid-parental height [37,40,55]. Some authors have concluded that GH therapy was not needed because final height SD was not more than 2 SD below the mean, or because GH-treated children did not have significantly improved growth [36,37]. Others have reported, based on a retrospective study, that GH therapy does not benefit children, but most of the children had received less than 1 year of GH therapy [35]. One study reported on 13 children, eight of whom received prophylactic cranial radiation, who were given GH, which restored normal height velocity but without "catch-up" growth [53]. The final height SD scores remained impaired. In these children, a mean of 3.2 years had elapsed between TBI and initiation of GH therapy, which began at a mean age of 12.2 years (range 5.8–18.2 years).

The poor response to GH therapy has been attributed to factors such as spinal radiation, early puberty, suboptimal GH dosing

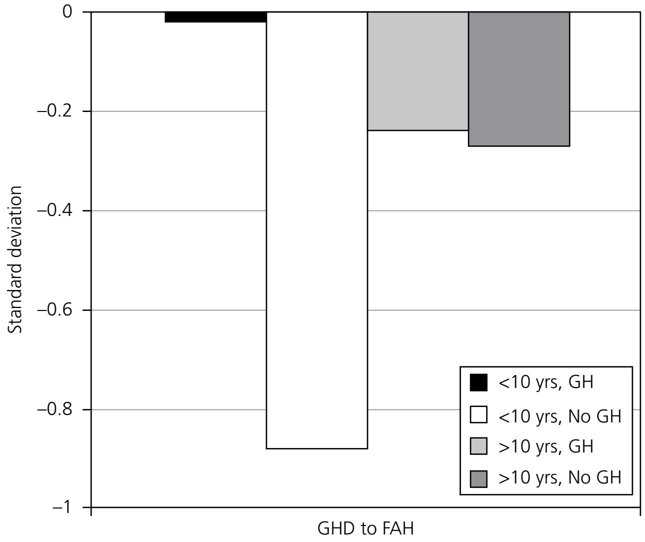

Figure 102.4 Final height SD score for 42 children with growth hormone deficiency (GHD) treated with GH when less than 10 years of age (black box) or more than 10 years of age (light gray box), and final height SD score for 48 children with GH deficiency not treated with growth hormone when less than 10 years of age (long white box) or more than 10 years of age (dark gray box). FAH, final adult height.

schedules, variable monitoring practices, and older age of most patients when GH therapy was initiated. However, improvements in growth and final height can be achieved with contemporary dosing regimens of about 0.3 mg/kg/week in prepubertal children with adequate pubertal dose increase to promote growth spurt [42,57,101,102]. In a more contemporary study of GH-deficient children transplanted at a single transplant center with fractionated TBI, GH treatment was associated with significantly improved final height [39]. Children who were treated with GH gained on average 0.9 SD in height compared with untreated children. The effectiveness of GH treatment was inversely associated with patient age at the time of GH treatment and positively associated with the duration of treatment. Younger patients (<10 years of age) responded better to GH treatment than older patients, and females responded better than males. Interestingly, a history of cranial radiation did not have a significant impact on the response to GH therapy. Figure 102.4 shows how the final height for 42 children with GH deficiency treated with GH and 48 children with GH deficiency who were not treated with GH was influenced by age at the time of TBI [39]. A beneficial effect of GH treatment on either short-term growth [57] or growth up to final height [38] has also been reported by other authors.

Initially, treatment with human GH was restricted owing to the limited supply of the drug, but with the availability of recombinant GH, access no longer is a problem. Reports of leukemia occurring in patients treated with human GH have raised concern that there might be a causal relationship between GH therapy and development of leukemia [100,103–105]. The Lawson Wilkins Pediatric Endocrine Society and the Human Growth Foundation of the United States convened a workshop in 1988 to review known leukemia cases in 22,000 GH-treated patients in Europe, North America, Japan, and Australia since 1959 [106]. Of 15 cases of leukemia, four occurred after a brief period of GH therapy or there were compelling reasons to suspect another cause for the leukemia. The 11 suspect cases developed during 150,000 patient-years of risk, including the periods of treatment and follow-up, so that the incidence of leukemia was approximately one per 21,000 patient-years at risk. The expected annual leukemia incidence among treated patients with hypopituitarism was estimated to be one per 42,000 patient-years. After review of all the available data, workshop participants concluded that there might be a small increase in leukemia incidence associated with the GH treatment of GH-deficient patients, but it was not clear that this incidence was directly related to GH therapy. In absolute terms, a current estimate of individual risk, assuming a 10-year GH treatment course, would be one per 2400 (0.042%), which is no different from the Surveillance and End Results estimate of new cases of leukemia reported in the United States (1:2400 per year).

Sklar et al. studied 361 GH-treated cancer survivors from among 13,539 survivors enrolled in the Childhood Cancer Survivor Study and compared risk of recurrence, risk of second neoplasms, and risk of death between survivors who did and did not receive GH treatment [107]. With respect to disease recurrence, the data have been largely negative. Relative risk of a first recurrence was 0.83 ($p = 0.65$) for those treated versus not treated with GH when adjusted for age at diagnosis, radiation therapy, and chemotherapy. For CNS tumor survivors, the risk of disease recurrence was actually significantly lower for patients treated with GH than patients not treated with GH. The risk was not different between GH-treated and not treated patients for other diagnoses also, including acute leukemia [49]. However, the risk of second neoplasm was slightly increased in patients treated with GH (5.5% of those treated with GH developed a second neoplasm). All second neoplasms were solid tumors; there were no cases of secondary leukemia. The most prevalent types of tumors were osteosarcoma (particularly in leukemia survivors) and meningioma (particularly in patients with CNS tumors). The risk of death was similar between GH-treated and not treated patients. Osteosarcoma occurred in three of the 122 leukemia/lymphoma survivors treated with GH compared with only two cases in >4500 leukemia/lymphoma survivors who did not receive GH. The study by Sanders et al. showed no difference in the development of recurrent leukemia or secondary malignancies between those HCT recipients who received GH therapy and those who did not [39]. The incidence of osteochondroma/exostosis was higher among GH-treated patients [39].

Puberty

Puberty, a transitional stage from a sexually immature to a mature sexual state, is accompanied by significant changes in gonadal and growth hormonal activity, development of secondary sexual characteristics, and increased growth velocity. There is considerable variation in the timing of pubertal events. Pubertal development is usually closely correlated with osseous maturation as measured by bone age. An intact hypothalamic–pituitary–gonadal axis is required for initiation and completion of puberty. The normal pubertal growth rate is usually 1.5–2 times greater than prepubertal growth rate [108]. Sex hormones indirectly stimulate linear growth by increasing endogenous GH secretion [109]. This stimulation leads to increasing circulating and tissue levels of IGF-1, which activates growth at the level of bone and cartilage.

Hypogonadism is common after HCT [52,110]. Hypergonadotropic hypogonadism (primary gonadal failure) is more common than hypogonadotropic hypogonadism (due to hypothalamic–pituitary dysfunction). In girls, ovarian failure is diagnosed by increased plasma follicle-stimulating hormone (FSH) and/or luteinizing hormone (LH) concentrations [111]. Ovarian failure impairs both fertility and estradiol production. In boys, seminiferous tubule dysfunction (characterized by an increased plasma FSH concentration and smaller testicular size) is more common than Leydig cell dysfunction (manifesting as low testosterone level and increased plasma LH concentration) because the germinal epithelium of the testis is more vulnerable to chemotherapy and radiation than Leydig cells [9,112]. Thus, in males, fertility may be impaired, but testosterone production may be normal. Precocious puberty is much less common than delayed puberty [38].

Clinical presentation of impaired sex hormone production depends on the age and pubertal stage at the time of HCT. Younger patients may have delayed puberty, whereas older patients may show asynchronous or incomplete pubertal development, primary or secondary amenorrhea, and infertility due to azoospermia or premature menopause. Delayed or incomplete puberty occurs in about 57% of females and 53% of males [52,113–115]. In general, premature ovarian failure occurs in 65–84% of females after HCT [111–113,116–118] and testicular failure occurs in 48–85% males after HCT [52,111,112,119,120], although the incidence varies depending on the therapeutic regimen. Sex steroids are also required for the growth spurt during adolescence. In the absence of pubertal sex hormone secretion, the increased growth velocity associated with the pubertal growth spurt is substantially blunted, resulting in a decrease in final adult height [121]. Treatment with low to moderate doses of sex hormones promotes increased linear growth, but physiologic adult doses of sex hormones result in a greater influence on skeletal maturation, with resultant compromise in final adult height via the mechanism of premature epiphyseal closure.

Gonadal hormone production and germ cell viability are affected by high doses of alkylating agents and irradiation, with variables related to patient age, gender, and type and dose of therapy [122]. Impaired gonadal function affects 16% females and 14% males after CY alone, 72% females and 48% males after BU–CY, and 57–71% females and 58–81% males after TBI, depending on the dose [52,113–115]. Azoospermia develops in prepubertal boys who have received a cumulative dose of more than 350 mg/kg CY, whereas doses of 200 mg/kg or less result in minimal alteration of spermatogenesis. Irradiation to the prepubertal testes results in damage to the germinal epithelium that does not become apparent until the usual time of puberty [123]. Boys who have received more than 24 Gy of testicular irradiation have delayed or arrested development of secondary sexual characteristics, with elevated gonadotropin and low testosterone values. Primary ovarian failure usually occurs following total cumulative doses of more than 500 mg/kg CY to prepubertal girls [122]. Hence both ovaries and testes are sensitive to gonadotoxic effects of chemotherapy and radiation.

Preparative regimens containing only chemotherapy

Following 200 mg/kg CY and BMT for aplastic anemia, 31 of 32 girls and 28 of 31 boys who were prepubertal at the time of administration of CY were followed past the age of 12 years to evaluate pubertal development [52,112]. Nearly all of these children demonstrated normal age-appropriate progression through puberty. Three girls with delayed pubertal development had Fanconi anemia and eventually developed normal secondary sexual characteristics and normal LH, FSH, and estradiol levels, and two girls showed delay due to chronic GVHD therapy. Among the 26 girls with normal age-appropriate development, menarche occurred at a median of 12.5 years of age (range 11–16 years), and the five with delayed development had menarche occur between 16 and 19 years of age. Thus, prepubertal patients are more resistant to gonadotoxic effects of CY and are more likely to retain or recover ovarian function [124]. The three boys with delayed pubertal development had chronic GVHD and did ultimately develop normal secondary sexual characteristics with normal LH, FSH, and testosterone values. Younger age is also somewhat protective in boys [124].

Gonadal function after 14 mg/kg BU plus 200 mg/kg CY and allogeneic BMT for thalassemia has been reported for 64 prepubertal patients (31 girls and 33 boys) who ranged in age from 9.3 to 17.2 years [125–128]. Twenty-five girls had evidence of primary ovarian failure, with elevated gonadotropin levels, and six girls had hypogonadotropic hypogonadism. All girls had low estradiol levels both before and after transplantation and required hormone supplementation for development of secondary sexual characteristics. Among the 33 boys, post-transplant LH and FSH concentrations were within normal limits for 23 boys, but abnormal with either elevated LH and FSH or low LH and FSH in 10 boys. However, after gonadotropin-releasing hormone stimulation, three had normal responses, two had elevated FSH responses, and 10 had low responses. These gonadal function results must be interpreted with caution, however, because patients with thalassemia treated with chelation and transfusion therapy frequently show delayed or absent puberty.

Pubertal development has been evaluated among children transplanted for malignancy who received 16 mg/kg BU plus 120–200 mg/kg CY [125,129,130]. These data demonstrate that BU is highly toxic to prepubertal, particularly female, gonads. Probability of recovery of ovarian function is extraordinarily low or non-existent in patients who received BU–CY [9,76,112]. Boys who were exposed to CY-only conditioning regimens are more likely to recover testicular function (24–90%) than those exposed to BU–CY (6.5–50%) [112,119].

Preparative regimens containing radiation

The development of secondary sexual characteristics among children who were prepubertal at the time of TBI administration but more than 12 years of age at follow-up was evaluated in 24 girls and 31 boys after 10 Gy single-fraction TBI, and 77 girls and 77 boys after 12–15.75 Gy fractionated-exposure TBI [52,112]. Following 10.0 Gy single-exposure TBI, 71% of girls and 83% of boys had delayed development of secondary sexual characteristics, elevated LH and FSH levels, and low sex hormone levels. After fractionated-exposure TBI, 49% of girls and 58% of boys had delayed development. The majority of these girls and boys have received appropriate sex hormone therapy for promotion of secondary sexual characteristic development.

The amount of testicular radiation received and patient age at the time of TBI are important factors in subsequent normal spontaneous pubertal development (Table 102.1). Thirty of the 36 (84%) boys who had received more than 10 Gy testicular irradiation for testicular leukemia in addition to 12–15.75 Gy TBI have primary gonadal failure and require testosterone therapy to promote the development of secondary sexual characteristics. However, about half of the boys who receive 400 cGy prophylactic testicular radiation in addition to fractionated TBI develop normally through

Table 102.1 Pubertal development in boys given testicular irradiation

		Testicular irradiation	
		4.0 Gy	**≥10 Gy**
Current age	≥12 years	51	36
Development	Normal	28 (55%)	6 (16%)
	Delayed	23 (45%)	30 (84%)

Data source: J.E. Sanders, unpublished data, 2007.

puberty (J.E. Sanders, unpublished data). Spermatogenesis can be impaired after 8 Gy, whereas Leydig cells are resistant to radiation doses up to 24 Gy [131].

Among children receiving 14.40 Gy fractionated TBI, one study reported that, among 17 boys evaluated, those with increased levels of LH were significantly younger at HCT (5.4 ± 0.8 versus 7.8 ± 0.8 years; $p = 0.024$), but among the 16 girls evaluated, those with ovarian failure were significantly older at the time of TBI than those with spontaneous puberty (8.6 ± 2.3 versus 6.1 ± 1.8 years; $p = 0.03$) [29]. Another study did not observe an effect of age on subsequent gonadal function [55]. A third analysis suggested that an effect of age may be linked to the total dose of TBI [125]. Among 30 evaluable children given 12.0 Gy TBI, 15 of 16 (94%) less than 10 years of age and 11 of 14 (78%) more than 10 years of age developed normally. However, among the 46 evaluable children given 14.40–15.75 Gy TBI, 23 of 30 (77%) less than 10 years of age and three of 16 (19%) more than 10 years of age developed normally. Larger numbers of patients are needed in order to be able to draw firm conclusions regarding the impact of patient age and total dose of TBI administered on the development of puberty.

It is recommended that the development of secondary sexual characteristics be monitored carefully after patients reach 10–11 years of age and that the Tanner stage of pubertal development be determined annually. Children with evidence of gonadal failure should be referred to a pediatric endocrinologist for consideration of sex hormone treatment. Patients with normal pubertal development, gonadotropins, and sex hormone production should receive appropriate sexual behavior counseling as pregnancy may occur.

Conclusion

Evaluations of endocrine function following HCT demonstrate that the occurrence of abnormalities that may influence subsequent growth and development are related to the type of HCT preparative regimen received. Children who receive CY only in their preparative regimen are at low risk for endocrine function abnormalities. These children typically have normal thyroid function, normal growth rates, and normal development through puberty. Individuals who develop normally through puberty or in whom gonadal function returns to normal may be fertile. Offspring of these patients, in general, do not differ from the general population.

Children who receive BU plus CY preparative regimens have normal thyroid function, and the majority have normal prepubertal growth rates although pubertal growth may be affected. Data from the patients evaluable for pubertal development suggest that a substantial proportion of these children may not develop secondary sexual characteristics at a normal age, which is particularly true for girls but not boys. Gonadal function, however, is likely to be abnormal in both boys and girls.

In contrast, HCT preparative regimens that contain TBI frequently result in multiple endocrine function abnormalities that affect normal growth and development. Patients are at risk for development of thyroid function abnormalities for many years after TBI, and also the risk for development of thyroid malignancy. Growth rates are usually blunted, especially if the child has received prior CNS irradiation. GH deficiency, which frequently occurs, and growth failure should be diagnosed and treated early with GH for the best response. Without GH treatment, young children have significantly compromised final adult heights. Gonadal damage results in a significant fraction of prepubertal patients having delayed pubertal development. Children with delayed development of secondary sexual characteristics may benefit from careful administration of appropriate sex hormone therapy. Children who do develop normally through puberty are potentially fertile.

All patients who receive HCT should continue to undergo long-term follow-up evaluations. Table 102.2 contains recommendations

Table 102.2 Selected screening recommendations for selected endocrine late effects after HCT in pediatric patients

Effect	Children's Oncology Group (COG) recommendations [133]	Joint Transplant Society recommendations [134]	Expert Panel recommendations [132]	
			Screening	Management
Thyroid dysfunction	Risks: XRT ≥10 Gy, thyroid in field. Yearly screening, more frequently during rapid growth	Thyroid function testing yearly post-HCT, or if relevant symptoms develop	TSH and FT4 annually: 1. for 10 years after busulfan; 2. for at least 30 years after TBI PE of thyroid yearly to screen for tumors after TBI	If TSH is high and FT4 normal, either treat or repeat in 2 months. Replace thyroid as indicated for low FT4 levels Rare secondary thyroid tumors post-TBI can be cured with surgery
Growth impairment	Risks – young age, TBI ≥10 Gy single fraction, ≥12 Gy fractionated. Screen with nutritional assessment, height/weight/BMI every 6 months until growth completed. Refer to endocrine for height <3rd percentile, drop ≥2 percentile rankings on growth chart, growth velocity <4–5 cm/year, lack of pubertal growth spurt	Pediatric recipients: monitor growth velocity in children annually; assessment of thyroid, and growth hormone function if clinically indicated	Accurate measure of growth yearly through full growth (age 17 years girls, 19 years boys). Bone age as needed	Bone age and referral to endocrine for patients not growing appropriately. GH therapy may unmask hypothyroidism
Reproductive risks	Risks: combined doses of alkylators, heavy metals, DTIC, temazolamide with XRT to cranium or gonads. Screen FSH/LH/testosterone, Tanner staging ages 13–14 and as clinically indicated for delayed puberty, irregular menses. Semen analysis, repeat as indicated as resumption can occur 10 years after therapy	Consider referral to appropriate specialists for patients who are contemplating a pregnancy or are having difficulty conceiving. Counsel sexually active patients in the reproductive age group about birth control post-HCT	Women: monitor ovarian function (FSH, estradiol, assess cycling). Men: testosterone, FSH, LH, semen analysis	Women: AMH may assess ovarian reserve. Treat ovarian failure with hormone replacement therapy. Men: if oligospermia noted, consider intracytoplasmic sperm injection

AMH, anti-Mullerian hormone; DTIC, dacarbazine; FT4, free thyroxine; PE, physical examination; TSH, thyroid-stimulating hormone; XRT, radiation therapy.
Recommendations from the Center for International Blood and Marrow Transplant Research (CIBMTR), the American Society of Blood and Marrow Transplantation (ASBMT), European Group for Blood and Marrow Transplantation (EBMT), Asia-Pacific Blood and Marrow Transplantation Group (APBMT), Bone Marrow Transplant Society of Australia and New Zealand (BMTSANZ), East Mediterranean Blood and Marrow Transplantation Group (EMBMT) and Sociedade Brasileira de Transplante de Medula Ossea (SBTMO) [132].

adapted from the Children's Oncology Group Long-Term Follow-up Guidelines, the Joint Transplant Society Recommendations published in 2012, and the consensus recommendations from an expert panel convened in 2011 from a National Cancer Institute (NCI), National Heart, Lung, and Blood Institute (NHLBI), and Pediatric Blood and Marrow Transplant Consortium (PBMTC)-sponsored consensus conference of international experts in clinical and biological research into late effects after HCT in children [132]. These recommendations form the basis for the comprehensive follow-up of thyroid function, growth, and development after HCT in children.

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CHAPTER 103

Delayed Non-malignant Complications After Hematopoietic Cell Transplantation

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Introduction

Over 70% of those who survive the first 2 years after hematopoietic cell transplantation (HCT) are now expected to become long-term survivors [1–3]. However, HCT survivors are at risk of developing a variety of long-term complications, such as endocrinopathies, musculoskeletal disorders, cardiopulmonary compromise, and subsequent malignant neoplasms (SMNs) (summarized in Table 103.1) [4–14]. These complications directly impact the burden of morbidity borne by HCT survivors, and have provided the rationale for standardized follow-up of HCT survivors at high risk for these complications. This chapter summarizes the magnitude of risk of key delayed non-malignant complications experienced by HCT survivors, identifying patients at increased risk because of host characteristics and therapeutic exposures. Some of these complications are described in greater detail elsewhere [gastrointestinal and hepatic complications (Chapter 94), lung injury (Chapter 95), kidney and bladder complications (Chapter 96), endocrine complications (Chapter 97), growth, development, and fertility issues (Chapter 102), and neurologic complications (Chapter 105)] and will therefore be summarized only briefly here, with reference to the relevant chapters where a detailed account on the specific issues can be found. Additionally, this chapter describes the overall burden of morbidity (physical and psychosocial) and the late mortality experienced by HCT survivors. Finally, it describes the current recommendations for follow-up, patterns of healthcare utilization by the HCT survivors, and adherence to these recommendations.

Cardiovascular risk factors and cardiac disease

HCT survivors are at risk for cardiovascular risk factors and also cardiovascular complications because of the combined effect of pre-HCT therapeutic exposures, transplant-related conditioning, and post-HCT graft versus host disease (GVHD). Furthermore, HCT recipients are at a 2.3–4.0-fold increased risk of death due to cardiac causes compared with the general population [1,10]. Late cardiotoxicity includes cardiomyopathy, congestive heart failure (CHF), valvular dysfunction, arrhythmia, and pericarditis. The association between anthracyclines and the subsequent development of cardiomyopathy is well established, and is primarily due to anthracycline-induced myocyte damage. Mediastinal radiation produces inflammation and fibrosis resulting in restrictive cardiomyopathy and valvular defects. Fibrosis also affects the electrical conduction pathways, causing arrhythmias. Late cardiovascular complications include the development of cerebrovascular disease and coronary artery disease after HCT [5,15–18], commonly reported together as cardiovascular disease (CVD), and often associated with pre-HCT cranial, neck, and mediastinal radiation.

Anthracycline-related cardiotoxicity is dose dependent and, in non-transplant patients, the incidence exceeds 25% in patients treated in the conventional setting with doses exceeding 550 mg/m^2 [19]. Among anthracycline-exposed childhood cancer survivors, female gender and younger age at exposure to anthracyclines and mediastinal radiation are known to increase the risk [20,21]. Evidence regarding the magnitude of risk of cardiovascular disease in HCT recipients is emerging. Risk factors unique to the HCT procedure that could potentially increase the risk of cardiomyopathy include conditioning with high-dose chemotherapy, total body irradiation (TBI), and GVHD. Furthermore, HCT survivors are at increased risk of de novo cardiovascular risk factors (CVRFs) such as diabetes, hypertension, and dyslipidemia due, in part, to exposure to TBI, immunosuppressive therapy, or other conditions such as growth hormone deficiency or hypothyroidism [5,22], and these could potentiate anthracycline-related cardiomyopathy

In a large cohort of autologous HCT recipients, the cumulative incidence of CHF was 9.1% at 15 years after HCT, and increased to 14.5% at 15 years [23]. The cohort was at a 4.5-fold increased risk compared with the general population. The risk increased substantially for patients receiving ≥250 mg/m^2 of cumulative anthracycline therapy [odds ratio (OR) 9.9, $p < 0.01$]. Presence of hypertension among recipients of high-dose anthracyclines (≥250 mg/m^2) resulted in a 35-fold increase in risk of CHF, and the risk was nearly 27-fold for high-dose anthracycline-exposed individuals with diabetes, providing evidence that hypertension and diabetes were critical modifiers of anthracycline-related myocardial injury after HCT. Pre-HCT exposure to anthracyclines in doses exceeding 250 mg/m^2 was also demonstrated to increase the risk of CHF in autologous and allogeneic HCT recipients [4]. In this study, anthracycline dose ≥250 mg/m^2 (OR 3.2, $p = 0.05$), number of pre-HCT chemotherapy cycles (OR 1.2, $p < 0.01$), and two or more comorbidities (OR 4.3, $p = 0.01$) were independently associated with late CHF.

CVD has emerged as a late complication after HCT, due in part to the more mature follow-up of the HCT survivors, allowing observation and reporting of complications with long latency.

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Table 103.1 Commonly occurring long-term complications in survivors of hematopoietic cell transplantation

Adverse outcome	Therapeutic exposures associated with increased risk	Factors associated with highest risk
Cataracts	Cranial irradiation, TBI, steroids	Higher radiation dose, combination of steroids and radiation, single daily fraction
Cardiomyopathy/congestive heart failure	Pre-HCT anthracyclines	High cumulative doses, females, chest irradiation, post-HCT comorbidities (diabetes and hypertension)
Myocardial infarction	Pre-HCT radiation to chest	Underlying risk factors for coronary artery disease (smoking, hypertension, hyperlipidemia, obesity, etc.)
Pulmonary fibrosis/interstitial pneumonitis	Pre-HCT bleomycin, radiation to chest, carmustine, TBI, chronic GVHD	Younger age at treatment, bleomycin dose >400 U/m^2
Hypothyroidism	Pre-HCT radiation to the thyroid gland (neck, mantle, etc.), TBI	Increasing dose, female gender, older age at treatment
Hypogonadism	Pre-HCT alkylating agents, craniospinal irradiation, abdomino-pelvic irradiation, gonadal irradiation, TBI	Treatment during peripubertal or postpubertal period in girls, higher cumulative doses of alkylators
Renal insufficiency	Pre-HCT platinum-based therapy, ifosfamide, high-dose methotrexate, abdominal radiation, surgery, TBI, calcineurin inhibitors	High-dose chemotherapy, younger age, abdominal radiation and chemotherapy
Short stature	Pre-HCT cranial irradiation, corticosteroids, TBI	Younger age at treatment, cranial radiation dose >18 Gy, unfractionated (10 Gy) TBI
Osteopenia/osteoporosis	Pre-HCT craniospinal irradiation, gonadal irradiation, TBI, corticosteroids	Associated hypothyroidism, hypogonadism, growth hormone deficiency
Avascular necrosis	Corticosteroids, high-dose radiation to any bone, calcineurin inhibitors	Dexamethasone, adolescence, female gender

Late CVD following HCT has been classified as coronary artery disease [i.e. myocardial infarction, angina pectoris, or symptomatic atherosclerotic heart disease (coronary artery narrowing >50%)] or cerebrovascular disease [i.e. stroke, transient ischemic attacks, symptomatic lacunar infarct, or symptomatic carotid artery occlusion (>50% narrowing) necessitating surgical intervention], in accordance with the American College of Cardiology's established case definitions and clinical data standards for coronary artery and cerebrovascular disease. In the general population, increasing age, hypertension, diabetes, dyslipidemia, high body mass index (BMI), and male gender are known to modify the risk of CVD [24,25]. Among HCT survivors, the presence of multiple CVRFs (two or more of the following: obesity, dyslipidemia, hypertension, and diabetes) were associated with a 5.2-fold increased risk of late CVD ($p < 0.01$) [26]. Pre-HCT chest radiation was associated with a 9.5-fold greater risk of coronary artery disease ($p = 0.03$). Thus, pre-HCT exposure to chest radiation and the presence of comorbidities were primarily responsible for the risk associated with late CVD after HCT.

Several studies have demonstrated that HCT recipients are at an increased risk of CVRFs. A higher prevalence of self-reported diabetes and hypertension among HCT survivors compared with a sibling comparison group has been documented [5]. In a large cohort study, the 10-year cumulative incidence of hypertension, diabetes, and dyslipidemia was 37.7, 18.1, and 46.7%, respectively [27]. The prevalence of CVRFs was significantly higher among HCT recipients than the general population, contributed to largely by allogeneic HCT recipients. Older age and obesity at HCT were associated with an increased risk of CVRFs. History of grade 2–4 acute GVHD was associated with an increased risk of hypertension [relative risk (RR) 9.1, $p < 0.01$], diabetes (RR 5.8, $p < 0.01$), and dyslipidemia (RR 3.2, $p < 0.01$); conditioning with TBI was associated with an increased risk of diabetes (RR 1.5, $p = 0.01$) and dyslipidemia (RR 1.4, $p < 0.01$). Most importantly, this study demonstrated that the high incidence of CVRFs in HCT survivors was directly responsible for the subsequent development of CHF and CVD. Thus, the 10-year cumulative incidence of CHF and CVD increased with the number of CVRFs present in the patients prior to the diagnosis of CHF or CVD (no CVRFs, 4.7%; 1 CVRF, 7.0%; ≥2 CVRFs, 11.2%, $p < 0.01$). The risk was especially high (15%) for patients with multiple CVRFs and pre-HCT exposure to anthracyclines and chest irradiation.

These studies demonstrate that the risk of CHF and CVD is increased after HCT, and is related primarily to pre-HCT exposure to anthracyclines (CHF) or chest irradiation (coronary artery disease). Furthermore, the risk of CVRFs is increased after HCT, especially among older individuals exposed to TBI and among those who develop acute GVHD. Finally, the risk of anthracycline-related CHF or chest irradiation-related coronary artery disease is increased substantially among patients with one or more CVRFs. These data form the foundation for identifying high-risk individuals who should undergo targeted surveillance, in addition to providing a rationale for interventional strategies in the form of aggressive management of CVRFs.

Recommendations for screening

Patients exposed to anthracyclines should undergo monitoring for late-onset cardiomyopathy using serial non-invasive testing (echocardiogram) and physical examination. The intensity of echocardiographic screening can range from annual to every 5 years, depending on cumulative anthracycline dose, age at exposure, treatment with mediastinal radiation, and presence of CVRFs. Survivors who received radiation involving the heart field should also be monitored for early-onset atherosclerosis. Lifestyles that promote good heart health should be recommended to all survivors, including smoking cessation, a regular exercise program, dietary recommendations, and screening for (and aggressive management of) dyslipidemia, hypertension, and diabetes.

Delayed pulmonary complications

Delayed pulmonary complications are frequent after HCT, and typically include infectious complications related to the compromised immune system and non-infectious complications. Non-infectious pulmonary complications include the following: bronchiolitis obliterans (BO), bronchiolitis obliterans organizing pneumonia (BOOP), and idiopathic pneumonia syndrome (IPS) [28]. A detailed account of lung injury after HCT can be found in Chapter 95.

Non-infectious complications usually appear 3 months to 2 years after HCT, and are closely related to chronic GVHD. However, the functional consequences persist for years. The cumulative incidence of non-infectious pulmonary complications approaches 10% at 2 years after HCT [29]. Both the magnitude and severity of non-infectious pulmonary complications are worse after unrelated donor HCT. The cumulative incidence is 15.6% among those diagnosed with chronic GVHD. The overall survival at 5 years is significantly inferior among those with pulmonary complications compared with those without.

BO is characterized by a non-specific inflammatory injury affecting the small airways, with an incidence approaching 8% [30]. BO is strongly associated with chronic GVHD; additional factors associated with increased risk include use of peripheral blood stem cells, exposure to methotrexate for GVHD prophylaxis, older age of the recipient and/or donor at HCT, exposure to busulfan for conditioning, prior history of respiratory infection, and low serum immunoglobulin levels. The onset of BO is protracted, and typically occurs approximately 1 year after HCT. Manifesting as an obstructive defect in the initial stages, it progresses to peribronchiolar fibrosis, with emergence of restrictive changes [31]. Patients present with a dry cough, progressive dyspnea, and wheezing. High-resolution computed tomography (CT) images of the chest in inspiration and expiration are considered to be the radiologic procedure of choice to measure the structural alterations in the lung. A characteristic finding in patients with BO is a mosaic image, and carries a sensitivity of 74–91% and a specificity of 67–94% [32]. Criteria used to make a clinical diagnosis of BO include (i) $FEV_1/FVC < 0.7$ and FEV_1 <75% of predicted value, (ii) evidence of air trapping or small airway thickening or bronchiectasis on high-resolution CT, and (iii) absence of respiratory infection [33].

BOOP is a clinicopathologic syndrome involving bronchioles, alveolar ducts, and alveoli. The syndrome has a cumulative incidence of less than 2%. It typically presents as an interstitial pneumonia and usually occurs within the first 12 months following HCT. The clinical presentation in patients is acute, with the sudden onset of a dry cough, and also dyspnea and fever. Pulmonary function tests suggest a restrictive pattern and observations from chest X-ray show peripheral patchy consolidation, ground-glass attenuation and nodular opacities [34]. Definitive diagnosis requires histologic confirmation. Patients with BOOP are more likely to have acute or chronic GVHD.

IPS usually occurs within the first 4 months after HCT; risk factors include exposure to TBI and pre-HCT pulmonary-toxic chemotherapy and the presence of GVHD, with the risk increasing with age at HCT [35]. Late-onset interstitial pneumonitis occurs years after HCT, typically in patients with severe chronic GVHD of the sclerodermatous cutaneous variety. A restrictive defect is found on pulmonary function tests [36].

Recommendations for screening

Monitoring for pulmonary dysfunction in HCT survivors should include the assessment of symptoms such as chronic cough or dyspnea. Patients should be counseled about the risks of smoking and exposure to second-hand smoke. It is recommended that pulmonary function tests and chest X-rays are carried out upon entry into long-term follow-up for at-risk patients, and should be repeated as clinically indicated in symptomatic patients and in those with subclinical abnormalities identified at screening. Influenza and pneumococcal vaccines are recommended in survivors at risk for pulmonary compromise.

Endocrine complications

Endocrine complications are among the most common chronic health conditions encountered after HCT, and include thyroid dysfunction, osteoporosis, growth impairment, and gonadal dysfunction. The risk of these complications is influenced by pre-HCT therapeutic exposures, transplantation-related conditioning, and post-transplantation management of GVHD. Endocrinopathies and their consequences are described in greater detail in Chapters 97 and 102.

Thyroid abnormalities primarily include subclinical and overt hypothyroidism. Subclinical or compensated hypothyroidism is defined as elevated thyroid-stimulating hormone (TSH) accompanied with normal T4 levels. The incidence of compensated hypothyroidism ranges from 25 to 30%, with a median latency of 2 years. Overt hypothyroidism is characterized by low T4 levels accompanied by elevated TSH; the cumulative incidence ranges from 3.4 to 9% with a latency of 2.7 years. Hypothyroidism is directly related to radiation to the thyroid gland (either as part of neck/mediastinal radiation or TBI). Younger age at exposure to radiation increases the risk [37,38].

HCT recipients are at risk for osteopenia and osteoporosis. The decreased bone mineral density is due to the use of steroids in the treatment of GVHD [39], growth hormone deficiency [40], hypogonadism [41,42], physical inactivity, and a diet low in calcium [43]. The incidence of osteopenia in adults is reported to approach 50% at 4–6 years after HCT, while the incidence of osteoporosis approaches 20% at 2 years [44–49]. The average decrease in bone mineral density (BMD) has been reported to be 6–10% in the femoral neck region and 2–6% in the lumbar region, and the most significant loss in BMD occurs during the first 6 months after HCT [48–50]. Spine and total body BMD have been shown to return to baseline at month 48 [42]. Bone mineral loss increases the risk of fractures in the HCT population, as it does in the general population. Non-traumatic fractures have been observed in 10.6% of the population within 3 years after HCT [44].

Growth impairment is a frequent complication in children after HCT [49]. While central damage to the hypothalamic–pituitary axis due to cranial radiation or TBI is the primary mediator, several factors modify the risk, including nutritional and hormonal (gonadal failure, hypothyroidism) factors, exposure to corticosteroids for the management of GVHD, and peripheral lesions of the bones, cartilage, and epiphyseal growth plate [50]. Short stature after exposure to TBI could be due to the impairment of growth directly, by damaging epiphyseal growth plates, or indirectly, by decreasing growth hormone secretion, or by causing hypogonadism or hypothyroidism. The final height standard deviation (SD) score of the patient conditioned with TBI is significantly lower than that of the patient conditioned without TBI [51].The loss of height is more severe in boys than girls. Patient age at exposure to TBI is a significant factor in predicting adult height. Children younger than 10 years of age at HCT are at greatest risk for short stature [52]. Patients conditioned with chemotherapy alone attain final height values within predicted ranges. The final height in patients treated with hyperfractionated TBI is similar to that in patients receiving standard fractionation; however, the reduction in height in this group is due to a reduction in sitting height, suggesting impairment of spinal column growth [50]. Growth hormone insufficiency is the leading cause of growth impairment – the prevalence ranges from 20 to 85%. Maximum benefit to final height is achieved with growth hormone replacement for patients with documented growth hormone deficiency who were transplanted before 10 years of age [53].

Gonadal failure is a frequent endocrine complication after HCT. Pubertal disturbances after HCT are caused by radiation-related perturbations of the hypothalamic–pituitary axis and/or by chemoradiotherapy-related damage to the gonads. Testicular germinal epithelium (Sertoli cells) is responsible for spermatogenesis and is more sensitive to radiation and chemotherapy than the testicular Leydig cell component, which is involved in testosterone secretion. The probability of developing gonadal failure increases with cumulative doses of gonadotoxic therapies. Recovery of spermatogenesis seems to occur more frequently in patients receiving lower doses of cyclophosphamide (120 mg/kg) than in those treated with higher doses (200 mg/kg). Males who are long-term survivors have a considerable chance of recovering some degree of sperm production even when treated with TBI, provided that they are under 25 years of age at HCT and do not develop chronic GVHD [54,55].

Ovaries are more vulnerable to radiation and chemotherapy. It has been shown that whereas approximately 50% of prepubertal girls who receive fractionated TBI enter puberty spontaneously and achieve menarche at a normal age, almost all female patients who are more than 12 years old when undergoing HCT show ovarian failure, probably as a result of decreased reserve of primordial follicles [56]. High-dose busulfan is a significant cause of ovarian failure even when it is administered in the prepubertal period [57]. Irreversibility of ovarian function after HCT in most patients highlights the necessity for timely hormonal replacement therapy to prevent osteoporosis and other complications resulting from lack of hormones.

Recommendations for screening

Thyroid

Screening for thyroid dysfunction relies on a good history and physical examination, and also annual thyroid function tests (free thyroxine, thyroid-stimulating hormone). Survivors with abnormalities on screening evaluation should be referred to an endocrinologist for hormone replacement therapy.

Growth

Growth and growth velocity should be measured every 6 months during childhood. Any deviation from the SD score from age- and gender-expected means should prompt a consultation with the endocrinologist.

Gonadal function

For males, screening for hypogonadism should include an age-appropriate history and physical examination, with attention to issues related to libido, impotence, or fertility. Measurement of serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone levels has been recommended as a baseline at age 11 years and in boys whose puberty appears to be delayed. Abnormalities in testicular function should prompt a consultation with the endocrinologist. For fmales, screening for ovarian dysfunction involves history (primary or secondary amenorrhea, menstrual irregularity, and pregnancies or difficulties in becoming pregnant), physical examination, and serum gonadotropin and estradiol levels. For patients with absence of clinical evidence of puberty, baseline studies should be obtained at the expected time of onset of puberty to assess the need for hormone therapy to induce puberty.

Fertility

Permanent gonadal damage and infertility are known toxicities of preparative regimens used for patients undergoing HCT [13,58–60]. HCT survivors were found to be 36 times more likely to not report a conception compared with age- and gender-matched siblings [61]. Older age at transplantation (≥30 years: OR 4.8), female gender (OR 3.0), and exposure to TBI (OR 3.3) are associated with increased risk for infertility. Infertility should be regarded as a complication of significant concern as it has the potential to influence various medical and quality of life (QOL) aspects of survivorship. In fact, some survivors have reported that the potential loss of fertility was as painful as confronting cancer [62,63]. Survivors under the age of 40 years and those without children before HCT express significantly elevated infertility concerns [64]. Although HCT survivors are at an extremely high risk for infertility secondary to gonadotoxic myeloablative therapy, survivor fertility status cannot be definitely known without testing.

The high prevalence of infertility, and the high rate of infertility-related concerns among cancer survivors, increase the need for transplanters to discuss fertility-preserving options prior to HCT with prospective HCT recipients, and for healthcare providers taking care of long-term HCT survivors to provide family-building options and psychosocial support to those at risk.

Osteonecrosis

Osteonecrosis is a debilitating and painful condition that develops when the blood supply to the bone is compromised, usually in areas with terminal circulation. Osteonecrosis has been reported as a complication of HCT, and can cause significant morbidity and often requires surgery. The cumulative incidence reported in the literature has ranged from 3 to 10% at 5 years after HCT [65–67]. The risk of osteonecrosis is highest among unrelated donor recipients, approaching 15% at 10 years after HCT (Figure 103.1) [68]. Risk factors for osteonecrosis include GVHD therapy with corticosteroids or calcineurin inhibitors and the use of TBI for conditioning [65,66,69]. Among recipients of allogeneic HCT, males (RR 2.1), those with chronic GVHD (RR 2.2), and those exposed to three of the four drugs cyclosporine, FK506, prednisone, and MMF (RR 9.2) are at increased risk [68]. The association with corticosteroids increases with increasing dose. When compared with no corticosteroid exposure, exposure to 1–3869 mg of prednisone was found to be associated with a 4.0-fold increased risk of osteonecrosis, exposure to 3870–9735 mg with a 5.6-fold increased risk, and exposure of >9735 mg with an 8.6-fold increased risk [70]. Pathogenesis of osteonecrosis includes local vascular damage that leads to

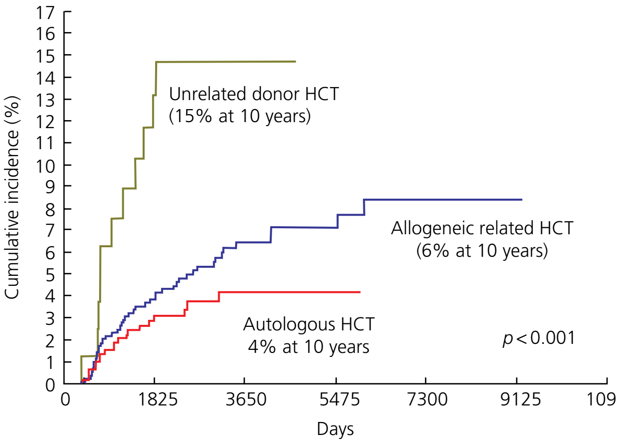

Figure 103.1 Cumulative incidence of avascular necrosis by type of HCT. Source: adapted from Campbell et al. 2009 [68].

increased marrow edema and ischemia, ineffective osteoblastic repair processes due to metabolic factors, and mechanical stresses [71]. The femoral head is the most commonly affected area; others include the knee and shoulder joints.

Recommendations for screening

Clinical anticipation in at-risk survivors guiding a careful history and a thorough physical examination are the cornerstone of screening. Prompt radiologic screening with appropriate interventions can reduce the morbidity related to pain and lack of mobility.

Chronic kidney disease

Renal dysfunction has long been recognized as a complication of HCT [72–79], and is detailed in Chapter 96. Conventionally, chronic kidney disease is defined as a persistently (≥3 consecutive months) low glomerular filtration rate (GFR) of <60 mL/min/1.73 m^2. However, direct measurement of GFR is logistically difficult in routine clinical practice, and has therefore precluded reports on chronic kidney disease with large samples. This limitation was overcome in a study by applying the endogenous filtration marker creatinine to assess GFR, in the Modification of Diet in Renal Disease (MDRD) Study equation endorsed by the National Kidney Foundation [7]. Using this definition, the cumulative incidence of chronic kidney disease was found to approach 4.4% at 5 years for the entire cohort; the cumulative incidence was 3.8% among autologous HCT recipients, 4.5% for matched sibling HCT recipients, and 10% for unrelated donor HCT recipients. For every 5-year increment in age at HCT, the risk of chronic kidney disease increased by 33%. In addition, exposure to cyclosporine without tacrolimus (RR 1.9) or with tacrolimus (RR 4.6) and a primary diagnosis of multiple myeloma (RR 2.5) were associated with an increased risk of delayed chronic kidney disease. Older patients exposed to calcineurin inhibitors were at the highest risk (Figure 103.2).

Recommendations for screening

Patients at risk for chronic kidney disease should be monitored with evaluation of serum creatinine, blood urea nitrogen, and serum chemistries at entry into long-term follow-up; these tests should be repeated as clinically indicated. Urinalysis and measurement of blood pressure should be performed at baseline and annually thereafter. Patients at risk for chronic kidney disease should be cautioned to avoid nephrotoxic agents (e.g. ibuprofen, aminoglycosides) and to seek early intervention for urinary tract infections.

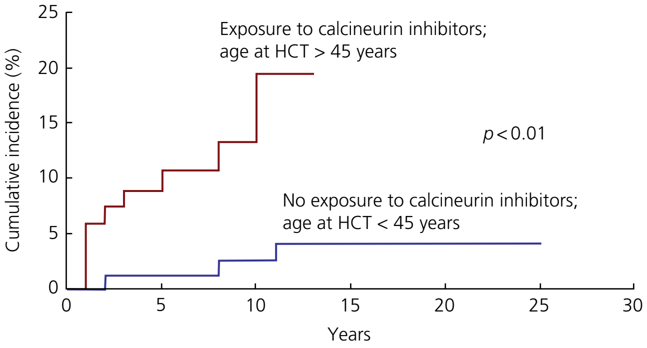

Figure 103.2 Cumulative incidence of chronic kidney disease by age at exposure to calcineurin inhibitors among allogeneic HCT recipients. Source: adapted from Choi et al. 2008 [7].

Visual impairments

The association between cataract formation and exposure to ionizing radiation has been recognized since the late 19th century. Following HCT, it is well known that TBI-based conditioning regimens are more cataractogenic [80–82]. The major risk factors for development of cataracts include TBI (including total dose, fractionation, or dose rate) [80,83–86] and pre-HCT cranial irradiation [81,84].

The cumulative incidence of cataracts approaches 36% at 15 years after HCT performed in childhood [87]. Data from the European Bone Marrow Transplant Registry [88] that included adults demonstrated the overall 10-year estimated cataract incidence to be 50%. It was 60% in the patients who had received single-dose TBI, 43% among those who had received six or fewer fractions, and 7% among those who had received more than six fractions. Furthermore, the cumulative incidence of cataracts was significantly lower (30%) among patients who had received a low dose rate of radiation (≤0.04 Gy/min) compared with those who had received a high dose rate (>0.04 Gy/min) (59%). Patients who had received heparin for prophylaxis against venocclusive disease had fewer cataracts (cumulative incidence 33% versus 53%). Multivariate analysis revealed older age (>23 years), higher dose rate, allogeneic HCT, and steroid administration (>100 days) to be associated with an increased risk of cataract formation.

Recommendations for screening

Patients at risk for cataracts should undergo annual fundoscopic examination and assessment for visual acuity. Surgical interventions (cataract extraction, photocoagulation) may be indicated, with preservation of the sight being the overall goal.

Chronic disease burden after hematopoietic cell transplantation

As described above, HCT survivors are at risk for developing a variety of long-term complications. Most studies conventionally focus on individual complications, providing insight into their etiology and pathogenesis and identifying patients at increased risk, so that targeted surveillance and interventions can be instituted to reduce morbidity and mortality. However, the burden of morbidity resulting from the cumulative impact of these individual complications needs to be examined, so that care can be tailored accordingly. Health problems experienced by 10-year survivors of HCT have been documented by Syrjala et al. [89]. Survivors reported twice as many medical problems when compared with controls ($p < 0.001$). Survivors were more likely to report musculoskeletal symptoms, cataract surgery, liver problems, sexual problems, memory and attention concerns ($p = 0.003$), urinary symptoms, and use of psychotropic medication. In another study, investigators described the risk of chronic health conditions in HCT survivors and siblings [90]. The Common Terminology Criteria for Adverse Events, Version 3.0, was used to assign severity score [grades 1 (mild) through 4 (life-threatening)] to each health condition. Two-thirds of the HCT survivors and 39% of the siblings developed at least one chronic health condition ($p < 0.0010$). Whereas one-fifth of the HCT survivors developed severe/life-threatening conditions, only 8% of the siblings did so ($p < 0.001$). The cumulative incidence of a chronic health condition was 59% at 10 years after HCT; for severe/life-threatening conditions, the 10-year cumulative incidence approached 35% (Figure 103.3). HCT survivors were 3.5 times more likely to develop severe/life-threatening conditions than the siblings, and HCT survivors with chronic GVHD were

4.7 times more likely. HCT survivors were more likely to report gastrointestinal, musculoskeletal, and cardiovascular problems compared with an age-, gender- and race-adjusted sibling group.

The burden of morbidity reported after HCT represents the cumulative toxicities resulting from exposure to pre-HCT chemotherapy and radiation, transplantation-related conditioning regimens, post-HCT GVHD, and medications used to manage acute and chronic GVHD, and the development of comorbidities after HCT. The long-term morbidity ascribed to the HCT procedure was examined recently [91]. In this study, the investigators compared the risk of chronic health conditions and adverse health in survivors of HCT performed in childhood with survivors of childhood cancer treated conventionally. About 25% of the HCT survivors reported severe/life threatening conditions. HCT survivors were 3.9 times more likely to report severe/life-threatening conditions ($p < 0.01$) than conventionally treated patients. Furthermore, they were 3.5 times more likely ($p <0.01$) to report functional impairment and 5.8 times more likely ($p < 0.01$) to report activity limitation than conventionally treated patients. Unrelated donor HCT recipients were at the highest risk. Understanding the burden of morbidity after HCT is important for a variety of reasons. It is important to the healthcare providers and policy makers in identifying and procuring resources for the long-term care of persons with a high burden of morbidity, to the researchers in identifying common etiologic pathways that increase the risk of overall morbidity, and to the HCT survivors in making informed decisions regarding the QOL concerns long-term after HCT.

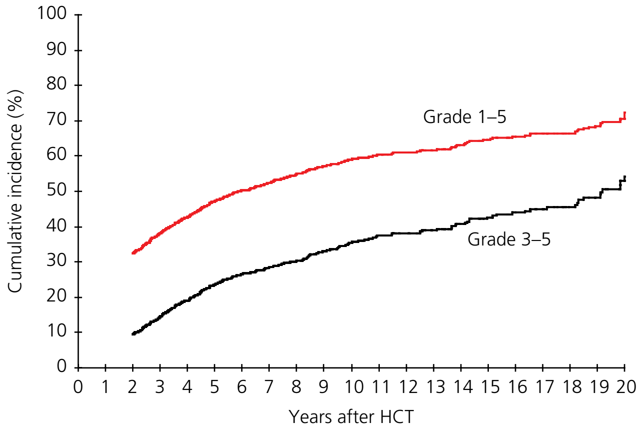

Figure 103.3 Cumulative incidence of any chronic health conditions (grades 1–5) and severe or life-threatening chronic health conditions (grade 3 or 4) or death due to a chronic health condition (grade 5) after HCT. Source: adapted from Sun et al. 2010 [90].

Late mortality

The high burden of morbidity carried by HCT recipients can result in premature death. Several studies have examined late mortality in patients undergoing HCT, and these are summarized here.

Late mortality experience was evaluated in a cohort of autologous HCT recipients who had survived 2 years or longer after HCT for hematologic malignancies [2]. Overall survival was 68.8% at 10 years (Figure 103.4a), and the cohort was at a 13-fold increased risk for premature death compared with the general population. Relapse

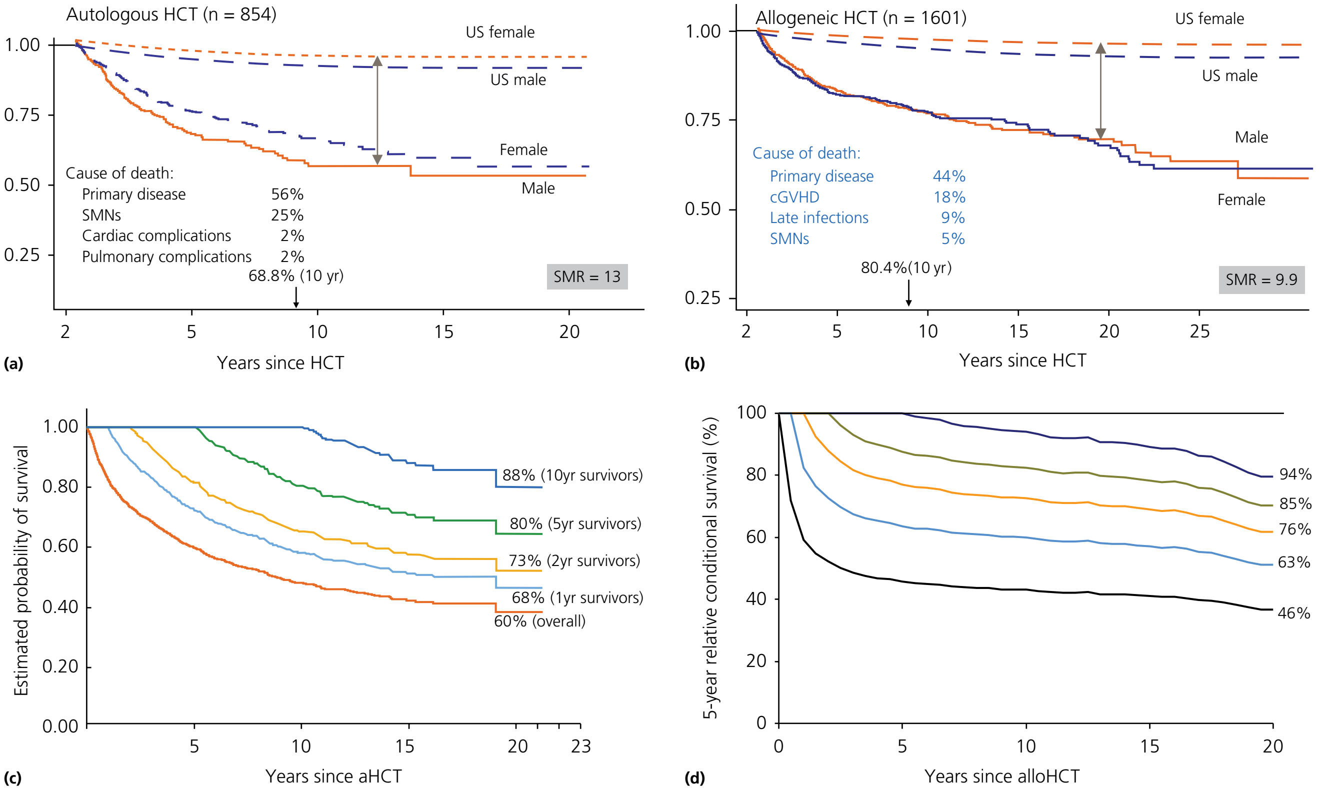

Figure 103.4 (a) Late mortality after autologous HCT. Source: adapted from Bhatia et al. 2005 [2]. (b) Late mortality after allogeneic HCT. Source: adapted from Bhatia et al. 2007 [1]. (c) Conditional cause-specific survival after autologous HCT. Source: adapted from VanderWalde et al. 2013 [94]. (d) Conditional cause-specific survival after allogeneic HCT. Source: adapted from Armenian et al. 2012 [95].

of primary disease (56%) and SMNs (25%) were leading causes of premature death. Compared with patients with AML, relapse-related mortality was increased among patients with Hodgkin lymphoma (RR 3.6), non-Hodgkin lymphoma (RR 2.1), and acute lymphoblastic leukemia (RR 6.5). TBI provided a protective effect (RR 0.6). Non-relapse-related mortality was increased after carmustine (RR 2.3) and with use of peripheral blood stem cells (RR 2.4). Subsequent malignancies accounted for a 12-fold higher mortality rate than that expected in the general population, pulmonary dysfunction 5.6-fold, and cardiac compromise 4.4-fold.

Late mortality experience was also evaluated in individuals who had survived 2 years or longer after allogeneic HCT [1]. It was demonstrated that the survival probability at 15 years after HCT was 80.2% for those individuals who had survived 2 years after HCT (Figure 103.4b), with a relative mortality of 9.9. Relapse of primary disease (29%) and chronic GVHD (22%) were the main causes of premature death. Non-relapse-related mortality was greater among patients who were over 18 years of age when undergoing HCT, and also among those with chronic GVHD (RR 2.7), and was reduced among individuals who received methotrexate for GVHD prophylaxis (RR 0.5). Compared with the general population, allogeneic HCT recipients were 15.1 times more likely to have died of a pulmonary dysfunction, 3.6 times more likely to have died of a subsequent new malignancy, and 3.6 times more likely to have died of cardiac compromise. In another cohort of allogeneic HCT recipients who had survived 2 years or longer after HCT, the probability of being alive 10 years after HCT approached 85% [92]. Relapse was the major cause of death; the major cause for non-relapse-related mortality was GVHD.

In a recent cohort study of patients who had survived for at least 5 years after allogeneic or autologous HCT without recurrence of the original disease, the estimated survival of the cohort at 20 years of follow-up after HCT was 80.4% [93]. Mortality rates were 4–9-fold higher than in the general population for at least 30 years following HCT, producing an estimated life expectancy that was 30% lower compared with normal subjects, regardless of current age. Leading causes of excess deaths were SMNs and recurrent diseases, followed by infections, chronic GVHD, and respiratory and cardiovascular disease.

The probability of survival is conventionally calculated from the date of HCT, despite the fact that the probability of survival changes for patients who have survived for a specified length of time after transplantation. Conditional survival is the survival probability of a cohort that takes into consideration the fact that the cohort has already survived a given length of time. The hazard of mortality changes with time from HCT, since the risk of relapse decreases, while the risk of chronic health conditions increases – but these occur at different rates. The concept of "conditional probability" takes into account that hazard rates change in a measurable way over time. Conditional survival applies this concept to determine the probability that a patient who has survived for a designated period will be alive at another fixed interval. A recent study determined disease- and cause-specific conditional survival for autologous HCT recipients [94] (Figure 103.4c). Estimated probability of relative survival was 62% at 5 years and 50% at 10 years from autologous HCT. On the other hand, 5-year relative survival was 70, 75, 81, and 88% after having survived 1, 2, 5, and 10 years after HCT, respectively. The risk of death approached that of the general population for 10-year survivors [standardized mortality ratio (SMR) 1.4, 95% confidence interval (CI) 0.9–1.9], with the exception of female Hodgkin lymphoma patients transplanted before 1995 at age 40 years or younger; these individuals were at a sixfold increased risk of premature death compared with the general population. Among those who had survived 10 years, non-relapse-related mortality rates exceeded relapse-related mortality rates. Cause-specific conditional survival in allogeneic HCT recipients was also examined [95] (Figure 103.4d). Conventionally computed probability of survival was 44.6% at 5 years and 41.2% at 10 years from allogeneic HCT. On the other hand, conditional 5-year survival rates at 6 months and 1, 2, and 5 years after allogeneic HCT were 62, 75, 83, and 93%, respectively. Among patients followed for 15 years or longer after allogeneic HCT, the risk of all-cause mortality was 2.6-fold that of the general population. Individuals who survived the first 5 years had negligible (≤5%) risk of relapse- and GVHD-related mortality over the subsequent 5 years. On the other hand treatment-related mortality increased over time; among those who survived the first 5 years, treatment-related mortality rates exceeded relapse-related mortality.

In summary, patients who have survived for 2–5 years after HCT without recurrence of the original disease have a high probability of surviving an additional 15 years. However, life expectancy is not fully restored to the level seen in the normal population in most patients, although the cause of death shifts from disease-related to treatment-related with increasing time after HCT.

Health behaviors of long-term survivors of hematopoietic cell transplantation

The high burden of morbidity borne by HCT survivors necessitates that they engage in health promotion, prevention, and screening behaviors after HCT. A recent study examining health behaviors and cancer screening practices [96] found that HCT survivors were more likely to have received a mammogram (OR 2.8, $p = 0.05$) than their siblings. Furthermore, HCT survivors were less likely to indulge in high-risk behavior (OR 0.5, $p < 0.01$) than their siblings. In the analysis restricted to HCT survivors, patients younger than 35 years at study participation (OR 4.7, $p < 0.01$) and those with lower education (below college level: OR 2.1, $p < 0.01$) were more likely to demonstrate high-risk behavior. Another study confirmed these findings, demonstrating that HCT survivors had similar health and screening behaviors to matched controls [97].

In summary, HCT survivors had comparable cervical and testicular cancer screening practices, were more likely to have had breast cancer screening by mammography, and were less likely to be engaged in high-risk behaviors compared with healthy controls. However, despite potential long-term risks, certain subsets of survivors continue to engage in high-risk behaviors such as smoking and excessive alcohol intake, indicating the need for targeted interventions for these high-risk populations. Continued vigilance in encouraging appropriate cancer screening and healthy behaviors for HCT survivors is critical.

Quality of life after hematopoietic cell transplantation

The psychologic health of HCT survivors has been evaluated by several investigators, using both cross-sectional and longitudinal study designs. Adverse psychologic outcomes in long-term HCT survivors were evaluated using a healthy comparison group composed of siblings [98]. Psychologic health status was evaluated using the Brief Symptom Inventory-18. About 22% of the HCT survivors reported adverse psychologic outcomes, compared with 8% of siblings ($p < 0.01$); 15% of HCT survivors reported somatic distress, representing an almost threefold higher risk compared with siblings. Among survivors, exposure to prednisone was associated with psychologic distress across all domains (anxiety, depression, and somatic distress). In addition, low annual household income and self-reported poor health, having severe/life-threatening chronic health conditions, and the presence of active chronic GVHD were associated with a twofold increased risk for somatic distress. Of HCT survivors, 7% expressed suicidal ideation; patients with higher scores on depression subscale were most vulnerable.

In addition to cross-sectional studies describing long-term psychologic wellbeing, investigators have utilized a prospective longitudinal study design to determine the trajectory of QOL from before HCT to several years after. Physical, psychologic, social, and spiritual wellbeing were assessed before HCT and at 6 months and 1, 2, and 3 years after HCT [99]. For all HCT patients, physical QOL was stable from before to after HCT, and psychologic, social, and spiritual wellbeing improved at 6 months. Allogeneic HCT recipients reported worse physical and psychologic wellbeing. Older patients reported worse physical but better social wellbeing regardless of HCT type. Two or more domains were affected by race/ethnicity, household income, and education in autologous HCT recipients; and by BMI, decline in BMI, primary diagnosis, and chronic GVHD in allogeneic HCT recipients. At 3 years, 74% of HCT recipients were employed full or part time. Older autologous HCT recipients with lower pre-HCT income were less likely to return to work; among allogeneic HCT recipients, patients with chronic GVHD were less likely to return work.

In another prospective longitudinal study, function was assessed from pre-HCT to 5 years post-HCT for adults who had received myeloablative HCT for leukemia or lymphoma [100]. The investigators studied physical limitations, return to work, depression, and distress before transplantation, at 90 days and 1, 3, and 5 years after HCT. It was found that physical recovery occurred earlier than psychologic recovery or return to work. Only 19% recovered with respect to all outcomes at 1 year. The proportion without major limitations increased to 63% at 5 years. Among survivors in complete remission, 84% returned to full-time work by 5 years. Patients with chronic GVHD ($p = 0.01$), those with less social support before HCT ($p = 0.001$), and women ($p < 0.001$) were more depressed after transplantation. Transplant-related distress was slower to recover for allogeneic HCT recipients and those with less social support before HCT ($p < 0.01$).

Sexual wellbeing is an important component of QOL, and information regarding the longitudinal trajectory of sexual wellbeing in HCT cohorts is emerging. The prevalence and predictors of sexual dysfunction in adult HCT recipients were assessed pre-HCT and at 1 and 3 years after [101]. Men and women did not differ in sexual satisfaction prior to transplantation. At 1 and 3 years post-HCT, women reported significantly more sexual dysfunction than men; 80% of the female participants and 29% of the male participants reported at least one sexual problem by 3 years after HCT. Among men, older age at HCT, poorer psychologic functioning prior to HCT, not being married, and lower sexual satisfaction prior to HCT predicted sexual dissatisfaction at 3 years after HCT. On the other hand, among women, lack of receipt of hormone replacement therapy and less sexual satisfaction at 1 year after HCT were associated with sexual dissatisfaction at 3 years post-HCT.

A second study delineated the trajectory of sexual wellbeing from before HCT to 3 years after in allogeneic and autologous HCT recipients and identified predictors of diminished sexual functions [102]. The participants completed the Derogatis Interview for Sexual Function Self Report and the Derogatis Global Sexual Satisfaction Index prior to HCT and at 6 months and 1, 2, and 3 years post-HCT. It was found that two-thirds of men were sexually active at the pre-HCT time point, declining to 51% at 3 years post-HCT ($p = 0.01$). On the other hand, one-third of women were sexually active at the pre-HCT time point, increasing to 48% at 3 years post-HCT ($p = 0.02$). Both men and women experienced a significant and persistent decline in sexual satisfaction after transplantation ($p < 0.001$). Among men, the sexual functioning scores for the Orgasm ($p = 0.002$) and Drive/Relationship ($p < 0.001$) domains declined after HCT, whereas among women, the scores for the Cognition/Fantasy ($p = 0.01$) and Behavior/Experience domains ($p = 0.01$) improved. Despite these gender-specific longitudinal trends, sexual function was worse in women than in men at all times and across all domains ($p \leq 0.001$). Older age negatively impacted sexual function in both men and women after HCT ($p < 0.01$). Chronic GVHD was associated with lower scores for the Cognition/Fantasy ($p = 0.003$) and Orgasm ($p = 0.006$) domains in men and the Arousal domain ($p = 0.05$) and sexual satisfaction ($p = 0.005$) in women. Finally, men exposed to TBI experienced a significant decline in sexual function across all domains ($p < 0.05$).

These studies demonstrate the gender-specific sexual concerns after HCT, and the need for a multidisciplinary approach to intervention in the vulnerable subpopulations.

Neuropsychologic wellbeing

Impaired cognition is an increasingly recognized consequence in HCT survivors, with the potential to impact societal reintegration, such as return to work. Decline in memory, learning, attention, concentration, and psychomotor and executive functioning have been reported after HCT. Neurotoxic agents (cranial and total body irradiation; high-dose chemotherapy, intrathecal methotrexate, and steroids) place HCT survivors at risk for cognitive impairment. Utilizing a prospective longitudinal study design [103], allogeneic HCT recipients completed standardized neuropsychologic tests including information processing speed, verbal memory, executive function, and motor dexterity and speed after 80 days and 1 and 5 years after HCT. Case-matched control subjects received testing at the 5-year time point. It was demonstrated that survivors recovered significant cognitive function from the 80-day time point to 5 years in all tests ($p < 0.001$) except verbal recall. Between 1 and 5 years, improvements were noted in verbal fluency ($p = 0.0002$) and executive function ($p < 0.01$). However, motor dexterity did not improve, remaining more than 0.5 SD below population norms. Using the Global Deficit Score as a summary of overall impairment, 41.5% of the survivors and 19.7% of the controls had mild or greater deficits ($p = 0.007$).

Long-term follow-up

Exposure to high-intensity therapy combined with prolonged immune suppression after HCT increases the risk of adverse sequelae, and hence the healthcare needs, among survivors. Healthcare utilization by long-term HCT survivors [104] revealed that 98% of allogeneic HCT recipients and 94% of autologous HCT recipients reported medical contact a decade after HCT. Whereas cancer-related visits decreased with time from HCT, the prevalence of general physical examinations increased. This study demonstrates that primary physicians assume increasing responsibility for providing healthcare for long-term HCT survivors, thus making it critical that there be standardized guidelines for follow-up of these survivors. The overall goal of standardized guidelines is early identification of treatment-related complications, potentially allowing for early intervention with resultant reductions in morbidity and mortality and attendant reductions in healthcare costs.

To accomplish early detection and prevention of life-threatening complications, HCT survivors should undergo annual comprehensive physical examination that includes screening for functional and psychosocial consequences of treatment and education regarding key aspects of health promotion. The Center for International Blood and Marrow Transplantat Research (CIBMTR), European Group for Blood and Marrow Transplantation (EBMT), and American Society for Bone Marrow Transplantation (ASBMT) have developed recommendations for screening and prevention practices for HCT survivors [105]. These recommendations were recently updated [106], and concentrate on risks faced by patients beyond 6 months following transplantation. Most of these recommendations are derived from studies that identified specific complications in long-term survivors and the risk factors associated with them. The recommendations are organized by tissue/organ system, with details regarding potential late effects, the known risk factors, and recommendations for monitoring tests and preventive measures. The recommendations are also organized by time after HCT, detailing suggested recommendations at 6 months, 1 year, and then annually.

Comprehensive guidelines for follow-up of pediatric cancer survivors (including patients who have been treated with HCT) have been developed by the Children's Oncology Group (COG) (www.survivorshipguidelines.org). The COG guidelines are evidence based (utilizing established associations between therapeutic exposures and late effects to identify high-risk categories) and consensus based (matching the magnitude of risk of complications with intensity of screening). Since treatment exposures vary by primary diagnosis, HCT type, patient age, and treatment era, *a therapy-based approach* was chosen. These guidelines have been developed as a resource for clinicians who provide ongoing healthcare to HCT survivors. The COG Guidelines are intended for use beginning 2 years or longer after completion of therapy (or date of HCT), and provide a framework for monitoring of late effects in survivors. The recommendations for periodic screening evaluations provided in these guidelines are appropriate for asymptomatic survivors who present for routine medical follow-up. Table 103.2 gives an overview of the key content areas in the guidelines. The major difference between the COG guidelines and those developed by EBMT/CIBMTR/ASBMT is the fact that unlike the latter, the former utilize the cumulative therapeutic exposures to generate a tailored list of potential late effects and the recommended screening for early detection of those late effects. The intensity of screening is determined by whether the patient is at standard risk or increased risk due to cumulative therapeutic exposures and/or host/demographic characteristics. This provides a greater level of granularity and specificity to the screening that the patient undergoes, and therefore eliminates the possibility of over- or under-screening.

Table 103.2 Key content areas in guidelines for long-term follow-up of survivors

Therapeutic agent	All major therapeutic agents used in the treatment, including pre-HCT chemotherapy, radiation, surgery, and transfusions, and HCT-related preparative regimens
Potential late effects	Sequelae observed after treatment; potentially resulting from pre-HCT therapy, preparative regimens used for HCT, or as a result of post-HCT events (psychosocial, dermatologic, ocular, audiologic, oral, craniofacial, cardiovascular, pulmonary, hepatic, gastrointestinal, renal, musculoskeletal, neurologic, endocrine, reproductive consequences, immune reconstitution and risk of infection, and second malignant neoplasms)
Potential risk factors	Host factors (gender, age at therapeutic exposure, genetic predisposition, ethnicity); medical conditions (premorbid/comorbid conditions); therapeutic exposures (cumulative dose); health behaviors (diet, smoking, exercise)
Highest risk categories	Host factors; treatment factors; medical conditions; genetic predisposition
Periodic evaluations	Health history; clinical examinations; laboratory analysis; diagnostic imaging; psychosocial assessments
Minimum recommended frequency	Baseline and periodic testing and recommendations based on risk factors and magnitude of risk (supported by literature and clinical experience)
Health protective counseling	To prevent/reduce risk of complications and promote early detection
General healthcare	Linked to recommendations per US Preventive Services Task Force
Cancer screening guidelines	Based on ACS recommendations for standard risk. For increased risk of SMNs (therapeutic exposures, family history, or premorbid/comorbid conditions) modified guidelines tailored to the magnitude of risk will be available

The high burden of morbidity suffered by HCT survivors necessitates that strategies be developed for prevention or early detection of these complications. However, many survivors are no longer under the care of transplant centers, and community healthcare providers may be unfamiliar with the unique needs of the HCT survivors. Despite the differences in approach used by the two sets of guidelines described above, having a set of consensus-based guidelines represents a huge first step in standardizing the long-term follow-up of HCT survivors. The next important step is to ensure that these guidelines are disseminated and accepted by the healthcare providers and also by agencies that reimburse the costs of the screening tests. Adherence to these guidelines, the yield from such recommendations, and the cost-effectiveness of standardized follow-up of the HCT survivors will provide evidence for the refinement of these guidelines.

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CHAPTER 104

Subsequent Malignant Neoplasms After Hematopoietic Cell Transplantation

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Introduction

The number of patients undergoing hematopoietic cell transplantation (HCT) for malignant or non-malignant disorders has increased steadily over the past four decades. Improvement in survival after HCT and extended follow-up of the growing population of HCT survivors have resulted in an increasing focus on issues related to long-term complications (see Chapter 103). An important and potentially devastating complication of HCT is the occurrence of subsequent malignant neoplasms (SMNs) [1–3]. The magnitude of risk of SMNs after HCT has ranged from 4- to 11-fold that of the general population. The estimated actuarial incidence is reported to be 3.5% at 10 years, increasing to 12.8% at 15 years among recipients of allogeneic HCT (Table 104.1) [4].

Risk factors associated with the development of SMNs include: exposure to chemotherapy and radiation prior to HCT; use of total body irradiation (TBI) and high-dose chemotherapy for myeloablation; human leukocyte antigen (HLA) non-identity; immunodeficiency after HCT aggravated by the use of immunosuppressive drugs for prophylaxis and treatment of graft-versus-host disease (GVHD) such as monoclonal and polyclonal antibodies and T-cell depletion; and infection with viruses such as Epstein–Barr virus (EBV) and hepatitis B and C viruses. However, assessment of risk factors for all SMNs in aggregate is somewhat artificial because of the heterogeneous nature of these malignancies, with differing clinicopathologic features, distinct pathogenesis, and hence very distinct risk factors associated with their development. It has therefore become conventional practice to classify SMNs after HCT into three distinct groups:

1. therapy-related myelodysplastic syndrome (t-MDS) and acute myeloid leukemia (AML) (t-MDS/t-AML);
2. lymphoma (including lymphoproliferative disorders);
3. solid tumors.

Leukemia and lymphoma develop relatively early in the post-transplantation period. On the other hand, solid tumors have a longer latency period and are being increasingly described because of improved survival after HCT and longer follow-up (Figure 104.1). In this chapter, we discuss the three types of SMNs seen among patients undergoing HCT. The emphasis is on clinical presentation of these malignancies, the magnitude of risk and risk factors associated with their development, insights into their pathogeneses, and the treatment options and outcome of patients with these malignancies. The chapter concludes with a brief summary regarding surveillance recommendations for early detection of SMNs.

MDS and AML after autologous HCT

Autologous HCT is now the treatment of choice for patients with Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) who have a suboptimal response to initial therapy, those with refractory or relapsed disease, and those at high risk for relapse after conventional therapy. Autologous HCT is also being increasingly used in specific clinical situations among patients with multiple myeloma.

With improvement in survival following autologous HCT, t-MDS and t-AML are emerging as the major cause of non-relapse mortality [2,5–7]. The cumulative probability of t-MDS/t-AML reported in the literature has ranged from 1.1% at 20 months [8] to 24.3% at 43 months [9] after autologous HCT, depending on the size of the population studied and the completeness of follow-up. The median time to development of t-MDS/t-AML is 12–24 months (range 4 months to 6 years) after HCT. The magnitude of risk of t-MDS/t-AML is higher and the time to development of t-MDS/t-AML is shorter after HCT compared with conventional chemotherapy and radiation. However, the difference in the magnitude of risk may arise, in part, because there are fewer long-term survivors of conventional therapy who have been exposed to multiple salvage therapies.

Clinicopathologic syndromes

Two types of t-MDS/t-AML are recognized in the World Health Organization classification depending on the causative therapeutic exposure: an alkylating agent/radiation-related type and a topoisomerase II inhibitor-related type [10]. Recent reports suggest that patients with multiple myeloma exposed to lenalidomide may be at increased risk for t-MDS/t-AML. However, many cases fail to fall into distinct categories because often patients are exposed to multiple classes of drugs.

Alkylating agent/radiation-related t-MDS/t-AML

Alkylating agent-related t-MDS/t-AML usually appears 4–7 years after exposure to the mutagenic agent. Approximately two-thirds of patients present with MDS and the remainder with AML with myelodysplastic features [11,12]. Patients frequently present with cytopenias, often pancytopenia. Multilineage dysplasia is often present. There is a high frequency of the multidrug-resistance

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Table 104.1 Magnitude of risk and populations at increased risk of subsequent malignant neoplasms after hematopoietic cell transplantation

Study	Study design	Sample size/number of SMNs	Primary diagnoses; type of HCT	Magnitude of risk and risk factors
Witherspoon et al. [92]	Retrospective cohort study	2246/35 leukemia, lymphoma, solid malignancies	Autologous or allogeneic HCT	6.7-fold increased risk compared with general population Acute GVHD treated with ATG or antiCD3 antibody, TBI was associated with increased risk of SMNs
Bhatia et al. [2]	Retrospectic cohort study	2150/53 leukemia, lymphoma, solid malignancies	All hematologic malignancies, inborn diseases of immune system Autologous and allogeneic HCT	11.6-fold increased risk compared with general population Cumulative probability: 9.9% at 13 years for all SMNs; 1.6% at 4 years for PTLD; 5.6% at 13 years for solid malignancies. T-cell depletion, use of ATG, unrelated donor HCT, primary immune deficiency associated with increased risk of PTLD
Deeg et al. [129]	Retrospective cohort	700/23 leukemias (ALL), solid tumors and lymphomas	Severe aplastic anemia Allogeneic HCT	Cumulative probability: 14% at 20 years Azathioprine; diagnosis of Fanconi anemia; irradiation and age at HCT in the non-Fanconi cohort
Kolb et al. [130]	Retrospective cohort study	1036/53 leukemia, lymphoma, solid malignancies	Leukemia, lymphoma, inborn diseases of hematopoietic and immune systems, severe aplastic anemia Autologous and allogeneic	Cumulative probability: 3.5% at 10 years 3.8-fold increased risk compared with general population Older age at HCT and treatment of chronic GVHD with cyclosporine associated with increased risk
Baker et al. [131]	Retrospective cohort study	3372/147 leukemia, lymphoma and solid malignancies	All malignancies and metabolic disorders; immune deficiency; aplastic anemia Autologous and allogeneic	8.1-fold increased risk compared with general population Cumulative incidence: 6.9% at 20 years for any malignancy; 1.4% at 10 years for PTLD; 1.4% at 10 years for MDS/AML; 3.8% at 20 years for solid tumors

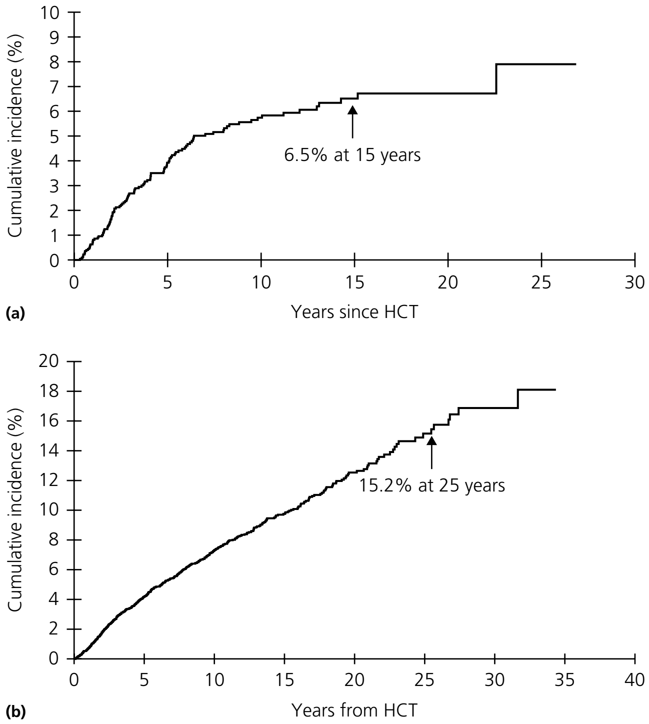

Figure 104.1 (a) Cumulative probability of therapy-related myelodysplasia and acute myeloid leukemia in a cohort of patients undergoing autologous hematopoietic cell transplantation (HCT) for Hodgkin lymphoma or non-Hodgkin lymphoma – a single institution experience. (b) Cumulative probability of solid malignancies after HCT in patients undergoing autologous or allogeneic HCT at a single institution.

phenotype. In addition, there is a high incidence of abnormalities involving chromosomes 5 [–5/del(5q)] and 7 [–7/del(7q)].

Topoisomerase II inhibitor-related AML

Studies in which primary human CD34$^+$ cells were exposed to etoposide in vitro provide evidence that etoposide can directly induce *MLL* rearrangements in hematopoietic cells. Stable genome rearrangements originating within the *MLL* translocation breakpoint hotspot were readily detected in etoposide-treated cells. The spectrum of illegitimate repair included frequent *MLL* partial tandem duplications and translocations, and minor populations containing deletions or insertions. Several clones had breakpoints that localize to *MLL* bcr sequences identified at rearrangement junctions in therapy-related leukemias [13].

AML secondary to topoisomerase II inhibitors often does not have a preceding myelodysplastic phase, and presents as overt acute leukemia, often with a prominent monocytic component [14,15]. The latency period between the initiation of treatment with topoisomerase II inhibitors and the onset of leukemia is brief, ranging from 6 months to 5 years, with a median of 2–3 years [15]. Most often, this type of t-AML is associated with balanced translocations involving chromosome bands 11q23 or 21q22 [15]. Other translocations including inv(18)(p13q22) or t(17,19)(q22;q12) have been reported [14,16].

Lenalidomide-related t-MDS/t-AML

There is emerging evidence that lenalidomide may be associated with an increased occurrence of t-MDS/t-AML. Two of three recent phase III, placebo-controlled, randomized trials reported a higher incidence of t-MDS/t-AML; however, the number of events was small [17–19]. An analysis of pooled data from 11 industry-sponsored trials suggested that there was no correlation between the development of t-MDS/t-AML and the cumulative dose or duration of lenalidomide received [20]. These observations suggest that large numbers of patients exposed to lenalidomide with comprehensive and complete follow-up are needed before the risk of t-MDS/t-AML can be described in this population with precision.

Clinical diagnosis

Cytopenias and dysplastic changes on marrow examination can often be seen in isolation in many patients after autologous HCT, many of whom may not subsequently develop t-MDS/t-AML. Therefore, the Dana-Farber Cancer Institute group has proposed [21] that the diagnosis of t-MDS/t-AML after HCT be based on the presence of:

1. significant marrow dysplasia in at least two cell lines;
2. peripheral cytopenias without alternative explanations;
3. blast counts in marrow defined by the French–American–British classification, or the presence of clonal cytogenetic abnormality.

Risk factors for t-MDS/t-AML after HCT

Factors associated with an increased risk of t-MDS/t-AML include host factors (older age at HCT) [2,22], pre-transplantation therapy with alkylating agents, topoisomerase II inhibitors, and radiation therapy [6,9], method of hematopoietic cell mobilization (etoposide) [2,6], and transplantation conditioning with TBI [23,24] (Table 104.2). Some other factors include a lower number of $CD34^+$ cells infused at HCT [25] and a history of multiple transplantations [24]. Therefore, t-MDS/t-AML appears to be related to perhaps the cumulative effect of pre-transplantation chemotherapy and radiotherapy, transplantation-related factors such as the hematopoietic cell priming, and transplantation conditioning regimens.

The significant impact of primary chemotherapy and radiotherapy on the risk of t-MDS/t-AML after HCT points to an origin of events prior to HCT [6,9]. Moreover, the nature of the pre-transplantation cytotoxic exposure (alkylating agent versus topoisomerase II inhibitor) has a significant impact on the type of t-MDS/t-AML that evolves in the post-transplantation period, reinforcing this observation. A role for pre-transplantation exposures is further supported by the observation that specific cytogenetic abnormalities observed post-transplantation have also been observed in the pre-transplantation marrow or peripheral blood graft among patients who develop t-MDS/t-AML after HCT [26,27]. Abruzzese et al. [27] reported that nine of 12 cases of t-MDS/t-AML studied demonstrated abnormal cells in pre-transplantation bone marrow samples using fluorescence in situ hybridization (FISH). Lillington et al. [26]. reported that significant levels of clonally abnormal cells could be detected in samples obtained prior to high-dose therapy in 20 of 20 patients with t-MDS/t-AML using single-locus specific FISH probes to detect loss of chromosomal material from 5q31, 7q22, and 13q14. In comparison, only three of 24 patients who had not developed t-MDS/t-AML had abnormal clones in pretreatment samples. These studies support a role for genetic abnormalities induced by prior cytotoxic chemotherapy in the etiology of t-MDS/t-AML. However, prospective studies of larger groups of patients are warranted to determine the significance of these observations.

An association of TBI with increased risk for t-MDS/t-AML [23,24]. raises the possibility that the disease may arise from residual hematopoietic cells that persist in the patient despite treatment with high-dose chemotherapy, rather than from reinfused hematopoietic cells, although it is also possible that TBI-induced alteration in the hematopoietic microenvironment may contribute to the development of t-MDS/t-AML. Therefore, it is unclear whether t-MDS/t-AML arises from the graft, from residual cells in the patient, or as a result of a damaged microenvironment.

Table 104.2 Magnitude of risk and populations at increased risk of therapy-related leukemia after hematopoietic cell transplantation

Study	Study design	Sample size/ number of SMNs	Primary diagnoses; type of HCT	Magnitude of risk and risk factors
Krishnan et al. [6]	Retrospective cohort study Nested case–control study	612/22 MDS/AML	Hodgkin lymphoma Non-Hodgkin lymphoma Autologous HCT	Cumulative probability of MDS/AML: 8.6% at 6 years Stem-cell priming with VP-16, pre-HCT radiation associated with increased risk of t-MDS/t-AML
Milligan et al. [24]	Retrospective cohort design	4998/66 MDS/AML	Hodgkin lymphoma Non-Hodgkin lymphoma Autologous HCT	5-year cumulative probability: 4.6% for HL and 3% for NHL. Older age at HCT, diagnosis of HL, exposure to TBI, multiple HCT, years between diagnosis were associated with increased risk of MDS/AML

A higher risk of t-MDS/t-AML has been demonstrated among recipients of CD34-enriched cells isolated from peripheral blood after chemotherapy priming and growth factors, as compared with autologous transplantation using $CD34^+$ cells from the bone marrow without pretreatment [2,6]. Potential explanations offered for this observation include harvesting of hematopoietic precursor cells damaged by chemotherapy at a time before they have completed DNA repair or an overrepresentation of damaged cells in the mobilized product [28]. Supporting this hypothesis is a study by Krishnan et al. [6] demonstrating an increased risk of t-AML with 11q23 abnormalities among patients with HL and NHL mobilized with high doses of etoposide for collection of hematopoietic cells prior to autologous HCT.

Friedberg et al. [25] reported an increased risk of t-MDS/t-AML among patients who received a significantly smaller number of cells re-infused per kilogram of body weight. In the setting of low hematopoietic cell numbers, reconstitution of bone marrow clearly may result in a greater proliferative stress, which may increase susceptibility to irreversible DNA damage associated with t-MDS. These findings are consistent with in vitro data suggesting an increased proliferative stress placed upon committed progenitors at the expense of the primitive progenitors, as shown later in the chapter [29]. Alternatively, reduced ability to harvest cells could indicate an existing defect in marrow function. Kalaycio et al. demonstrated that patients who had difficulty harvesting adequate numbers of hematopoietic cells were at an increased risk of developing t-MDS/t-AML independent of the risk conferred by increased exposure to prior cytotoxic therapies [30].

Pathogenesis of t-MDS/t-AML

t-MDS and t-AML are clonal hematologic disorders that are the consequence of an acquired somatic mutation induced by cytotoxic therapy in hematopoietic stem and progenitor cells which confers a proliferative and/or survival advantage. Improved understanding of the molecular pathogenesis of t-MDS/t-AML may allow the development of strategies to identify populations at risk and modify therapies in order to decrease the morbidity and mortality associated with this complication. Furthermore, t-MDS/t-AML offers a unique perspective on mutagen-induced carcinogenesis and the role of genetic susceptibility to cancer in humans.

t-MDS/t-AML after autologous HCT appears to result from genetic damage to the stem and/or progenitor cell from pretransplant cytotoxic treatment, which may be potentiated by the transplant process itself through several mechanisms, including hematopoietic cell mobilization, collection, and storage, chemotherapy and radiation used for high-dose therapy, and the stress of engraftment and hematopoietic regeneration on the hematopoietic precursors [28].

Genetic lesions associated with t-MDS/t-AML

Loss of chromosome 5 or del(5q) and loss of chromosome 7 and del(7q) are recurring abnormalities in t-MDS/t-AML. These abnormalities are also seen in AML evolving from MDS and de novo AML in elderly subjects. This has led to a search for candidate tumor suppressor genes in these regions. Several groups have attempted to identify commonly deleted segments and to derive transcript maps of these segments [31,32]. The majority of patients with 5q deletions exhibit losses at the 5q31 locus, with deletions in 5q33 being seen in some patients. Chromosomal segment 7q22 is a common site of chromosome 7 deletions. This observation has led to a search for candidate tumor suppressor genes in these chromosomal regions. Identification of a commonly deleted tumor suppressor gene has been elusive thus far. It is possible that haploinsufficiency and reduced gene dosage for critical genes involved in hematopoiesis may sufficiently alter the balance between growth and differentiation to induce dysplastic hematopoiesis [33]. The role of specific genes has been evaluated. For example, the *APC* gene located in the commonly deleted 5q region has been studied. Mice with the *Apcmin* allele that results in a loss of APC function showed no abnormality in steady-state hematopoiesis but had reduced long-term stem-cell function and developed an MDS/myeloproliferative phenotype over time [34], supporting a potential role for *Apc* haploinsufficiency in the MDS phenotype in patients with del (5q).

Molecular analysis of a 2.5-Mb commonly deleted segment of chromosome band 7q22 has failed to uncover a candidate tumor suppressor gene. Abnormalities in chromosome 7 are associated with myeloid leukemias in genetically predisposed individuals, such as Fanconi anemia or neurofibromatosis type 1 [35,36], raising the possibility that similar predisposition may be present in patients with t-MDS/t-AML with loss of chromosome 7. Further, 5q and 7q deletions in familial platelet disorder with leukemia are associated with mutations or deletions of a single *AML1* allele [37]. Balanced translocations involving the *AML1* gene have also been associated with t-MDS/t-AML in patients exposed to topoisomerase II inhibitors [38,39]. Transgenic expression of AML1-ETO leads to immortalization of murine myeloid progenitors but not overt leukemia; additional mutations are required for leukemogenesis [40]. Therefore, altered AML1 function may have a major role in dysregulation of hematopoietic growth and genomic instability and predispose to leukemia through a multistep process.

The *MLL* gene located at chromosome band 11q23 is frequently involved in translocations associated with topoisomerase II inhibitors. *MLL* has a major role in developmental regulation [41]. Altered MLL function, as with AML1, may dysregulate hematopoietic growth and genomic instability, and predispose to leukemia through a multistep process. The long latency period to onset of leukemia in the knock-in mouse suggests that additional genetic changes are required for evolution of t-AML [42].

Genetic susceptibility: polymorphisms in drug-metabolizing enzymes

The interindividual variability in risk of t-MDS/t-AML is likely related to common polymorphisms in low-penetrance genes that regulate the availability of active drug metabolite, or those responsible for DNA repair.

Drug metabolism: Allan et al. utilized a candidate gene approach to examine associations between polymorphisms in the glutathione *S*-transferase genes (*GSTM1, GSTT1*, and *GSTP1*) and t-MDS/t-AML [43]. Relative to de novo AML, the *GSTP1* codon 105 allele occurred more often among t-MDS/t-AML patients with prior exposure to chemotherapy [odds ratio (OR) 2.7, 95% confidence interval (CI) 1.4–5.1], particularly among those with prior exposure to known GSTP1 substrates (OR 4.3, 95% CI 1.4–13.2) and not among t-MDS/t-AML patients with exposure to radiation alone.

Drug transport: P-glycoprotein (encoded by MDR1) traps hydrophobic drugs in the plasma membrane of cells and effluxes them using an ATP-dependent process; many chemotherapeutic drugs are substrates of this protein. A number of polymorphisms exist in the *MDR1* gene, some proposed to be functional and those with loss of function have been evaluated as risk factors for t-MDS/t-AML [44].

DNA repair: Individuals with altered DNA repair mechanisms are likely susceptible to the development of genetic instability that drives the process of carcinogenesis as it relates to t-MDS/t-AML.

Mismatch repair (MMR) functions to correct mismatched DNA base pairs that arise as a result of misincorporation errors that have avoided polymerase proofreading during DNA replication [45]. Defects in the MMR pathway result in genetic instability or a mutator phenotype, manifested by an elevated rate of spontaneous mutations characterized as multiple replication errors in simple repetitive DNA sequences (microsatellites) – functionally identified as microsatellite instability (MSI). Approximately 50% of t-MDS/t-AML patients have MSI, associated with methylation of the MMR family member MLH1 [46,47], low expression of MSH2 [48], or polymorphisms in MSH2 [49].

Double-strand breaks (DSBs) in DNA may lead to loss of genetic material, resulting in chromosomal aberrations. High levels of DSBs arise following radiation and chemotherapy exposures. Cellular pathways available to repair DSBs include homologous recombination (HR), non-homologous end-joining (NHEJ), and single-strand annealing (SSA). RAD51 is one of the central proteins in the HR pathway, functioning to bind to DNA and promote ATP-dependent homologous pairing and strand transfer reactions. *RAD51-G-135C* polymorphism is significantly over-represented in patients with t-MDS/t-AML compared with controls (C allele: OR 2.7) [50]. XRCC3 also functions in the HR DSB repair pathway by directly interacting with and stabilizing RAD51 [51,52]. XRCC3 is a paralog of RAD51, also essential for genetic stability [53,54]. A polymorphism at codon 241 in the *XRCC3* gene results in a Thr → Met amino acid substitution [55]. The variant *XRCC3–241Met* allele has been associated with a higher level of DNA adducts compared with cells with the wild-type allele, implying aberrant repair [56], and has also been associated with increased levels of chromosome deletions in lymphocytes after exposure to radiation [57]. Although *XRCC3-Thr241Met* was not associated with t-MDS/t-AML (OR 1.4, 95% CI 0.7–2.9), a synergistic effect resulting in an eightfold increased risk of t-MDS/t-AML (OR 8.1, 95% CI 2.2–29.7) was observed in the presence of *XRCC3–241Met and RAD51–135C* allele in patients with t-MDS/t-AML compared with controls [50]. The NHEJ pathway joins broken DNA ends containing very little homology. This process is not always precise and can result in small regions of non-template nucleotides around the site of the DNA break, potentially relevant in MLL translocation associated with t-MDS/t-AML [58].

The *base excision repair (BER)* pathway corrects individually damaged bases occurring as a result of ionizing radiation and exogenous xenobiotic exposure. The XRCC1 protein plays a central role in the BER pathway and also in the repair of single-strand breaks, by acting as a scaffold and recruiting other DNA repair proteins [59,60]. The protein also has a BRCA1 C-terminus (BRCT) domain – a characteristic of proteins involved in DNA damage recognition and response. The presence of variant *XRCC1–399Gln* has been shown to be protective for t-MDS/t-AML [61].

Nucleotide excision repair (NER) removes structurally unrelated bulky damage induced by radiation and chemotherapy. The NER pathway is linked to transcription, and components of the pathway comprise the basal transcription factor IIH complex (TFIIH), which is required for transcription initiation by RNA polymerase II. One of the genes involved in the NER pathway (*ERCC2*) is a member of the TFIIH complex. The polymorphic Gln variant (*ERCC2 Lys751Gln*) is associated with t-MDS/t-AML [62].

Neither polymorphism demonstrated a significant association. However, an interactive effect was detected such that *MDM2* TT

TP53 Arg/Arg double homozygotes, and individuals carrying both an *MDM2* G allele and a *TP53* Pro allele, were at increased risk of chemotherapy-related t-MDS/t-AML. Knight et al. conducted a genome-wide association study (GWAS) in patients who had developed t-MDS/t-AML and healthy controls [63]. They identified three SNPs [rs1394384 (OR 0.3, 95% CI 0.2–0.6), rs1381392 (OR 2.1, 95% CI 1.3–3.4), and rs1199098 (OR 0.5, 95% CI 0.3–0.8)] to be associated with t-MDS/t-AML with chromosome 5/7 abnormalities. rs1394384 is intronic to *ACCN1*, a gene encoding an amiloride-sensitive cation channel that is a member of the degenerin/epithelial sodium channel; rs1199098 is in linkage disequilibrium with *IPMK,* which encodes a multikinase that positively regulates the prosurvival AKT kinase and may modulate Wnt/beta-catenin signaling; rs1381392 is not near any known genes, miRNAs, or regulatory elements, although it lies in a region recurrently deleted in lung cancer. The investigators were able to confirm findings in an independent replication cohort.

The *p53* gene has a critical role in DNA damage response signaling, affecting cell cycle, cell death, and DNA repair pathways. Abnormal *p53* activity could lead to a reduced ability to repair DNA damage, resulting in genomic instability and increased susceptibility to leukemogenesis. In patients with de novo MDS and AML, *p53* mutations are seen in fewer than 10% of patients. Ben-Yehuda et al. [64] identified *p53* mutations in 38% of 21 t-MDS/t-AML patients. Mutations were non-germline and restricted to leukemic cells. Horiike et al. [49] identified *p53* mutations in six of 12 patients with t-MDS and chromosome 5 and/or 7 losses, but did not observe any *p53* mutations in nine other patients without chromosome 5 and/or 7 involvement. Christiansen et al. [65] observed mutations in *p53* in 21 of 77 patients (27%) with t-MDS/t-AML; 19 of these 21 patients with mutations had received alkylating agents. *p53* mutations were associated with deletion or loss of 5q and a complex karyotype, were more common in elderly patients, and were associated with an extremely poor prognosis [65]. These studies indicate that *p53* mutations may be observed in certain cytogenetic and prognostic subsets of patients with t-MDS/t-AML, but do not identify a clear role for *p53* mutations in the pathogenesis of this disorder.

Mouse strains resistant or susceptible to t-AML induced by the alkylator ethyl-*N*-nitrosourea (ENU) were analyzed to identify genes that regulate t-AML susceptibility. Thirteen quantitative trait loci (QTL) were significantly associated with ENU-induced leukemogenesis. These QTL contained several genes that could contribute to leukemogenesis, including p53, DNA repair, and apoptosis-regulating genes. These results suggest that susceptibility to alkylator-induced leukemia may be a complex trait related to multiple genes [66].

Genetic instability

Genetic instability is hypothesized to be an early event in the development of malignancy, allowing the accumulation of multiple mutations in the same cell over time, and evolution of a clonal malignant population. Telomeres are non-coding regions of DNA that provide a cap at the ends of chromosomes and prevent dicentric fusion and other chromosomal aberrations [67]. Each somatic cell division is associated with a loss of telomere length. Cumulative telomere shortening can impose a limit on cell divisions and lead to cell senescence. Telomere shortening is also associated with genetic instability [68]. In hematopoietic tissues, there is progressive shortening of telomere length through life, with considerable variability between age-matched individuals. Following HCT, the increased replicative demand on hematopoietic cells associated with hematopoietic regeneration can lead to accelerated telomere shortening. The extent of telomere shortening correlates with the number of cells transplanted. In autologous HCT recipients, telomere length in the transplanted cells is already short because of prior chemotherapy, older age at HCT, increased replicative stress on the hematopoietic cells because of a small number of cells transplanted, or other unknown causes. Patients who developed t-MDS/t-AML after autologous HCT showed accelerated telomere shortening compared with matched controls who did not develop t-MDS/t-AML [69]. Telomere alterations preceded the onset of t-MDS and were independent of other known risk factors for t-MDS/t-AML on multivariate analysis. Constitutional hypomorphic telomerase mutations are seen with increased frequency in patients with AML, suggesting that inherited mutations in telomerase reverse transcriptase (TERT) that reduce telomerase activity may be risk factors for AML.

Hematopoietic abnormalities

Autologous HCT for HL and NHL has been reported to be associated with hematopoietic abnormalities including marked and possibly permanent reduction in primitive progenitor long-term culture-initiating cells and committed progenitor colony-forming cell numbers, altered progenitor expansion potential, and microenvironmental defects [29,70–72]. These abnormalities may be related in part to damage to hematopoietic cells from pre-transplant chemotherapy because hematopoietic defects can also be seen in pre-transplant samples [29,71,72]. A longitudinal study of patients undergoing autologous HCT for lymphoma revealed a significant reduction in primitive and committed progenitors in patients prior to HCT compared with healthy controls. Further profound and persistent reduction in primitive progenitors, but only transient reduction in committed progenitors, was seen after HCT, consistent with extensive proliferation and differentiation of the committed progenitors and subsequent depletion of primitive progenitors during hematopoietic regeneration post-HCT. Patients within this cohort who developed t-MDS/t-AML had reduced recovery of committed progenitors and poorer telomere recovery post-HCT, possibly indicating a functional defect in primitive hematopoietic cells [29]. In a large population-based study, the use of growth factor was independently associated with a 1.5-fold increased risk of developing t-MDS/t-AML. Extensive proliferation of hematopoietic cells bearing genotoxic damage post-transplant may have a role in the establishment and amplification of an abnormal clone. Alternatively, the numerous replication cycles imposed on hematopoietic stem cells after HCT may result in excessive shortening of telomeres in descendent cells (as discussed above). Telomeric shortening may be associated with genomic instability and chromosomal abnormalities, and could contribute to the pathogenesis of t-MDS/t-AML [68].

$CD34^+$ cells in the graft from lymphoma patients also show reduced migration compared with normal $CD34^+$ cells. The in vitro migratory capacity of $CD34^+$ cells in the graft correlated with the speed of hematopoietic recovery after transplantation [73]. Therefore, pre-transplant chemotherapy may also lead to a reduced engraftment potential of primitive progenitor cells. This observation is consistent with experimental data showing that exposure to chemotherapeutic agents results in long-lasting damage to the repopulating ability of hematopoietic stem cells as measured in competitive repopulating unit assays in mice [74]. HCT may also be associated with defects in the marrow hematopoietic microenvironment, including reduction in stromal precursor growth and reduced capacity to support the growth of myeloid progenitors and B cell progenitors [71,75,76]. Microenvironmental defects may contribute to hematopoietic abnormalities after transplantation. Recent studies support the importance of the hematopoietic microenvironment in MDS pathogenesis. Repeated sub-lethal irradiation of mice resulted in bone marrow (BM) dysfunction with features of MDS including low white blood cell (WBC) counts, macrocytic anemia, and reduced platelets and megakryocytes. BM dysfunction was associated with increased BM angiogenesis, vascular endothelial growth factor (VEGF), and activation of the transcription factor nuclear factor-kappa B (NFκB). Tumor necrosis factor (TNF)-α-deleted mice were protected from irradiation effects [77]., suggesting that radiation-mediated TNF-α induction may contribute to BM dysfunction and t-MDS. Interestingly, de novo MDS patients also express high levels of TNF-α [78]. Deletion of the microRNA processing factor Dicer1 in mouse osteoblast progenitors resulted in hematopoietic abnormalities leading to the development of MDS and AML, associated with reduced expression of the *Sbds* gene that is mutated in Schwachman–Bodian–Diamond syndrome, an inherited human marrow failure disorder [79]. Deletion of *Sbds* in osteoblast progenitors also induced marrow dysfunction and myelodysplasia. These results support a role for perturbation of microenvironmental cells in hematopoietic defects in MDS.

Gene expression profiling

Yeoh et al. [80] reported that the gene expression profile of ALL cells at diagnosis was predictive of therapeutic outcomes, including the risk of development of t-MDS/t-AML. Qian et al. [81]. performed gene expression profiling of CD34$^+$ hematopoietic progenitor cells from t-AML patients. This analysis identified different subtypes of t-AML with characteristic gene expression patterns. Common to each subgroup were gene expression patterns characteristic of arrested differentiation in early progenitor cells. Extension of such studies may enhance our understanding of the molecular pathways involved in t-AML. Li et al. performed microarray analysis of gene expression in patients who developed t-MDS after autologous HCT for HL or NHL (cases) and patients who did not develop t-MDS after autologous HCT (aHCT) (controls) [82]. To evaluate gene expression changes pre-HCT, peripheral blood stem cell (PBSC) samples obtained from t-MDS cases and controls before aHCT were studied. Patients who later developed t-MDS showed significantly altered gene expression compared with control subjects, related to mitochondrial function, ribosomes, DNA repair, and hematopoietic stem-cell regulation. Additional alterations in cell-cycle regulatory genes were seen on progression to overt t-MDS/t-AML. PBSC CD34$^+$ cells from patients who developed t-MDS demonstrated altered mitochondrial function, increased reactive oxygen species (ROS), reduced ROS detoxification, and enhanced DNA damage after therapeutic exposure. These results indicate that genetic programs associated with t-MDS/t-AML are perturbed long before disease onset, and are consistent with previous studies indicating a potential role for abnormalities in stem-cell growth, DNA repair, and checkpoint response in t-MDS/t-AML pathogenesis.

Outcome of patients with t-MDS/t-AML after autologous HCT

The response to chemotherapy is inferior for t-MDS/t-AML compared with de novo leukemia; The average complete remission rate ranges between 35 and 40%, and overall long-term survival is poor (reviewed in [83]). As a result, allogeneic transplantation has been evaluated as a therapeutic modality for t-MDS/t-AML. There are few data about outcome after allogeneic HCT for t-MDS/t-AML arising after aHCT, since all prevous reports have combined combined t-MDS/t-AML arising after conventional therapy with that arising after aHCT [84–87]. All these studies emphasize the important role of cytogenetics in determining outcome.

Yakoub-Agha et al. identified predictive factors of outcome for 70 patients with t-MDS/t-AML undergoing allogeneic HCT between 1980 and 1998 [85]. With a median follow-up of 7.9 years after HCT, the estimated 2-year overall survival and event-free survival rates were 30 and 28%, respectively. Age greater than 37 years, male gender, positive recipient cytomegalovirus serology, absence of complete remission at HCT, and intensive schedules used for conditioning were associated with poor outcome.

Armand et al. examined the contribution of cytogenetics to survival, relapse, and non-relapse mortality for 80 patients with t-MDS/t-AML [84]. Cytogenetics was the strongest prognostic factor for outcome. The three categories included the following: (i) favorable [t(8;21), inv(16) or t(16;16), t(15;17)]; (ii) intermediate [normal, +8, del(9q), 11q23, t(9;22), t(6;9), other]; and (iii) unfavorable [−5, del(5q), or −7, abnormal 3q, complex, not obtained/not available]. After accounting for cytogenetics, patients with t-MDS/t-AML had an equivalent outcome to those with de novo disease.

Kroger et al. described the outcome in 461 patients with t-MDS/t-AML treated with allogeneic HCT between 1981 and 2006 [86]. The cumulative incidence of non-relapse mortality and relapse at 3 years was 37 and 31%, respectively. Relapse was significantly higher in patients not in complete remission at the time of HCT ($p = 0.002$), abnormal cytogenetics ($p = 0.005$), older age ($p = 0.03$), and t-MDS ($p = 0.04$). Using age (<40 years), abnormal cytogenetics, and not being in complete remission at the time of transplantation as risk factors, three different risk groups with overall survival rates of 62, 33, and 24%, respectively, could be easily distinguished.

Litzow et al. analyzed outcomes in 868 persons with t-AML ($n = 545$) or t-MDS ($n = 323$) receiving allogeneic transplants from 1990 to 2004 [87]. A myeloablative regimen was used for conditioning in 77%. Disease-free survival (DFS) and overall survival (OS) were 32% (95% CI 29–36) and 37% (95% CI 34–41) at 1 year and 21% (95% CI 18–24) and 22% (95% CI 19–26) at 5 years, respectively. In multivariate analysis, four risk factors had adverse impacts on DFS and OS: (1) age older than 35 years; (2) poor-risk cytogenetics; (3) t-AML not in remission or advanced t-MDS; and (4) donor other than an HLA-identical sibling or a partially or well-matched unrelated donor. Five-year survival for subjects with none, one, two, three, or four of these risk factors was 50% (95% CI 38–61), 26% (95% CI 20–31), 21% (95% CI 16–26), 10% (95% CI 5–15), and 4% (95% CI 0–16), respectively ($p < 0.001$).These data show that allogeneic HCT provides potentially curative therapy for t-MDS/t-AML as it does for de novo MDS. The major risk factors for inferior outcome appear to be disease stage and cytogenetics, together with patient age and donor HLA match or mismatch. Treatment of t-MDS/t-AML should include consideration of the likelihood of success of achieving remission with chemotherapy and, depending on the availability of an appropriate donor, the likelihood of a successful outcome with an allogeneic transplant approach. It is important to follow patients at risk for development of t-MDS/t-AML closely to identify the early development of this complication. Prompt transplantation should be considered after diagnosis of t-MDS/t-AML or, if possible, high-risk MDS, particularly in patients with low peripheral blast counts. It is clear, however, that innovative transplant strategies are needed to reduce the high risks of relapse and non-relapse mortality.

Prediction of risk of t-MDS/t-AML

Because of the poor prognosis associated with t-MDS/t-AML, there is interest in identifying predictors or early biomarkers of risk, to decrease the morbidity associated with this disease. Assessment of risk of t-MDS/t-AML after autologous HCT is complicated by the lack of a single underlying genetic abnormality. The development of t-MDS/t-AML appears to require the acquisition of more than one mutation. Moreover, t-MDS/t-AML is a heterogeneous disorder with multiple subtypes characterized by different genetic abnormalities. Therefore, the identification of a single genetic abnormality may not necessarily have predictive value for development of t-MDS/t-AML. Identification of early biomarkers would allow the timely use of appropriate measures to treat the disorder, such as reduced-intensity conditioning (RIC) or other novel agents, rather than waiting for the t-MDS/t-AML to present in the clinically overt form, when the disease burden would require higher-intensity therapy, with a greater risk of resultant morbidity. In fact, identification of patients at high risk for t-MDS/t-AML prior to transplantation would allow treatment strategies other than autologous transplantation – such as reduced-intensity allogeneic transplantation – to be considered for such patients to avoid the risk of t-MDS/t-AML.

Standard cytogenetics and FISH

Abnormal clones are frequently detected on cytogenetic analysis after autologous HCT for lymphoma. Traweek et al. [88]. reported the risk of developing a clonal cytogenetic abnormality typical of MDS to be 9% at 3 years. Five of 10 patients with the abnormal clone developed t-MDS. Stone [89] reported that 50% of sporadically tested post-transplant patients who were hematologically normal had clonal cytogenetic abnormalities. However, less than 30% of these patients developed t-MDS [21]. Evaluation by FISH may enhance sensitivity of detection of chromosomal abnormalities. Significant levels of clonally abnormal cells were detected by FISH prior to high-dose therapy in samples obtained from 20 of 20 patients who developed t-MDS/t-AML, but only in three of 24 patients who did not develop t-MDS/t-AML [26]. However, this technique is locus specific and requires prior selection of markers for analysis and the predictive value of clonal cytogenetic abnormalities for subsequent development of t-MDS/t-AML needs to be studied in a prospective fashion.

Polymerase chain reaction (PCR) assays for point mutations

Mutations in genes such as *MLL* or *AML1* or gene rearrangements involving the 11q23 gene may be useful markers for risk of subsequent t-MDS/t-AML. This method is highly sensitive, but is locus specific, and the specificity and predictive value of such assays are unknown at present. This test may be most helpful if performed using quantitative techniques that would allow assessment of increasing levels of abnormality [21].

Gene expression analysis

Gene expression microarray analysis of PBSC CD34$^+$ cells from patients who developed t-MDS after aHCT for HL or NHL and control subjects who did not develop t-MDS was used to derive a cross-validated t-MDS gene signature. This 38-gene signature when applied to an independent test set of patients who developed t-MDS after autologous HCT for NHL or HL, and matched controls correctly classified 14 of 16 subjects who developed t-MDS/t-AML, and 19 of 20 subjects who did not. Clonal abnormalities characteristic of t-MDS/t-AML were not seen on cytogenetic analysis of bone marrow samples obtained prior to autologous HCT, nor were they seen in FISH analysis of PBSC CD34$^+$ cells, indicating that the gene expression profile of cytogenetically normal hematopoietic cells can potentially identify patients at risk for t-MDS/t-AML post-aHCT.

Reducing risk of t-MDS/t-AML after autologous HCT

It is possible to consider potential strategies to reduce the risk of t-MDS/t-AML, based on our understanding of the risk factors and pathogenesis of t-MDS/t-AML. Such strategies may include minimizing pre-transplantation cytotoxic exposure, possibly by bringing high-risk patients to HCT earlier in the course of disease, prior to exposure to multiple treatment regimens. Alteration in autologous hematopoietic cell procurement regimens and the conditioning regimens could be considered to eliminate factors associated with increased risk of this complication. Standardized screening of patients in the immediate pre-HCT period with marrow pathology and cytogenetics could potentially help identify high-risk populations that would then benefit from an allogeneic rather than autologous HCT. If strategies to develop predictors for patients at high risk prior to HCT are realized, alternative treatment approaches such as allogeneic transplantation or non-transplant modalities may be worth considering for patients identified as being at increased risk of this complication. Finally, strategies for chemoprevention may be worth exploring in this population. While each of these risk reduction strategies holds validity, perhaps the one with the highest yield will be identifying patients at the highest risk of developing t-MDS/t-AML and minimizing leukemogenic exposures in that populations. Finally, the primary goal should be optimizing management of primary disease while attempting to reduce the risk of complications such as t-MDS/t-AML.

Lymphomas

Post-transplantation lymphoproliferative disorder

Lymphoproliferative disorders are the most common SMN in the first year after allogeneic T-cell-depleted HCT. Most of these cases are related to a compromised immune status and EBV infection. A large majority of the post-transplantation lymphoproliferative disorders (PTLDs) have a B-cell origin, although some T-cell PTLDs have been described.

B-cell PTLD

B-cell PTLD is a clinically and morphologically heterogeneous group of diseases. B-cell PTLD usually develops within the first 6 months after HCT, with most of the events occurring within the first 3 months. The cumulative incidence of PTLD has been reported to range from 1.0 to 2.0% at 10 years [2,90]. The incidence is highest in the first 5 months after HCT (120 cases per 10,000 per year), with a steep decline thereafter to fewer than five cases per 10,000 per year among HCT recipients surviving 1 year or longer.

Risk factors for B-cell PTLD

Risk factors found to be independently associated with an increased risk of PTLD include in vitro T-cell depletion of the donor marrow, unrelated or HLA-mismatched related donor, use of antithymocyte globulin or anti-CD3 monoclonal antibody for acute GVHD prophylaxis or in the preparative regimen, TBI, and primary immunodeficiency (Table 104.3) [2,91]. Patients transplanted for congenital immunodeficiency are at a particularly high risk for PTLD, because of both the underlying immunodeficiency and the use of T-cell

Table 104.3 Magnitude of risk and populations at increased of lymphoproliferative disorders after hematopoietic cell transplantation

Study	Study design	Sample size/ number of SMNs	Primary diagnoses; type of HCT	Magnitude of risk and risk factors
Landgren et al. [132]	Retrospective cohort study	26,901/127 PTLD	Hematologic malignancies and severe aplastic anemia Allogeneic HCT	T-cell depletion, use of ATG, unrelated donor HLA-mismatched grafts Cumulative risk increased to 8.1% with more than three risk factors
Curtis et al. [90]	Retrospective cohort study	18,014/78 PTLD	Hematologic malignancies Allogeneic HCT	Cumulative incidence 1% at 10 years Unrelated donor HCT, T-cell depletion, use of ATG, use of anti-CD3 monoclonal antibody

depletion of the donor graft [2,90,92]. Moreover, the risk of PTLD also depends on the method of T-cell depletion, being considerably higher where specific monoclonal antibodies are used for T-cell depletion (11–25%) rather than in patients where techniques removing both T- and B-lymphocytes, such as soybean agglutinin or alemtuzumab (<1%), are used [90]. However, the incidence of PTLD is higher in the latter group (6–18%) after exposure to immunosuppressive therapy such as steroids or antithymocyte globulins. The use of RIC coupled with highly immunosuppressive therapy needs close observation for the development of PTLD [93].

Pathogenesis of B-cell PTLD

B-cell PTLD is commonly associated with T-cell dysfunction, occurs in the presence of EBV infection, and is thought to develop because of a combination of depressed EBV-specific cellular immunity and the inherent transforming capacities of EBV. EBV is a ubiquitous herpesvirus that infects 95% of individuals by adulthood (see Chapter 90). The virus persists as a latent infection in B-lymphocytes, where reactivation and replication occur intermittently [94]. The latent membrane protein-1 is one of the EBV-encoded proteins believed to have an important role in B-cell immortalization by inducing the expression of *bcl-2*, which inhibits programmed death of the infected cells. *LMP-1* is also considered to be an oncogene and deletions near the 3′-end of the *LMP-1* gene, in a region that affects the half-life of the LMP-1 protein, have been reported in some lymphoproliferative disorders [95]. Infection of B cells by EBV also induces high levels of cytokines such as interleukin (IL)-1, IL-5, IL-6, IL-10, CD23, and tumor necrosis factor. Some of these factors have been shown to act as autocrine growth factors, stimulating the proliferation of EBV-transformed B cells and inhibiting their susceptibility to apoptosis.

Studies exploring susceptibility to EBV–PTLD have shown that cytotoxic T-lymphocyte precursor frequencies are low at 3 months after allogeneic HCT, but appear to normalize at 9–12 months, thus correlating with the period when B-cell PTLD is most frequently observed. Moreover, the EBV-specific cytotoxic T-lymphocytes home preferentially and induce selective regression of autologous EBV-induced B-cell lymphoproliferative lesions in xenografted severe combined immunodeficiency syndrome (SCID) mice [96]. These studies have formed the basis for clinical trials using adoptive transfer of EBV-specific cytotoxic T-lymphocytes [97]. The studies demonstrated long-term persistence of gene-marked EBV-specific cytotoxic T-lymphocytes in vivo, which not only restore cellular immunity against EBV but also provide a population of cytotoxic T-lymphocyte precursors that respond to in vivo and ex vivo challenges with the virus for as long as 18 months [98]. These studies have demonstrated that a combination of granulocyte–macrophage colony-stimulating factor and low-dose IL-2 therapy can prevent the immunodeficiency that leads to fatal EBV lymphoproliferative disease in xenografted SCID mice depleted of murine natural killer cells, and thus support a critical role for several human cellular subsets in mediating this protective effect.

Prediction of risk of B-cell PTLD

Quantitative competitive PCR has been demonstrated to be an effective technique in allowing frequent monitoring of the DNA load to predict the development of PTLD. Rapid increases in peripheral blood EBV DNA load predicted PTLD [99]. EBV-specific T-lymphocytes can be monitored through tetramer technology, which allows the detection of small quantities of antigen-specific T cells. Thus, it has been demonstrated that EBV-specific CD8 T cells are rapidly established following unmanipulated matched sibling allogeneic HCT, and HLA class I tetramers complexed with viral peptides can provide direct and rapid assessment of pathogen-specific immunity.

Treatment of B-cell PTLD

Patients considered to be at an increased risk for development of PTLD (primary immunodeficiency, HLA-mismatched HCT, and GVHD prophylaxis with T-cell depletion) should be monitored closely, so that appropriate therapy can be instituted early, prior to the development of overt disease. Therapeutic approaches that have been used include interferon-α, B-cell-specific monoclonal antibodies, and cellular therapy.

Anti-CD20 monoclonal antibody (rituximab) is effective in the treatment of PTLD [100]. The drug is well tolerated by all age groups and is more efficacious in patients without mass lesions. This observation forms the basis for recommendations to initiate treatment at an early stage, based on increasing EBV load, and before the development of lymphomatous lesions, or even before evidence of transformation. EBV-associated lymphoproliferative disorder developing after HCT has been shown to result from T-cell dysfunction. Reconstitution of "at-risk" patients with EBV-specific cytotoxic T-lymphocyte lines that have been reactivated and expanded in vitro should prevent the development of PTLD or treat pre-existing disease. The cytotoxic T-lymphocyte-reconstituted cellular immune responses to EBV have been shown to persist for up to 80 months. Cytotoxic T-cell therapy has been shown to be efficacious in controlling PTLD, with a decrease in the EBV DNA concentrations, and remission of clinical signs and symptoms [101].

Thus, over the past few years, the administration of in vitro-generated EBV-specific cytotoxic T cells or anti-B-cell monoclonal antibodies has provided an effective option for the prophylaxis or treatment of PTLD. Advances in quantitative PCR-based assays allow both the precise measurement of EBV load in peripheral blood samples and the identification of high-risk patients for early initiation of therapy. Patients should be monitored weekly by quantitative competitive PCR because viral load is a significant predictor of PTLD. Prevention of EBV PTLD by preemptive therapy based on molecular monitoring of EBV load may be used to decrease the occurrence of PTLD. Preemptive therapy with a single dose of rituximab has been reported to lead to the prevention of PTLD, and was first demonstrated by van Esser et al. [102]. The cut-off value of over 1000 EBV genome copies in 10^5 peripheral blood mononuclear cells demonstrates a sensitivity and specificity of 100% and a positive and negative predictive value of 1.00 [103].

Outcome of B-cell PTLD

Before the current methods of anti-EBV therapy were introduced, the mortality from EBV–PTLD was >80%. Preemptive use of rituximab and EBV-cytotoxic T-lymphocytes significantly reduced the risk of death due to EBV–PTLD in HCT recipients, with survival rates of 89.7 and 94.1%, respectively. Therapy of established PTLD also reduced the risk of fatal outcomes. However, the overall success rates were lower than after preemptive therapy, reaching 63 and 88.2% of total EBV–DNA clearance with rituximab and cytotoxic T-lymphocyte therapy, respectively. A reduction of immunosuppression and/or donor lymphocyte infusion might also reduce the risk of death due to EBV–PTLD. The responses to these modalities can be estimated to be 56.6 and 41%, respectively.

T-cell lymphoproliferative disorders

A few cases of T-cell lymphoproliferative disorders have been reported after HCT [104]. There is no association with EBV, human T-cell lymphotropic virus-1, human immunodeficiency virus or human herpesvirus 6. The T-cell lymphoproliferative disorders tend to occur much later than the EBV-associated B-cell PTLD.

Late-onset lymphoma

Late-occurring lymphomas represent an entity that is distinct from the early-occurring B-cell PTLD. In a large study of 18,000 HCT recipients, the only risk factor associated with the development of the late-occurring lymphoma was extensive chronic GVHD [90]. HL developing among HCT recipients has also been described. [105]. Most of the reported cases were of the mixed cellularity subtype, and most of the cases contained the EBV genome. These cases differed from the EBV-associated PTLD by the absence of risk factors commonly associated with EBV-associated PTLD, by a later onset (>2.5 years), and by a relatively good prognosis. The increased incidence of HL among HCT recipients could possibly be explained by exposure to EBV and overstimulation of cell-mediated immunity.

Solid tumors

Solid tumors have been described after HCT. The reported cumulative incidence of solid tumors following allogeneic HCT ranges from 1.2 to 1.6% at 5 years, from 2.2 to 6.1% at 10 years and from 3.8 to 14.9% at 15 years post-HCT [3,106,107]. There is no plateau in the incidence rate. Compared with an age- and sex-matched general population, the magnitude of increased risk of solid tumors is twofold [3]. Solid tumors reported in excess among HCT recipients are those typically associated with exposure to radiation therapy, and include melanoma, cancers of the oral cavity and salivary glands, brain, liver, uterine cervix, thyroid, breast, bone, and connective tissue. The risk of solid tumors increases sharply over time [106]. TBI is associated with an increased risk of solid tumors. The risk of solid tumors increases with the dose of radiation compared with those who did not receive radiation therapy (Table 104.4); the risk is 3–4 times elevated at the highest dose levels [107]. The risk of developing a non-squamous cell carcinoma following TBI is typically dependent on age at exposure. Among patients irradiated at ages under 30 years, the relative risk of non-squamous cell carcinoma was nine times that for non-irradiated patients, while the comparable risk for older patients was 1.1 [3]. Majhail et al. evaluated the risk of solid SMNs after HCT using high-dose busulfan–cyclophosphamide conditioning in 4318 recipients of allogeneic HCT for AML or chronic myelogenous leukemia (CML) [108]. The cumulative incidence of solid cancers at 5 and 10 years after HCT was 0.6 and 1.2% among AML and 0.9 and 2.4% among CML patients, respectively. In comparison with the general population, HCT survivors were at a 1.4-fold increased risk. Significantly increased risks were observed for tumors of the oral cavity, esophagus,

Table 104.4 Magnitude of risk and populations at increased of solid tumors after hematopoietic cell transplantation

Study	Study design	Sample size/ number of SMNs	Primary diagnoses; type of HCT	Magnitude of risk and risk factors
Curtis et al. [107]	Retrospective cohort	19, 229/80 solid malignancies	Allogeneic HCT	2.7-fold increased risk of solid malignancies compared with the general population Cumulative incidence: 6.7% at 15 years; younger age at HCT associated with increased risk of solid malignancies; chronic GVHD and male gender associated with increased risk of squamous cell carcinoma
Bhatia et al. [106]	Retrospective cohort study	2129/29 solid malignancies	Hematologic malignancies Autologous and allogeneic HCT	2-fold increased risk compared with general population Cumulative probability: 6.1% at 10 years
Shimada et al. [133]	Retrospective cohort study	809/19 solid malignancies	All malignancies Autologous and allogeneic HCT	2.8-fold increased risk Cumulative incidence: 4.2% at 10 years Extensive chronic GVHD and older age were associated with increased risk
Gallagher and Forrest [134]	Retrospective cohort study	926/28 solid malignancies	Hematologic malignancies Allogeneic HCT	10-year cumulative incidence: 3.1% 1.85-fold increased risk compared with general population Older age at HCT and female donor associated with increased risk
Socié et al. [135]	Retrospective cohort	147/4 solid tumors	Severe aplastic anemia/ allogeneic HCT	Cumulative probability: 22% at 8 years
Socié et al. [91]	Retrospective cohort	3182/25 solid tumors; 20 PTLD	Acute leukemia in childhood	Cumulative risk of solid tumors: 11% at 15 years; Age <5 years at HCT; TBI was associated with increased risk of solid tumors; chronic GVHD associated with decreased risk Chronic GVHD, unrelated donor HCT, T-cell-depleted graft, use of ATG associated with increased risk of PTLD
Majhail et al. [108]	Retrospective cohort	4318/66 solid cancers	Acute myeloid leukemia Chronic myelogenous leukemia Allogeneic (matched related and unrelated) HCT	Cumulative incidence: 1.2% at 10 years 1.4-fold increased risk compared with general population Chronic GVHD associated with increased risk, especially oral cavity
Rizzo et al. [3]	Retrospective cohort	28,874/189 solid malignancies	All malignancies/allogeneic HCT	2.1-fold increased risk Age at exposure to TBI <30 years associated with 9-fold increased risk of non-squamous cell carcinoma Chronic GVHD and male gender associated with risk of squamous cell carcinoma

Table 104.5 Magnitude of risk and populations at increased of specific SMNs after hematopoietic cell transplantation

Study	Study design	Sample size/number of SMNs	Primary diagnoses; type of HCT	Magnitude of risk and risk factors
Rowlings et al. [105]	Retrospective cohort	18,531/8 Hodgkin lymphoma	Hematologic malignancies and severe aplastic anemia Allogeneic HCT	6.2-fold increased risk Grade 2–4 acute GVHD, treatment for chronic GVHD
Leisenring et al. [109]	Retrospective cohort study design	4180/237 non-melanoma skin cancer	All malignancies and metabolic disorders Allogeneic HCT	20-year cumulative incidence: BCC 6.5%, SCC 3.4% TBI and younger age at irradiation, light skin color, chronic GVHD associated with BCC Acute and chronic GVHD associated with SCC
Curtis et al. [121]	Case–control	58 squamous cell and 125 basal cell carcinomas 501 matched controls	Hematologic malignancies, severe aplastic anemia, hemoglobinopathies Allogeneic (matched related and unrelated) HCT	Prolonged and severe chronic GVHD and its therapy (azathioprine, corticosteroids, cyclosporine) were associated with squamous cell carcinomas
Schwartz et al. [110]	Retrospective cohort	6,306/282 Basal cell carcinomas	Hematologic malignancies; severe aplastic anemia; Autologous, matched related and unrelated donor HCT	TBI exposure; youngest age at HCT; increasing attained age; whites; more recent birth cohorts
Friedman et al. [111]	Retrospective cohort	3337 females/52 breast cancers	Hematologic malignancies Allogeneic HCT	Cumulative incidence: 11% at 25 years Longer time since HCT, use of TBI, younger age at HCT
Cohen et al. [112]	Retrospective cohort	78,914/32 thyroid cancer	All malignancies and severe aplastic anemia Autologous and allogeneic	3.26-fold increased risk Young age at HCT, irradiation, female gender, chronic GVHD

lung, soft tissue, and brain. Chronic GVHD was an independent risk factor for all solid cancers, especially cancers of the oral cavity. Although most studies have focused on allogeneic HCT recipients, there is emerging evidence for an increased incidence of new solid malignancies among patients conditioned with TBI and receiving autologous HCT [22].

The magnitude of risk and associated risk factors for some of the more commonly occurring second solid tumors among HCT recipients are detailed in Table 104.5 and summarized below.

Skin cancer: The 20-year cumulative incidence of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are reported to be 6.5 and 3.4% respectively, after allogeneic HCT [109]. TBI is a risk factor of BCC, particularly among patients younger than 18 years old at HCT. Acute GVHD increases the risk of SCC; chronic GVHD increases the risk of BCC and SCC. Schwartz et al. demonstrated that the risk of BCC associated with radiation is highest for the youngest ages at exposure, with relative risks exceeding 20 for those transplanted at ages less than 10 years, and decreasing with increasing age at exposure until age 40 years, above which no excess risk was identified [110]. Rizzo et al. demonstrated that chronic GVHD and male sex are the main determinants for risk of SCC [3].

Breast cancer: The 25-year cumulative incidence of breast cancer is reported to be 11% after allogeneic HCT [111]. Allogeneic HCT survivors are at a 2.2-fold increased risk of developing breast cancer compared with an age- and gender-matched general population. The median latency from HCT to diagnosis of breast cancer is 12.5 years. The incidence is higher among those exposed to TBI (17%) than among those who did not receive TBI (3%). The risk is increased among those exposed to TBI at a younger age.

Thyroid cancer: HCT recipients are at a 3.3-fold increased risk of thyroid cancer compared with an age- and gender-matched general population [112]. Age younger than 10 years at HCT, neck radiation, female gender, and chronic GVHD are associated with an increased risk of thyroid cancer. Thyroid cancer develops after a latency of 8.5 years and is associated with an excellent outcome.

Pathogenesis of solid tumors after HCT

There is limited information about the pathogenesis of solid tumors after HCT. An interaction of cytotoxic therapy, genetic predisposition, viral infection, and GVHD, with the consequent antigenic stimulation and use of immunosuppressive therapy, all seem to have a role in the development of new solid tumors.

Radiogenic cancers generally have a long latent period, and the risk of such cancers is frequently high among patients undergoing irradiation at a young age. A large series reported an increased risk of brain and thyroid cancers after TBI as part of high-dose conditioning, although most of these patients had received cranial irradiation prior to transplantation [87]. The increased risk of thyroid, breast, brain, bone, and soft tissue cancers seen after HCT appear to be related to cumulative doses of radiation exposure, as a result of both the pre-transplant treatment regimen and the conditioning regimen used for transplant.

Immunologic alterations may predispose patients to SCC of the buccal cavity, particularly in view of the association with chronic GVHD [109]. Themeli et al. demonstrated that chronic tissue stress due to interaction of alloreactive donor cells with host epithelium after allogeneic HCT might cause genomic alterations [113]. They analyzed buccal samples obtained from 71 allogeneic HCT recipients for microsatellite instability (MSI). MSI was observed in 52% of the allogeneic HCT recipients, but was not observed among the 31 healthy individuals or autologous HCT recipients. There was a trend for increasing risk of MSI for patients who experienced severe GVHD. SMNs were diagnosed in 14% of the MSI$^+$ and 3% of the MSI$^-$ patients. In an in vitro model of mutation analysis, there was a significant induction of frameshift mutations and DNA strand breaks in HaCaT keratinocytes cocultured with mixed lymphocyte cultures (MLCs) but not after their exposure to interferon-γ, TNF-α, transforming growth factor-β, MLC supernatant, peripheral blood mononuclear cells (PBMCs) or phytohemagglutinin-stimulated PBMCs. These findings suggest a reactive oxygen species-mediated mechanism. These data suggest that alloreactions after allogeneic HCT might induce genomic alterations in the epithelium [113]. Khan et al. examined genomic instability in oral and nasal mucosal specimens from allogeneic HCT recipients. The comparison group included autologous HCT survivors, patients treated with conventional therapy, and healthy control subjects. Genomic instability was detected in 60% of the oral versus 4% of the nasal specimens in allogeneic HCT survivors, and in none of the individuals from the comparison group. In the oral specimens, genomic instability was significantly associated with a history of oral chronic GVHD, suggesting a possible mechanism for the development of SCC in the oral cavity and not the nasal cavity [114].

The increased risk of new solid tumors after HCT is thus likely related to pre-HCT radiation for treatment of primary disease, TBI used for conditioning, altered immune function in association with viral infections [hepatitis B virus, hepatitis C virus, or human papillomavirus (HPV)], and genomic instability related to chronic stress after allogeneic HCT.

Treatment of patients with solid tumors after HCT

Treatment strategies for patients developing solid tumors after transplantation are not well defined. With respect to outcome, small case series indicate both ends of the spectrum: favorable outcome [115] and also aggressive tumor growth and early relapse after standard therapy [116]. A comprehensive study of a large number of patients with second solid tumors will help determine the nature of these tumors and their outcomes compared with de novo tumors. Until then, patients with solid tumors developing after transplant should be treated with the best available therapy for that tumor, unless there is compelling evidence that they will not be able to tolerate that therapy.

Screening for subsequent malignant neoplasms in HCT survivors

There is paucity of data regarding the clinical details at presentation of SMNs, in particular the clinical stage. Theoretically, if screening practices resulted in subsequent cancers being diagnosed at an earlier stage at time of presentation, then treatment would be more successful and survival rates higher. Additionally, less aggressive treatment may be required resulting in less toxicity. The issues of who to screen, how to screen, and when to do it are all important but largely unanswered questions. For the pediatric survivor population, the Children's Oncology Group has developed comprehensive long-term follow-up guidelines that provide risk-adapted recommendations based on therapeutic exposures and that are supported by the best available published literature [117]. For the HCT survivors, screening recommendations must incorporate risks associated with pre-HCT therapy and also from the transplant conditioning therapy and other HCT-associated conditions such as chronic GVHD. Joint recommendations for screening and preventative practices for long-term survivors after HCT from the European Group for Blood and Marrow Transplantation, the Center for International Blood and Marrow Transplant Research, and the American Society of Blood and Marrow Transplant were published in 2006 [118]. This reference provides general guidance for SMN screening for HCT survivors but recommendations are limited to risk awareness counseling and clinical assessments. General recommendations for colorectal, breast and cervical cancer are provided but are for the most part based on guidelines for the general population. Screening guidelines developed for use in the general population [119,120] may or may not be sufficient or applicable for use in HCT survivors, given the modest increase in risk of solid SMNs overall. For some individuals, additional risk factors either pre-HCT (exposure to chest irradiation, cranial irradiation, alkylating agents, topoisomerase II inhibitors) or associated with HCT (TBI, chronic GVHD, duration of immunosuppression, T-cell depletion) may lead to elevated risks and patients at significantly increased risk need to be identified for tailored screening strategies.

Risk-reduction strategies

Although strategies aimed at altering therapeutic exposures (chemotherapy and irradiation) will most likely result in a reduction in the risk of subsequent malignancies in some patients, it is unlikely that these exposures are the only factors that convey risk. In HCT recipients, the risk of SMNs is associated with duration of immunosuppression and also to chronic GVHD (likely from ongoing immunosuppression as opposed to an impact of GVHD itself), hence delayed immune recovery or persistent immunodeficiency may also contribute to the risk of subsequent malignancy after HCT [121]. Efforts to prevent GVHD and to improve immune reconstitution after HCT may be an effective strategy to reduce the risk of subsequent malignancy.

There is increasing concern related to the risk of cancer and cancer death associated with obesity and a sedentary lifestyle [122]. Functional and performance limitations and lower levels of physical activity have been reported in HCT survivors, particularly in those who have chronic GVHD [123,124]. Obesity (as determined by body mass index) is uncommon in HCT survivors [125]; however, preliminary studies of body composition reveal that they have a high percentage of fat mass and a reduction in muscle mass. This state of sarcopenic obesity contributes to the development insulin resistance, hyperinsulinemia, and chronic inflammation; factors that have been implicated in the causal pathway of obesity-associated cancer risk [126]. Hence interventions recommended for the general population aimed at improving nutrition and maintaining a physically active lifestyle are likely of even greater significance for HCT survivors as preventative measures for development of SMNs. Finally, as the pathogenesis of SMNs becomes more clearly elucidated, screening for known genetic susceptibility to cancer could possibly be undertaken and alternative therapeutic options presented to the vulnerable subpopulation. These strategies, and also those encouraged for all, such as avoidance of tobacco and alcohol, use of sunscreen, and HPV vaccination, may help to reduce the risk of SMNs in HCT survivors. Additionally, providing education to our survivors in order to raise awareness of the risk of SMNs and also to the primary care community who will be involved with the long-term follow-up and screening of these individuals will be very important.

Conclusion

HCT offers curative therapy for many patients with otherwise incurable disease. Currently, 60,000 transplants are performed annually (see Chapter 1), and most patients who do not experience a recurrence of their underlying disease within 1–2 years of transplantation do well and lead productive lives [127,128]. However, some complications do occur, and among those that have been described after HCT, malignant diseases are of particular clinical concern, because an increasing numbers of patients are surviving the early phase after transplantation and remaining free of their original disease.

The incidence of post-transplant malignancies appears to be low, although reliable estimates of the overall risk will require much longer follow-up. Despite the lack of randomized studies, the benefit of HCT, as compared with conventional therapy alone, in certain clinical situations outweighs the risk of late SMNs. However, it is imperative to follow this population of HCT recipients closely in order to screen them for the development of SMNs, and thus decrease the morbidity and mortality associated with this complication. Longitudinal monitoring of patients at multiple time points (before, during, and after HCT) is required to evaluate risk factors, study the evolution of genetic lesions, and identify biomarkers for SMNs. Improved understanding of risk factors may allow future modification of pre-transplant and transplant-related therapeutic exposures to minimize the risk for these complications. A better understanding of the pathogenesis of SMNs will allow for more effective screening to identify patients at risk prior to the HCT procedure, and allow more effective monitoring to detect early evolution of the malignancy post-transplantation. This may, in turn, allow for improved therapeutic decision-making while evaluating patients for HCT, and early institution of treatments directed at preventing and treating SMNs in patients at risk after HCT.

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CHAPTER 105

Neurologic Complications of Hematopoietic Cell Transplantation

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Introduction

Neurologic complications occur frequently after hematopoietic cell transplantation (HCT). Published rates vary from 3% in an autologous transplant series to 44% in matched unrelated donor transplants [1,2]. An even higher incidence of 46% was reported in pediatric transplants in the early transplant years [3], compared with an incidence of 15.8% in pediatric transplants carried out in the 1990s [4]. The wide variations of incidences may be explained by differences in patients selected for transplantation and also different time intervals sampled and varying criteria used for retrospectively identifying neurologic complications. A single prospective study of 115 consecutive transplants in the early 1990s reported an incidence of 17% occurring in the first 3 months after transplant [5]. In another approach, an incidence of 24% was recently estimated by recording all brain imaging ordered in consecutive allogeneic transplant patients in the first 12 months after transplantation [6].

Whatever the incidence, neurologic complications clearly have serious consequences. Autopsy series of allogeneic transplant patients in 1973–1998 reported gross brain pathologic abnormalities in almost 50% of cases; and central nervous system (CNS) pathology such as intracranial hemorrhage, abscess, and CNS metastases was considered the major cause of death in 17% of those autopsied [7,8]. The expectation is that improved standards in transfusion support and infectious disease prophylaxis have reduced the incidence of serious cerebrovascular and infectious neurologic complications; however, in a recent study of brain imaging carried out in 2004–2007, 15% of patients with neurologic complications had intraparenchymal cerebrovascular disease or CNS infection. The highest mortality was in those patients with cerebrovascular complications (estimated 0.10 12-month overall survival) [6].

A practical approach to the transplant patient requires an understanding of which problems are most likely at different times of the transplant course. As indicated in Table 105.1, neurologic complications may occur at three stages: (a) from the conditioning agents used for marrow ablation, (b) during pancytopenia, or (c) from immunosuppressive therapies and graft-versus-host disease (GVHD) in allogeneic transplant recipients. It is important to understand the patient's pre-transplant neurologic status, particularly in patients heavily pretreated with neurotoxic chemotherapy, and how this may influence post-transplant complications. Experienced practitioners narrow down the differential diagnosis by taking into account the hematologic diagnosis, therapy given (pre-transplant chemotherapy, conditioning agents, immunosuppressants, and supportive care drugs), and the time interval after transplant. This chapter is organized according to standard disease categories as given in the first column of Table 105.1.

Infectious complications

The neurologic presentation of CNS infection in HCT recipients is usually an alteration in mental status, delirium, or depression of sensorium, often without meningeal signs or obvious lateralizing neurologic signs. A spinal fluid examination is indicated once a mass lesion has been excluded. For a suspected brain abscess, a stereotactic biopsy under appropriate platelet support usually is diagnostic. Antimicrobial prophylaxis early in the transplant course is particularly effective for Gram-negative organisms, and bacterial meningitis at this stage is now unusual. Also unusual, but still a risk, is bacterial meningitis in long-term survivors with chronic GVHD (cGVHD), including *Listeria monocytogenes* meningitis and meningitis due to penicillin-resistant *Streptococcus pneumoniae* [9].

Aspergillus species accounted for 30–50% of CNS infections in autopsy series and remain a major problem in HCT. Figure 105.1a shows a typical *Aspergillus* abscess. Less common is *Aspergillus* endarteritis presenting as a stroke (Figure 105.1b). *Candida albicans*, the other serious fungal pathogen encountered after transplantation, infects the CNS in only 3% of transplant patients with systemic candidemia [10], although *Candida* species abscesses accounted for 15% of the CNS infections in a large autopsy series from Brazil [8]. Chronic fungal meningitides, for example from *Cryptococcus neoformans,* a frequent complication of immunosuppression in other disease processes, is rarely encountered in HCT recipients. More common is CNS infection with the protozoan *Toxoplasma gondii* early after engraftment, particularly in patients who do not receive trimethoprim and sulfamethoxazole prophylaxis (Figure 105.1c and 1d).

Herpes simplex virus (HSV) type 1 reactivates and is shed in the oral secretions of 80% of seropositive patients during the first few weeks after HCT [11]. As described in Chapter 88, acyclovir prophylaxis prevents viral shedding and is now used routinely in transplant centers. Despite the high incidence of viral reactivation, the clinical diagnosis of herpes simplex encephalitis is rarely made in transplant recipients. In immunocompetent individuals, herpes encephalitis usually occurs without any sign of mucocutaneous lesions. The brain infection is focal in the medial temporal lobes, most often presenting with seizures and psychiatric symptoms.

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Table 105.1 Neurologic complications of hematopoietic cell transplantation

	Conditioning	Pancytopenia	Graft-versus-host disease
Infectious		Bacterial meningitis Gram-negative sepsis	Fungal abscess Meningoencephalitis
Cerebrovascular		Intracranial bleed Thrombotic angiopathy	Septic embolism, mycotic aneurysm ← Ischemic stroke →
Metabolic		Sedative–hypnotic drugs Hepatic encephalopathy of SOS	Hypoxia of interstitial pneumonia Hepatic encephalopathy of GVHD Uremic encephalopathy
Toxic	Encephalopathy (BCNU, busulfan, ifosfamide) Neuropathy (VP-16, cisplatin, paclitaxel)	Calcineurin inhibitor neurotoxicity → Steroid toxicity → Leukoencephalopathy →	Thalidomide neuropathy
Immune-mediated			Polymyositis Myasthenia gravis Demyelinating polyneuropathy

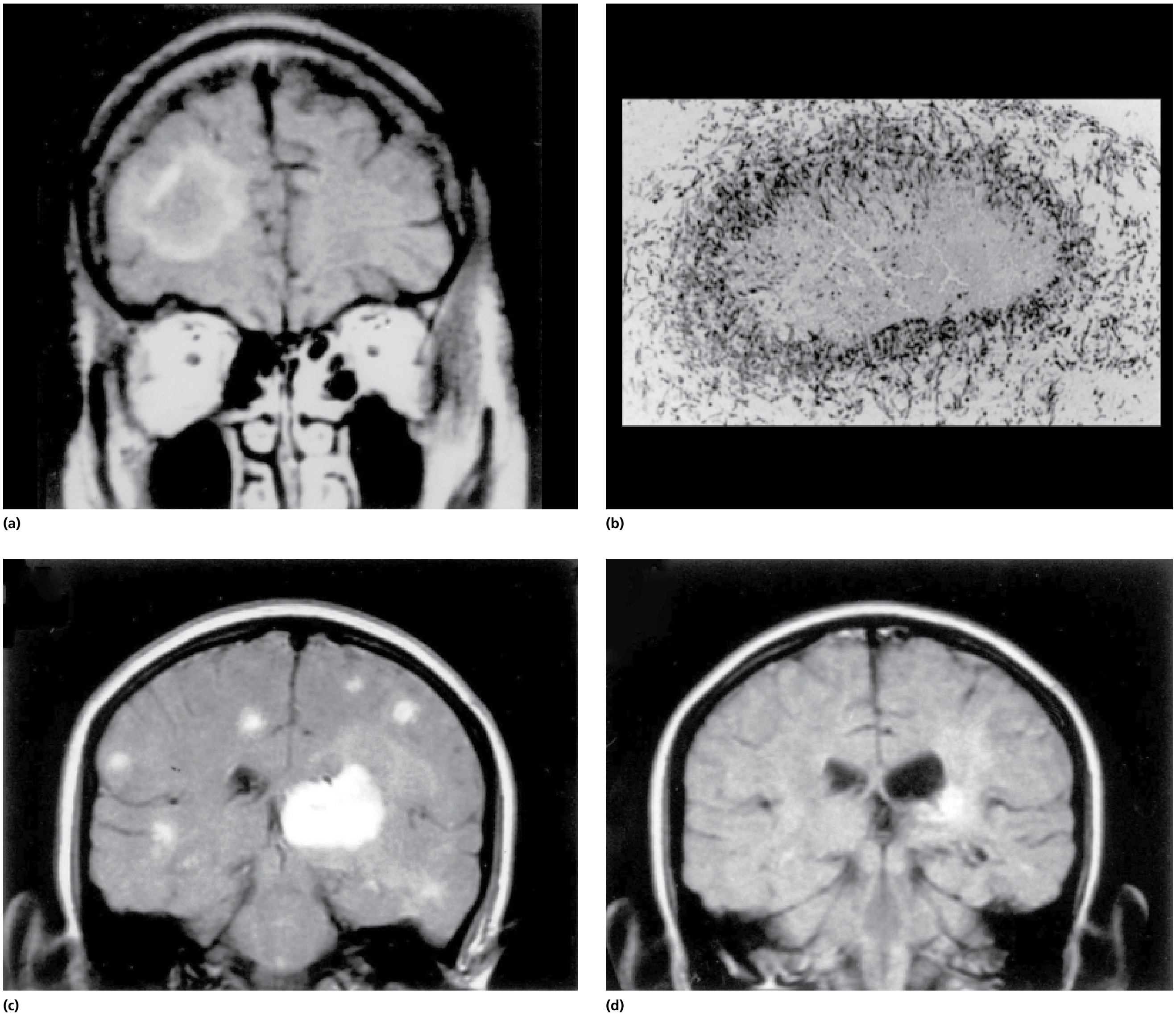

Figure 105.1 Fungal and parasitic CNS infections. (a) MRI of CNS *Aspergillus* infection. A 27-year-old man with Hodgkin disease developed pulmonary aspergillosis as documented by bronchoscopy 5 months after autologous transplantation. Gadolinium ring-enhanced lesions were documented in the right frontal lobe. CNS aspergillosis was confirmed at autopsy. (b) Post-mortem *Aspergillus* histopathology of a 45-year-old woman who developed a locked-in syndrome, rapidly evolving to coma and death 2 months after allogeneic transplantation for granulocytic sarcoma. Shown is a thrombosed branch of the basilar artery with mural invasion of the arterial wall by branching fungal hyphae (Gomori methanimine silver, ×100). (c) MRI of CNS *Toxoplasma gondii* infection. At 3.5 months after autologous transplantation for Hodgkin disease, a 21-year-old Hispanic woman developed right-sided upper motor neuron clumsiness with sensory symptoms, and MRI showed multiple gadolinium-enhancing lesions, the largest in the left parietotemporal area. (d) Neurologic recovery and almost complete resolution of the lesions on MRI occurred after 6 months of therapy with pyrimethamine and sulfadiazine.

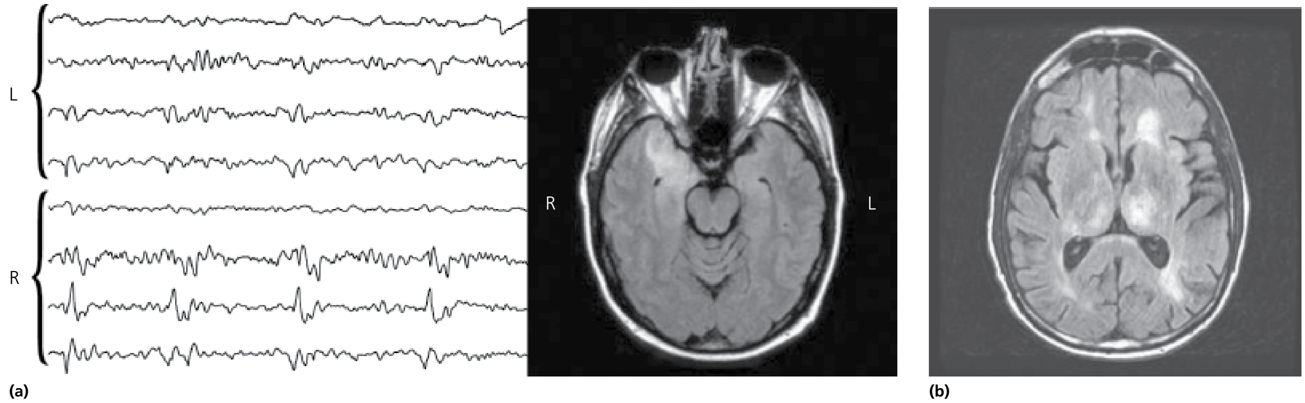

Figure 105.2 (a) Electroencephalogram (EEG) and brain MRI in herpes simplex encephalitis. A 46-year-old man whose acute myelogenous leukemia relapsed 8 months after autologous stem-cell transplant, and then underwent a non-myeloablative matched unrelated donor transplant with melphalan and fludarabine conditioning. On cyclosporine and prednisone for skin graft-versus-host disease, he developed abrupt confusion with visual agnosias 4 months after allogeneic transplantation. An EEG showed right-sided periodic sharp waves. Right frontotemporal increase signal developed on follow-up brain MRI FLAIR (fluid attenuated inversion recovery) sequence. The cerebrospinal fluid had 44 white blood cells/μL (87% lymphocytes) and 11 red blood cells/μL, and herpes simplex type 1 was cultured from the cerebrospinal fluid. Despite therapy with both acyclovir and foscarnet, there was neurologic progression with the patient developing an amnestic state, unable to form new memory traces. (b) Brain MRI (FLAIR sequence) in Epstein–Barr virus (EBV) encephalitis. A 63-year-old man 5 years after allogeneic transplant for AML with scleredermatous GVHD developed confusion, weakness with incoordination, and bilateral upper motor neuron signs. The cerebrospinal fluid had 39 white blood cells/μL (82% lymphocytes), and EBV sequences were detected in blood and cerebrospinal fluid. He was treated with rituximab and survived with neurologic disability.

Figure 105.2a shows an electroencephalogram (EEG) and magnetic resonance imaging (MRI) of a transplant patient who developed typical frontotemporal herpes simplex encephalitis. The EEG is helpful since periodic lateralized epileptiform discharges (as in Figure 105.2a) are characteristic for herpes encephalitis and may precede the MRI abnormality. Although HSV was cultured from the cerebrospinal fluid in the patient shown in Figure 105.2, viral cultures usually are negative in herpes encephalitis. Cerebrospinal fluid polymerase chain reaction (PCR) assay of HSV nucleic acid is helpful, but treatment should be initiated and continued in suspicious cases regardless of the PCR result.

Present about half as often as HSV, active varicella zoster virus (VZV) infection occurs later in the transplant course. The greatest incidence is during the fourth and fifth months after allogeneic transplantation (see Chapter 89). Facial nerve palsy and hearing loss can be associated with cranial zoster, arm weakness with cervical zoster, and neurogenic bladder with lumbosacral zoster. It is likely that a mild meningoencephalitis with or without pleocytosis and motor signs occurs fairly frequently in transplant patients with zoster; but the designation of varicella zoster encephalitis is usually reserved for the rare instances of diffuse encephalitis with a decrease in sensorium. Other serious manifestations include VZV vasculitis and retinal necrosis [12].

VZV and HSV have been implicated in peripheral facial weakness (Bell's palsy) in normal individuals without any skin or mucous membrane manifestations. Although a controlled study failed to show benefit of acyclovir added to prednisolone in normal individuals with Bell palsy [13], there has not been a similar study in transplant patients. It is probably prudent to start antiviral therapy with valacyclovir with or without corticosteroids in HCT patients who develop Bell palsy.

Recognized manifestations of cytomegalovirus (CMV) infection in transplant patients include pulmonary, hepatic, and gastrointestinal involvement. For reasons that are uncertain, CMV chorioretinitis, a very common problem in HIV-AIDS, is rarely diagnosed in HCT recipients [14]; similarly, CMV encephalitis is recognized at least histopathologically in HIV-AIDS but is rarely diagnosed or documented in marrow transplant recipients [15]. Fatal meningoencephalitis due to West Nile virus, adenovirus, and human herpesvirus type 6 (HHV6) have all been documented in marrow transplant recipients [16,17] and instances of HHV6 focal encephalitis in the medial temporal lobe, indistinguishable from HSV encephalitis, have been recognized (post-transplant acute limbic encephalitis). Use of conditioning regimens including alemtuzumab increases the risk of HHV6 encephalitis, even in patients receiving antiviral prophylaxis [18]. An example of Epstein–Barr virus (EBV) encephalitis in a transplant patient is presented in Figure 105.2b. Finally, progressive multifocal leukoencephalopathy, the slow virus infection caused by polyomavirus JC, has been reported in at least four patients after allogeneic HCT and 12 patients after autologous HCT [19,20]. PCR assays are now readily available for the detection of all of these viruses in cerebrospinal fluid, but particularly for progressive multifocal leukoencephalopathy (PML), brain biopsy may be necessary for diagnosis.

Cerebrovascular complications

Intracranial hemorrhage is most frequently associated with refractory thrombocytopenia and, except for subdural hematomas, large intracranial hemorrhages are usually fatal in HCT recipients. The characteristic clinical course of large supratentorial hemorrhages is abrupt onset of hemiparesis, or other neurologic deficit localized to the cerebral hemispheres, followed by rapid depression of the sensorium and development of brain-stem signs from transtentorial herniation (Figure 105.3a). A cerebellar hemorrhage is more difficult to recognize because of the subtlety of the initial signs – abnormalities of gait and eye movement – which then progress to gaze paresis and coma. Treatment of intracranial hemorrhage is the neurosurgical evacuation of the hematoma in those instances where this is feasible [21].

Subdural hematomas characteristically reduce the sensorium with no or mild lateralizing signs. Consequently, they can be

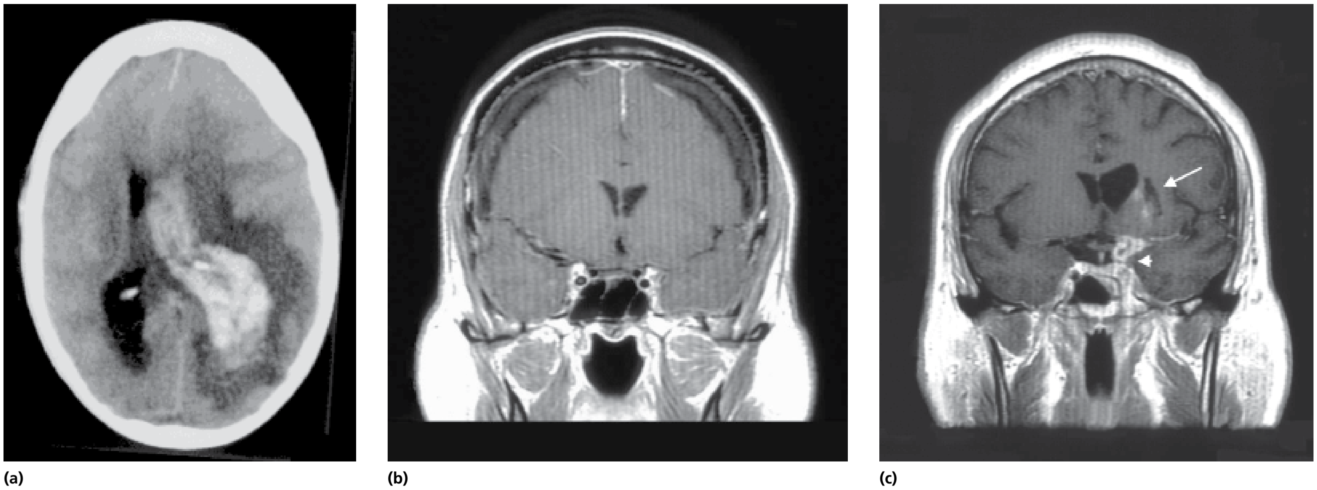

Figure 105.3 Cerebrovascular disease. (a) Brain CT of a fatal intracranial hemorrhage. A 34-year-old woman with biphenotypic leukemia underwent allogeneic bone marrow transplantation and 1.5 months later had a fatal massive left intraparenchymal hemorrhage with rupture into the left lateral ventricle. (b) Brain MRI of bilateral subdural hygromas resulting from multiple lumbar punctures and low cerebrospinal fluid pressure. A 48-year-old man with HIV-associated diffuse large cell lymphoma and leptomeningeal disease underwent three lumbar punctures for intrathecal therapy the week before high-dose BCNU and etoposide with autologous stem-cell transplantation. Two weeks after transplantation, there was confusion and reduced sensorium. The MRI showed bilateral subdural fluid collections, obliteration of cortical sulci, and balanced mass effect. After bilateral surgical drainage of the subdural hygromas, there was improvement in his sensorium. (c) Brain MRI of left sphenoid sinus infection with extension to the cavernous sinus (arrow head) and left hemisphere stroke (arrow). Sphenoid sinusitis developed in a 35-year-old man 5 months after autologous stem-cell transplantation for acute myelogenous leukemia. Despite repeated sphenoid surgeries, a left sixth nerve palsy developed followed by right hemiparesis and dysphasia. He was treated with amphotericin B and intraconazole for a presumed fungal infection. MRI shows sphenoid sinus opacification, a soft tissue mass within the left cavernous sinus occluding or narrowing the carotid artery, and an infarction involving the internal capsule, putamen, and head of caudate nucleus.

overlooked early in the course, particularly in patients who are sedated or encephalopathic for metabolic reasons. Non-hemorrhagic subdural fluid collections (hygromas) may develop over the cerebral convexities in patients who have low cerebrospinal fluid pressure as a consequence of repeated lumbar punctures (e.g., patients with acute lymphoblastic leukemia undergoing intrathecal treatment or prophylaxis for CNS leukemia) [22,23]. An epidural lumbar blood patch may help decrease the headache and shorten the time for the hygroma to resolve. However, with or without the blood patch, the hygroma may increase in size, as shown in Figure 105.3b, and surgical drainage may be necessary even if bleeding into the hygroma can be avoided. With or without neurosurgery, this complication may seriously compromise treatment of the leukemia by delaying further intrathecal therapy and the date of HCT.

Ischemic strokes in transplant recipients may be embolic from endocarditis or thrombotic associated with a hypercoagulable state. Vasospasm leading to ischemic symptoms or stroke has been suspected in rare patients at the time of cryopreserved stem-cell transfusion [24]. An endarteritis associated with meningeal infection, particularly with *Aspergillus* species, may occur and be difficult to distinguish clinically from primary cerebrovascular disease (see Figure 105.1b). Bacterial or fungal sphenoid sinusitis in transplant patients may result in a major hemisphere stroke by extension of the infection to the carotid artery in the venous cavernous sinus (Figure 105.3c). It is important that patients with severe sphenoid sinus infections undergo surgery and receive appropriate antibiotic or antifungal therapy to prevent cavernous sinus syndrome.

Non-bacterial thrombotic endocarditis occurs in transplant patients as a consequence of a hypercoagulable state [25]. Chemotherapy-induced endothelial damage and circulating immune complexes may also be contributing factors. In an autopsy series of 91 allogeneic transplant patients, there was a single case of bacterial endocarditis (group D *Streptococcus*) with stroke and seven cases of non-bacterial thrombotic endocarditis, with two of these patients having ischemic strokes.

Thrombotic microangiopathy, recognized in 5–15% of allogeneic transplant patients, may cause transient hemiparesis and seizures. Etiology probably is from endothelial cell damage from conditioning therapy or calcineurin inhibitors; diagnosis often requires a high degree of suspicion, identifying fragmented red blood cells (RBCs) and elevated serum lactate dehydrogenase (LDH) in the context of renal or hepatic abnormalities; treatment involves discontinuing calcineurin inhibitors. Plasma exchange has not been proven effective in transplantation-associated thrombotic microangiopathy [26,27].

Metabolic complications

The characteristic clinical feature of metabolic encephalopathy is toxic delirium, usually without lateralizing neurologic signs and with normal brain imaging. In transplant patients, toxic delirium is most often from sepsis, but it is also frequently iatrogenic from sedative–hypnotic drugs or calcineurin inhibitors. Less common is toxic delirium associated with viral encephalitis. The differential diagnosis here is often difficult, especially since brain imaging abnormalities of encephalitis are often absent or subtle. Clinical features that prompt spinal fluid analysis for viral PCR analysis or initiation of antiviral therapy include headache and psychiatric symptoms early in the course, absence of asterexis or myoclonus (frequent signs of metabolic encephalopathy and calcineurin inhibitor toxicity), persistent fever on antibiotics, seizures, worsening level of consciousness off calcineurin inhibitors, and no laboratory abnormality to account for worsening encephalopathy.

Hypoxic encephalopathy, carrying the risk of permanent neurologic disability, may occur from interstitial pneumonia or from hypoxemia associated with RBC lysis of thrombotic microangiopathy. Hepatic encephalopathy may occur from liver involvement in GVHD or from fulminant hepatic failure in sinusoidal obstruction syndrome (SOS) [28] (see Chapter 94). Renal failure with resultant uremic encephalopathy has been attributed to nephrotoxic drugs including the calcineurin inhibitors, the renal glomerulopathy that is rarely seen as a manifestation of GVHD, radiation nephritis, and thrombotic microangiopathy. Hypomagnesemia, associated with calcineurin inhibitors or as a residual from cisplatin nephrotoxicity, may cause convulsions in addition to cramps and muscle weakness. Reduced sensorium and seizures may occur from hyponatremia associated with the syndrome of inappropriate antidiuretic hormone secretion in viral encephalitis and intracranial mass lesions.

The occurrence of a severe metabolic abnormality within the first 100 days after transplant carries a poor prognosis with higher 100-day non-relapse mortality and a lower overall 5-year survival [29]. Multiple organ failure in HCT patients, as in other critically ill patients, is a poorly understood but generally fatal entity. SOS of the liver or pulmonary failure after transplant may herald multiorgan failure, including CNS failure. About 50% of HCT patients who develop a decrease in a standardized bedside mental status instrument in the first month of transplant go on to develop pulmonary or hepatic involvement and almost two-thirds of these patients do not survive 100 days after transplant [30]. An argument has been advanced that regardless of the initial organ system involved, the course in these patients reflects a systemic inflammatory response, the so-called multiple-organ dysfunction syndrome (MODS) [31]. As such, a systemic rather than an organ-specific approach to therapy is needed and such an approach will depend on a better understanding of the pathophysiology and initiating events of MODS and how these may be affected by high-dose chemoradiation.

Because of the increased risk of sepsis and shock, neurologic complications of critical illness may occur in transplant patients [32]. Critical illness polyneuropathy (CIPN) is thought to occur from inadequate perfusion of peripheral nerves and is often associated with encephalopathy, multiorgan failure, and prolonged mechanical ventilation. It is a predominantly motor axonal neuropathy with moderate to severe neurologic deficit interfering with respiratory weaning [33]. Recovery may be partial and slow, with survivors usually requiring months before they can resume ambulation. Another complication that interferes with respiratory weaning and may be confused clinically is acute critical illness myopathy [34]. Coincidence of both CIPN and acute critical illness myopathy probably occurs particularly in patients with multiorgan failure who have received neuromuscular blocking agents and corticosteroids.

Neurologic manifestations of recurrent disease

About 9% of neurologic problems encountered in the first year after transplant can be attributed to recurrence of CNS disease [6]. Leptomeningeal recurrences typically present as motor or sensory signs in the distribution of spinal nerve roots or cranial nerves. Clinical manifestations of hydrocephalus also can occur form cerebrospinal fluid absorption block. Although computed tomography (CT) can be used to monitor hydrocephalus, brain imaging with MRI is generally required to show leptomeningeal abnormalities: either gadolinium enhancement or patchy increased signal in the leptomeninges on fluid attenuated inversion recovery (FLAIR) sequences (Figure 105.4a, b). These MRI abnormalities may be present without motor or sensory abnormalities of leptomeningeal disease, but they are often subtle or absent. Cerebrospinal fluid analysis is needed to exclude an infectious process and confirm abnormal cytology.

Less common then leptomeningeal disease, CNS recurrence can manifest as a parenchymal mass, as shown Figure 105.4c. These recurrences can produce headache from increased intracranial pressure and upper motor neuron weakness or other signs from parenchymal mass lesion. Brain imaging is helpful in the differential diagnosis of cerebrovascular or infectious etiology; but brain biopsy may be required for definitive diagnosis. To avoid confusion from corticosteroid lympholytic effects, effort is generally made to delay dexamethasone in patients who are to undergo such diagnostic biopsy.

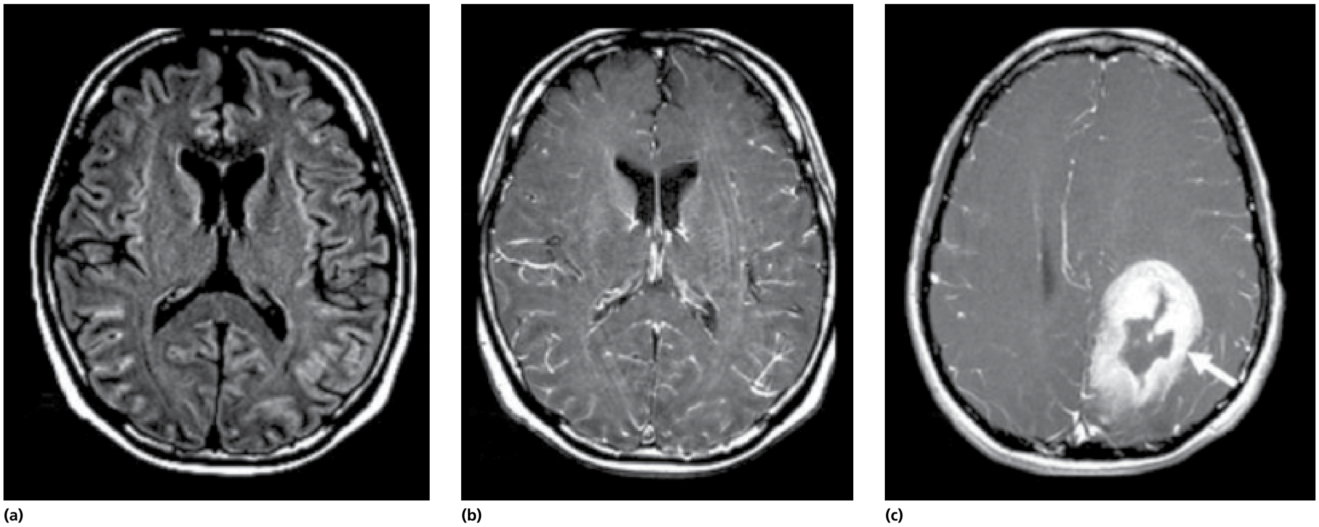

Figure 105.4 CNS recurrence of hematologic malignancy. (a, b) Brain MRI images of a 30-year-old woman with acute myelogenous leukemia who developed leptomeningeal disease 50 days after HCT. Axial FLAIR sequence (no contrast) shows abnormal hyperintensity diffusely throughout the cerebral sulci and along gyral surfaces (a), and the post-contrast T1-weighted image shows corresponding gadolinium enhancement (b). (c) Brain T1-weighted post-contrast image of a 17-year-old boy with altered mental status 105 days after HCT. The hemorrhagic enhancing mass was surgically removed and shown to be a chloroma.

Systemic recurrence of hematologic malignancy can also produce neurologic signs from a pressure effect on nerve root, plexus, or peripheral nerve. For example, bone marrow involvement in the mandible can result in numbness in the chin from involvement of the inferior alveolar nerve.

Immune-mediated complications of the central nervous system: a real entity?

In long-term HCT survivors, the incidence of MRI white matter abnormalities and abnormalities in neuropsychologic tests have been shown to correlate with cGVHD [35]. This correlation, however, probably results from complications of prolonged immunosuppression rather than a direct CNS affect of cGVHD.

A review of case reports and small series published between 1990 and 2007 found 18 reported cases of putative cGVHD of the CNS [36]. The time range of neurologic onset after HCT was 2–31 months (median 14.5 months) and the neurologic presentation was most consistent with angiitis and stroke in seven, immune-mediated encephalopathy in seven (four also with seizures), myelopathy in two, and progressive motor abnormalities in two. Etiologic factors other than GVHD, however, are possible in these patients. What would be helpful in evaluating these and subsequent cases is the establishment of diagnostic criteria which, if met in full or in part, would result in a consensus diagnosis of cGVHD of the CNS. Proposed criteria include (1) the presence of systemic cGVHD, (2) neurologic signs of white matter abnormality with no other explanation, (3) MRI abnormality corresponding to the neurologic signs, (4) abnormal cerebrospinal fluid studies (pleocytosis, elevated protein with relevant negative viral PCR studies), (5) brain biopsy or post-mortem examination abnormalities consistent with immune-mediated process, (6) and response to immunosuppressive therapy [36,37]. Cases that fall short may still be instances of cGVHD but more open to argument.

Immune-mediated abnormalities of the peripheral nervous system

Three immune-mediated diseases, all affecting the peripheral nervous system, can occur after HCT. Most common is polymyositis – a clinical syndrome characterized by proximal muscle weakness, elevated levels of creatine phosphokinase and other muscle enzymes, short-duration (myopathic) motor units on electromyography with signs of acute denervation (fibrillation potentials and positive sharp waves), and necrotic myofibers and mononuclear inflammatory cells on muscle biopsy. Less common is myasthenia gravis (MG), an immune-mediated disorder of the neuromuscular junction in which autoantibodies to the postsynaptic acetylcholine receptor produce a characteristic clinical syndrome of ptosis and extraocular muscle weakness, most often with proximal limb and facial muscle weakness. Less well accepted as a complication of HCT is immune-mediated demyelinating polyneuropathy, a condition producing neuropathic weakness that can be severe, sensory loss, and nerve conduction slowing on electrophysiologic tests. It is not difficult to differentiate polymyositis from MG and polyneuropathy. A potential problem arises when mild polymyositis goes unrecognized and accounts for a slower than usual functional recovery from transplantation.

A review article reported polymyositis in 3% of 318 patients with cGVHD [38]. Onset of weakness occurred 7–24 months after transplantation, and all patients showed improved strength with reinstitution or an increase in the dose of prednisone. There are rare reports in which polymyositis was the sole manifestation of GVHD and there is a case report of polymyositis occurring after autologous transplantation [39]. Muscle cramps are common in polymyositis, but disabling muscle cramps occasionally occur as a new problem in post-transplant patients without other neurologic findings or metabolic abnormalities.

It is important to exclude infectious causes of polymyositis and to exclude the occasional patient with drug-induced myositis. Zidovudine (AZT) as well as the combination of lovastatin and cyclosporine (CSP) can cause an inflammatory myopathy, and a fatal case of rhabdomyolysis occurred after a CSP-associated seizure in a transplant patient [40]. Muscle weakness in a patient with GVHD may also be from autoimmune hyperthyroidism [41] or, much more commonly, from steroid myopathy. A common treatment dilemma is whether steroids should be increased as therapy for polymyositis or decreased because of presumptive steroid myopathy. Further testing should help in this situation because unlike polymyositis, patients with steroid or other endocrine myopathies have neither electromyographic evidence of denervation nor inflammatory cells in muscle biopsy specimens.

The prevalence of post-transplantation MG is lower than that of polymyositis. Only three of 1800 allogeneic transplant recipients (<0.5%.) have been diagnosed at Seattle, and there is a similar low prevalence at other centers [42], Onset tends to be later than with polymyositis. Most patients are diagnosed after immunosuppressant drugs to prevent cGVHD have been decreased or discontinued. Patients transplanted for aplastic anemia appear to have a greater risk for acquiring MG, and many post-transplant MG patients have antiplatelet, antismooth muscle, antimitochondrial, and antinuclear antibodies and also anti-acetylcholine receptor antibodies. Thymoma, present in about 15% of patients with idiopathic MG, has not been identified in post-transplantation MG. Unlike polymyositis, which usually is self-limited, post-transplant MG tends to be a chronic, albeit sometimes fluctuating, problem requiring anticholinesterase drugs to control symptoms. Alternate-day low-dose prednisone is generally adequate if immunosuppressants are required. Return to higher dose immunosuppressants or treatment with plasma exchange is generally not necessary or recommended. It may be important to avoid, or use with caution, in these patients drugs that affect neuromuscular transmission, including aminoglycoside antibiotics, certain cardiac antiarrhythmic agents such as procainamide, and parenteral magnesium.

Because of the known increased risk of Guillain–Barré syndrome in Hodgkin disease and other disorders with reduced cellular immunity [43], it is not surprising that Guillain Barré-like neuropathies occasionally occur in transplant recipients [44]. Compared with MG and polymyositis, there is a greater clinical heterogeneity in these patients and less certainty as to the pathogenesis. Most are demyelinating but some are severe axonal neuropathies. The prevalence has been estimated at only 1% of allogeneic transplantations [45], but these neuropathies produce considerable morbidity because the deficit is predominantly motor and because they may occur early after transplantation when patients are medically most vulnerable. Prior CMV or *Campylobacter jejuni* infection probably triggers some instances of severe demyelinating neuropathy after transplant [46], and calcineurin inhibitors have also been implicated in some patients [45]. Although the pathogenesis is uncertain, an immune mechanism is suspected, and the transplant physician may face the dilemma of increasing the existing immunosuppressant drugs or discontinuing or switching calcineurin inhibitors. GVHD is present in most but not all patients, and the neuropathy has been thought to correlate with the course of GVHD in some patients. Necessary for the diagnosis of demyelinating neuropathy is the electrophysiologic demonstration of nerve conduction slowing or conduction block. It may be helpful to document cerebrospinal fluid protein elevation and myelin breakdown with reactive macrophages on nerve biopsy. Treatment of dysimmune polyneuropathy in these patients has included plasmapheresis with variable success; prednisone, which is effective in non-transplant-associated chronic inflammatory demyelinating polyneuropathy (CIDP) and intravenous γ-globulin. There may be a need for caution in considering a transplant for hematologic malignancy in patients with a pre-existing inflammatory demyelinating neuropathy, since abrupt worsening after conditioning involving radiation therapy has been reported in rare patients [47].

Complications from the calcineurin inhibitors cyclosporine and tacrolimus

Calcineurin inhibitors (CSP and tacrolimus) cause more neurologic problems in transplant patients than any other class of drugs [48]. Complications are diverse. Essential tremor is almost always present, and headaches of a vascular quality occur in up to 20% of patients. Seizures and encephalopathy are the most common serious complications. Typically these seizures follow complaints of headache, increased tremulousness, mild confusion, asterixis, and sometimes visual symptoms. Although usually single and generalized, multiple and partial seizures may also occur and there may be transient post-seizure deficits such as cortical blindness and behavioral abnormality. Phenytoin has been used after seizures induced by calcineurin inhibitors but recently some transplant physicians have shifted to levetiracetam, an anticonvulsant that unlike phenytoin does not affect the hepatic metabolism of calcineurin inhibitors. Most clinicians continue anticonvulsants until high-dose calcineurin inhibitor therapy is no longer required.

Patients who are encephalopathic from calcineurin inhibitors usually have elevated blood pressure and often have low serum magnesium. They may have abnormalities on MRI with multifocal areas of signal hyperintensity, most often in the occipital lobe white matter and sometimes associated occipital blindness [49] (Figure 105.5a). These abnormalities identified on MRI T2 or FLAIR sequences are consistent with posterior reversible encephalopathy syndrome (PRES), which is seen most often in the non-transplant setting in malignant hypertension, alone or in association with eclampsia [50]. Although most scan abnormalities from calcineurin inhibitors show predominant white matter lesions, there can be mixed cortical and subcortical involvement, sometimes associated with punctate cortical gadolinium enhancement (Figure 105.5b,c) [51]. Cerebellar and brain-stem MRI lesions exceptionally occur and transient eye movement abnormalities have been reported from calcineurin inhibitors in HCT patients [52] (Figure 105.5d). In contrast, clinical findings of hemiparesis or hemisensory loss would be considered highly atypical for calcineurin inhibitor toxicity; such lateralizing motor or sensory signs usually suggest an ischemic insult or a cerebral mass lesion.

Calcineuron inhibitor neurotoxicity is less often detected by CT than MRI, but marked CT abnormalities can occur, as shown in Figure 105.5e. The reversibility of the CT abnormality on follow-up scans (Figure 105.5f) argues against cerebral infarction and for vasogenic edema. Damage to the vascular endothelium from chemotherapeutic drugs, breakdown of the blood–brain barrier from radiation, or from CSP itself , may all be contributing factors. CSP leads to the release of endothelins, vasoactive neuropeptides that have been implicated in cerebral vasospasm [53]. Endothelin release may be triggered by or may cause microangiopathic hemolytic anemia, a condition found by multivariate analysis to have the highest correlation with CSP toxicity [48]. Vasospasm, shown to be present on an MR angiogram [51], may explain involvement at junctions of major vessels (watershed areas) or at the distal distribution of major vessels, such as the occipital pole. Neuropathology of CSP toxicity is limited, but reports indicate edema or demyelination. There is a report of vasculitis attributed to tacrolimus in a liver transplant patient [54].

Although these vascular abnormalities are now well recognized, they probably do not account for all instances of serious calcineurin inhibitor neurotoxicity. Sometimes there is severe neurotoxicity with encephalopathy prolonged for days, to near-coma, yet the MRI is normal. In other instances, there is relatively mild encephalopathy but with MRI abnormalities on FLAIR or T_2 sequences. In animals, CSP has a direct neurotoxic effect, independent of alterations in blood pressure or renal function [55]. A cell culture study showed that CSP can induce neuronal apoptosis and selective oligodendrocyte death [56]. The mechanism probably involves inhibition of the phosphatase activity of calcineurin, the same mechanism whereby CSP inhibits T-cell proliferation. CSP is bound by serum lipoproteins, metabolized in the liver cytochrome P450 enzyme system, and excreted in bile. Blood levels may become elevated in patients with hyperbilirubinemia, GVHD of the liver, or sinusoidal obstruction syndrome [57]. Erythromycin, ketoconazole, and calcium channel blockers decrease hepatic metabolism of CSP, whereas phenytoin enhances hepatic metabolism [48,58,59]. Hypocholesterolemia is associated with an increase risk of CSP neurotoxicity [60], probably because of an increase in unbound CSP; and it is likely that the low cholesterol levels in liver disease account at least in part for the twofold greater prevalence of CSP neurotoxicity in liver transplant patients compared with HCT patients. CSP does not penetrate the blood–brain barrier well under normal conditions, but damage to the barrier by conditioning agents, particularly irradiation, may increase the penetration of calcineurin inhibitors. Despite clinical experience suggesting a direct toxic role of CSP, neurotoxicity does not always correlate with steady-state drug levels. It has been proposed that high peak levels (e.g. at times of intravenous use) may predispose to neurotoxicity [48].

A considerable degree of clinical judgment often goes into decisions on when to reduce or hold calcineurin inhibitors to prevent the more serious instances of neurotoxicity. Even mildly encephalopathic patients who have worsening asterixis or develop myoclonus should be considered for supplementary intravenous magnesium, temporary discontinuation of the calcineurin inhibitor regardless of the drug level, and possibly a loading dose of intravenous fosphenytoin or phenytoin. Clinical experience in HCT suggests that temporary discontinuation of calcineurin inhibitors and then restarting at a lower dose are usually successful in preventing worsening or recurrence of neurotoxicity. Switching from CSP to tacrolimus or vice versa is seldom necessary, and is not always successful in preventing recurrence of neurotoxicity.

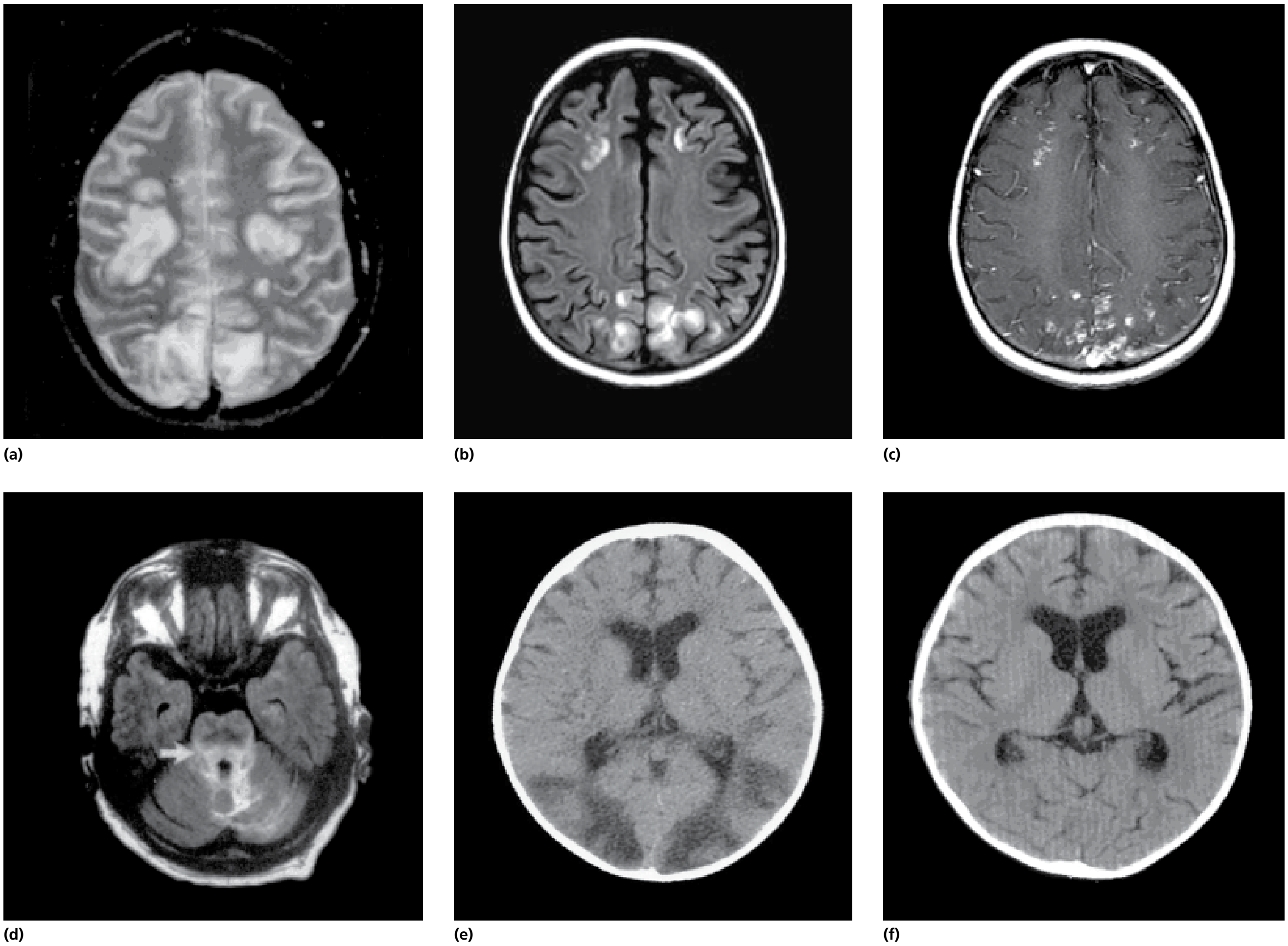

Figure 105.5 Calcineurin inhibitor neurotoxicity. (a) Brain MRI (T2 sequence) of a 20-year-old woman 5 weeks after allogeneic transplantation for chronic myeloid leukemia who developed abrupt onset of confusion and headache. Patches of bright signal on T_2-weighted images were present, especially in the parietal and occipital subcortical white matter. Later, a generalized seizure with encephalopathy occurred and CSP was stopped for 4 days and then restarted at a lower dose. Repeat MRI showed resolution of the lesions. (b, c) Brain MRI FLAIR sequence (b) and T1 sequence with gadolinium (c) in an 11-year-old girl with acute lymphoblastic leukemia on tacrolimus 4.5 months after cord blood transplant. (d) Brain MRI (FLAIR sequence) of a 50-year-old man who developed diplopia 2 months after allogeneic transplant for mantle cell lymphoma. The arrow shows signal abnormality included the area of the cranial nerve VI nucleus. Eye movements returned to normal 2 days after CSP was discontinued. (e) Brain CT in a 2-year-old girl with acute lymphoblastic leukemia and liver chronic GVHD who developed seizures and reduced sensorium 1 year after matched unrelated donor transplant. A follow-up scan (f) carried out 1 month later showed almost complete resolution of parieto-occipital edema. Source, panel (d): Openshaw 2001 [95]. Reproduced with permission of BMJ Publishing Group Ltd.

Complications from other immunosuppressive drugs

A sense of wellbeing and even euphoria often accompanies the initiation of corticosteroid treatment. With continued administration, psychotic depression, mania, or delirium may occur [61]. This toxicity is often dose dependent, but when a reduction in steroid dose is not feasible, neuroleptic or antianxiety medications may be necessary. Proximal muscle weakness due to muscle protein catabolism is experienced by virtually all transplant patients who receive the equivalent of 40 mg of prednisone per day for more than 3 weeks [62]. Typical symptoms include difficulty arising from a chair and difficulty washing hair. Muscle cramps and tenderness occur less often with steroid myopathy than with polymyositis. Unlike polymyositis, serum creatine phosphokinase levels are normal or only slightly elevated in steroid myopathy, and electromyography often shows only slight abnormalities with myopathic motor units. Treatment consists of switching from the more myotoxic fluorinated steroids such as dexamethasone to non-fluorinated agents, such as prednisone or methylprednisolone, tapering the steroids when this is possible, and instituting daily physical therapy. Regular exercise during corticosteroid treatment may reduce the catabolic effect on muscle.

Symptoms of thalidomide peripheral neuropathy can begin after 2 months of therapy at 100 mg per day, the starting dose sometimes used for cGVHD [63] and within the dose range sometimes used for maintenance after autologous transplant for multiple myeloma (see Chapter 55). Lower-limb numbness and paresthesias occur, often accompanied by burning and hyperesthesia of the feet. With continued exposure, leg cramps and a stocking-glove pattern of sensory loss develop involving superficial sensation more than proprioception or vibration. Patients with severe neuropathy may have muscle weakness. An unusual feature compared with other toxic neuropathies is the preservation of deep tendon reflexes well into the course of the neuropathy. There may be mild elevation of cerebrospinal fluid protein, and nerve conduction tests and morphologic analysis indicate a large-fiber, "dying-back" sensory neuronopathy (i.e. initial degeneration in the distal region of the axon and progression of the degeneration proximally to the nerve cell body) [64]. The extent of neurologic recovery after discontinuation of thalidomide depends on the severity of the symptoms and possibly the patient's age and duration of exposure. Motor signs revert more readily and completely than sensory symptoms and, in some patients, distressing sensory complaints may be permanent [65].

Low-dose intravenous methotrexate (MTX), frequently used for GVHD prophylaxis, causes only occasional and minor neurotoxicity of headache, dizziness, and rarely seizures when given to patients with rheumatoid arthritis [66]. High-dose MTX (5 g/m^2 per cycle), used mainly for osteogenic sarcoma, can trigger transient leukoencephalopathy, similar in appearance to but usually more extensive than the MRI abnormalities seen with calcineurin inhibitors [67], and acute, transient CNS toxicity often with hemiparesis, occurs rarely after intathecal MTX. Much less often seen now in transplant patients is the delayed-onset, chronic, and often fatal leukoencephalopathy resulting usually from the combination of intrathecal MTX and whole-brain irradiation [68]. Neurologic signs usually within 4–5 months after transplant include dysarthria, ataxia, dysphasia, spasticity and upper motor neuron weakness, seizures, confusion, and a decrease in sensorium. Leukoencephalopathy with severe neurologic sequelae and death has also been attributed to amphotericin B following total body irradiation in a transplant recipient [69].

Complications from conditioning agents

Neurologic complications from chemotherapeutic drugs in the conditioning regimen include encephalopathy and seizures as acute self-limited complications and peripheral neuropathy as delayed, longer lasting complications. Busulfan (BU) and carmustine [1,3-bis(2-chloroethyl)-1-nitrosourea; BCNU] are common causes of encephalopathy, and etoposide and cisplatin are common causes of peripheral neuropathy. Granulocyte colony-stimulating factor (G-CSF) may exacerbate autoimmune activity during peripheral blood hematopoietic stem-cell mobilization of patients with multiple sclerosis [70]. Long-term disabilities of conditioning agents that may involve cognition and quality of life are discussed in Chapter 103 and the long-term risks of primary brain tumors in Chapter 104.

Before routine use of prophylactic anticonvulsants, generalized seizures occurred overall in about 10% of patients receiving high-dose BU (4 mg/kg/day for 4 days) [71]. BU readily crosses the blood–brain barrier, and it is presumed that seizures occur as a direct neurotoxic effect. Although myoclonic twitching may be seen shortly before or after the ictus, BU seizures usually are not focal and seldom are they multiple or complicated. Because phenytoin induces the metabolism of BU, levetiracetam or another enzyme non-inducer is probably preferable anticonvulsant prophylaxis, particularly if there is no attempt to adjust the BU dose based on blood levels. Anticonvulsant prophylaxis also has generally been used with high-dose BCNU. Headache with flushing and paresthesias around the lips have been reported during high-dose BCNU infusion [72].

Neurologic manifestations of ifosfamide toxicity include seizures, acute confusional state, mutism, and disordered sensorium. Rare instances of extrapyramidal toxicities with opisthotonos have also been noted [73]. The toxicity may be related to a buildup of a chloral hydrate-like compound, chloroacetaldehyde, a major breakdown product of ifosfamide. There have been reports of treatment with methylene blue [74]. Fludarabine used now in reduced-intensity conditioning may cause encephalopathy and rarely progressive neurologic disability with CNS demyelination [75]. Seizures and transient encephalopathy have been observed with high-dose cytarabine (cytosine arabinosiude; Ara-C), although the most common neurologic problem is cerebellar dysfunction, occurring in 10% and leaving permanent ataxia from Purkinje cell dropout in 3% of treated patients [76] . There have also been a few published cases of peripheral neuropathy with high dose Ara-C, including patients with clinical and electrophysiologic features of acute demyelinating polyneuropathy consistent with the diagnosis of Guillain–Barré syndrome [77].

A sensory axonal neuropathy with mild or no motor disability developed in 4% of autologous transplant patients conditioned with high-dose etoposide (60 mg/kg intravenously for hematologic malignancy) [78]. Distal symmetrical sensory symptoms began within 2 months of the transplantation and improved slowly over months. One patient had a superimposed autonomic neuropathy and another had a peroneal neuropathy. A self-limited sensory neuropathy from etoposide conditioning also occurs in allogeneic transplant recipients. The delay in sensory symptoms for 1–2 months after etoposide is similar to the post-treatment worsening of neuropathy often seen with cisplatin and vincristine.

In high dose paclitaxel protocols for solid tumors, virtually all autologous transplant patients develop peripheral neuropathy, usually within 5 days of paclitaxel treatment [79]. Although reported as purely sensory in most affected patients, the neuropathy was sufficiently severe to require a walker in some patients.

Clinical features of cisplatin neuropathy are usually characteristic with large sensory fiber involvement, giving a proprioceptive deficit usually with preserved tactile and pin sensation and without muscle weakness [80]. By contrast, etoposide and paclitaxel neuropathies involve all sensory modalities [78,81]. Cisplatin neuropathy tends to correlate with cumulative dose rather than dose intensity. Symptoms usually occur after a cumulative dose of 300–600 mg/m^2. Also dose dependent is cisplatin ototoxicity, affecting high-frequency hearing initially and – with continued cisplatin courses – speech-frequency hearing loss, but very seldom producing vestibular toxicity [82]. In terms of cisplatin CNS toxicity, transient Lhermitte phenomenon occurs occasionally from an effect on the dorsal column of the cervical region of the spinal cord [83], and there are rare reports of cortical blindness and seizures [84].

Complications from supportive care drugs

Experienced transplant physicians tend to minimize the use of symptomatic medications early in the transplant course to avoid drug interactions, influence on the developing marrow, and untoward side effects. Extrapyramidal toxicity from antipsychotics, some antiemetic agents, and metoclopramide can interfere with rehabilitation progress after transplant.

Depression of the sensorium occurs very frequently in the first week after HCT from sedative–hypnotic drugs or analgesics. What can be especially misleading in lethargic or obtunded patients is unilateral pupillary dilatation from a scopolamine patch [85] used for drug-associated nausea. Similarly, ipratropium inhalation therapy given by mask has caused unilateral pupillary dilatation in transplant patients by inadvertent contact with the eye [86]. In these patients, pilocarpine 1% eye drops fail to produce miosis, confirming pharmacologic-induced mydriasis rather than pressure on the third cranial nerve from early transtentorial herniation. Recognition of this anticholinergic effect of scopolamine or ipratropium may prevent a needless head scan to exclude a cerebral mass lesion.

Many of the antibiotics used after transplantation have been associated with neurologic toxicity, for example, seizures from penicillin, piperacillin, metronidazole, and imipenem, encephalopathy from penicillin and metronidazole, hearing loss from aminoglycosides and vancomycin, peripheral neuropathy from metronidazole, and a myasthenia-like syndrome from aminoglycosides [87–90]. Since toxicity is often enhanced by renal insufficiency, special caution applies to those patients on calcineurin inhibitors and at risk for nephrotoxicity.

Acyclovir at high dose can cause tremor, agitation, or lethargy, occasionally with electroencephalogram epileptiform features [91]. In the setting of renal failure, reversible stupor and coma and occasionally generalized seizures have followed both oral and intravenous administration of acyclovir [92]. Delirium, which improved with dose reduction, was seen with ganciclovir treatment given at 10 mg/kg/day for CMV infection in a transplant patient [93]. Foscarnet most often causes renal and electrolyte disturbances, and low calcium and magnesium levels from foscarnet have been associated with paresthesias, muscle cramps, and rarely seizures [94].

Pre-transplantation neurologic screening

Pre-transplantation neurologic screening may identify patients particularly prone for neurologic complications, and in some cases this screening may lead to changes in treatment to minimize these complications. For example, anticonvulsant prophylaxis should be considered in transplant recipients with past seizures or a strong family history of epilepsy when these patients are treated with calcineurin inhibitors or other drugs known to lower the seizure threshold. Because of the cumulative toxicity of radiation therapy on the nervous system, it may be prudent to use preparatory regimens without fractionated total body irradiation in brain-damaged patients or in patients with degenerative CNS diseases. Because of the potential of producing disabling neuropathy, it is best to avoid high-dose etoposide or other peripheral nerve toxic conditioning agents in patients with severe diabetic neuropathy, hereditary neuropathies such as Charcot Marie Tooth, or significant pre-existing chemotherapy-associated neuropathies. Finally, it is prudent to screen donors carefully to avoid transfer of neurologic immune-mediated diseases such as multiple sclerosis.

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CHAPTER 106

Vaccination of Allogeneic and Autologous Hematopoietic Cell Recipients

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Introduction

Yearly, over 30,000 autologous and 20,000 allogeneic hematopoietic cell transplants (HCT) are performed worldwide (reviewed in [1]), resulting in increasing numbers of survivors returning to the community and traveling abroad. Following HCT, all patients are immunodeficient, the extent and duration of which are dependent primarily on patient age, stem-cell source, presence or absence of graft-versus-host disease (GVHD), the intensity of immunosuppression needed to control GVHD, and/or receipt of B-cell-depleting monoclonal antibodies, such as rituximab (reviewed in [2,3]). Unless reimmunized, all studies show that pre-transplant seroprotection wanes with time and any transferred donor immunity is temporary (reviewed in [2,4]). Infections due to pneumococcus [5], influenza [6] varicella zoster virus (VZV) [7,8], and *Bordetella pertussis* [9] can cause serious illness even in immunocompetent individuals, particularly the young and the elderly [10]. Many of these infections occur more frequently in HCT recipients, remaining a significant cause of morbidity, rehospitalization, and mortality after successful transplantation (reviewed in [2,11]).

Scope of the problem

Multiple studies have demonstrated the increased risk of invasive pneumococcal disease following allogeneic and autologous HCT (reviewed in [2,11]). A prospective study in adults over 18 years of age by Kumar et al. demonstrated a 30.2-fold increase in the incidence of invasive pneumococcal disease (IPD) in HCT recipients compared with the general adult population [12]. The yearly incidence of IPD was 11.5/100,000 non-transplanted adults, compared with 590/100,000 and 199/100,000 allogeneic and autologous HCT recipients, respectively. Mortality in this study was 12.2%, similar to that observed in a study by Youssef et al. in which 6/47 patients with IPD following HCT died [13]. Shingles and post-herpetic neuralgia (PHN) also occur more frequently in HCT recipients than in age-matched controls (reviewed in [2,11]). The yearly incidence of herpes zoster in healthy individuals ranges from 1.2 to 3.4 cases per 1000 persons, increasing to 3.9–11.8/1000 persons over 65 years of age [7]. In a 2009 report from Japan [14], the cumulative incidence of VZV infection at 2 years following an autologous or allogeneic HCT was 22 and 34%, respectively; 35% of patients who developed herpes zoster developed PHN. In a prospective study by Ljungman et al., analyzing the impact of the 2009 pandemic H1N1 virus in 288 recipients of an allogeneic ($n = 222$) or autologous ($n = 66$) HCT, age and lymphopenia ($<0.3 \times 10^9$/L) were risk factors for pneumonia, need for mechanical ventilation, and death. In allogeneic HCT recipients, transplantation from an unrelated donor or human leukocyte antigen (HLA)-mismatched donor was a risk factor for lower respiratory tract disease, presumably due to the delayed immune reconstitution observed in this patient population compared with HLA-matched sibling HCT recipients [15].

Although each of the above infections is a potentially vaccine-preventable disease, not all patients are vaccinated after HCT [16–18] and, if vaccinated, not all patients respond equally (reviewed in [2,4,11]). Infections such as those due to VZV often occur early after HCT, posing safety concerns regarding administration of a live preventative vaccine. A 2010 survey by the Pediatric Blood and Marrow Transplant Consortium found that compliance with the 2000 Centers for Disease Control and Prevention (CDC) guidelines for individual vaccines ranged from 22 to 93%, with <20% of centers consistently vaccinating patients according to the guidelines [17]. In the study by Kumar et al., only 21% of patients who developed IPD were vaccinated against pneumococcus post-HCT [12]; 100% of the pneumococcal serotypes isolated from these patients would have been covered by the 23-valent polysaccharide pneumococcal and 92% by the 13-valent protein conjugate vaccine (PCV13). Although the live attenuated shingles vaccine is approved for immunocompetent individuals over 50 years of age [7], the safety of this vaccine in HCT recipients is largely unknown, and therefore it remains contraindicated in current guidelines until adequately studied [4]. The influenza vaccine, although well studied, is unfortunately least effective in patients at highest risk for severe complications, namely patients early after HCT, those requiring intensive immunosuppression for the treatment of GHVD, and recipients of rituximab [19–21].

The 2009 influenza pandemic [22], the continued increase in pertussis [23], the rising incidence of pneumococcal disease due to antibiotic-resistant strains [24], and outbreaks of measles [25] and mumps [26] in immunocompetent individuals highlight the need for effective revaccination of HCT recipients. This chapter reviews vaccine guidelines published since 2009, the studies on which these guidelines were based, and the immunogenicity of vaccines in this patient population, and discusses areas in which additional data are needed.

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Loss of humoral immunity and vaccination guidelines

Unless revaccinated, the majority of patients will be susceptible to pertussis early after HCT (reviewed in [2,4,11,15]) and will have negative pneumococcal [27], *Haemophilus influenzae* [28], and tetanus [15] titers by 0.5–1, 1, and 1–2 years, respectively. Approximately 50% of patients will have titers against polio [28] and measles, mumps, rubella (MMR) [29] for at least 2 years after HCT, but almost all will become seronegative within 3–5 years [28,29]. The only patients who remain protected against measles for an extended period of time after HCT are those with a history of wild-type measles infection [29].

Following an autologous HCT, the time to lose immunity to vaccine-preventable diseases varies according to diagnosis, patient age, receipt of anti-CD20 monoclonal antibodies, and degree of immunity present prior to stem-cell harvest [4,30–33]. Whereas Horwitz et al. [30] showed that only 55% of patients with B-cell non-Hodgkin lymphoma (NHL) who received rituximab before and after an autologous HCT had protective tetanus titers 12–15 months post-HCT, Nørdoy et al. [32] found durable tetanus immunity in all rituximab-naïve patients evaluated 4–10 years after autologous HCT. Of 110 adult survivors of an autologous peripheral blood stem cell (PBSC) transplant performed primarily for Hodgkin lymphoma (HL) (*n* = 59) or NHL (*n* = 58), Small et al. found that at a median of 25.2 months after HCT, 90, 58, 40, 23, and 16% of patients lacked protective titers against pneumococcus, *H. influenzae*, measles, tetanus, and polio, respectively [34].

The European Group for Blood and Marrow Transplantation (EBMT) in 1995 [16] and 2005 [35] and the CDC in 2000 [36] developed standardized guidelines for the immunization of patients following HCT. These guidelines incorporated use of tetanus, diphtheria, polio, *H. influenzae*, pneumococcus, influenza, measles, mumps, and rubella vaccines. The EBMT and CDC guidelines, summarized in Table 106.1, differed in the number of doses of tetanus, polio, and *H. influenzae* recommended (two versus three) and PPV23 (one versus two) and the time to initiate revaccination (6 versus 12 months). In 2009, an international committee of infectious disease and transplant physicians met under the auspices of the Center for International Blood and Marrow Transplant Research (CIBMTR) to create an updated, unified international set of vaccine guidelines [4]. These guidelines were approved by the National Marrow Donor Program, the European Blood and Marrow Transplant Group, the American Society of Blood and Marrow Transplantation, the Canadian Blood and Marrow Transplant Group, the Infectious Diseases Society of America, the Society for Healthcare Epidemiology of America, the Association of Medical Microbiology and Infectious Diseases Canada, and the Centers for Disease Control and Prevention. In 2011, guidelines from the International Consensus Conference on Clinical Practice in Chronic GHVD were published specifically to address vaccination in this patient population [37].

Table 106.1 Summary of vaccine guidelines

Vaccine	2000 CDC	2005 EBMT	2009 CIBMTR
DTaP/Td/Tdap	2 doses each	3 doses each	3 doses each
IPV	12, 14 months	6–12 months	DTap <7 years, Tdap >7 years
HIB			6–12 months
Pneumococcus	PPV23	PPV23	13-valent
	12, 24 months	12 months	3 doses
			3–6 months
rHBV	Optional	Recommended	Country policy
MMR	24 months	24 months	24 months
Live attenuated varicella vaccine	No	Select patients	Select patients

The primary difference between the 2009 CIBMTR guidelines and those previously published was the recommendation to administer a series of three pneumococcal conjugate vaccines to all pediatric and adult transplant patients rather than primary immunization with one or two doses of the less immunogenic PPV23 [4]. In 2010, the guidelines were updated to include the newly licensed PCV13 vaccine, despite a lack of immunogenicity data in HCT recipients, owing to its broader coverage and the lack of availability of PCV7 [38]. PCV13 contains the same antigens included in PCV7 (4, 6B, 9V, 14, 18C, 19F, 23F) combined with antigens derived from serotypes 1, 3, 5, 6A, 7F, and 19A. The updated 2010 CIBMTR guidelines (Table 106.1) recommend (1) inclusion of three doses of PCV13 starting at 3–6 months post-HCT, (2) administration of PPV23 6–12 months following the third PCV13 in patients without chronic GVHD and consideration of a fourth dose of PCV13 in patients with chronic GVHD, (3) administration of the live varicella vaccine in select patient groups starting ≥24 months after HCT, and (4) the optional use of vaccines licensed since 2005 including the tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine (Tdap) [9], the recombinant human papilloma vaccine [39], and the protein-conjugated meningococcal vaccine [40].

The 2011 guidelines from the International Consensus Conference on Clinical Practice in Chronic GVHD [37] advocate immunization starting at 6 months after HCT. The guidelines recommend a potential delay of only 3 months in patients on >0.5 mg/kg prednisone or receiving an intensive three-drug regimen to treat GVHD [37]. The guidelines call for three doses of the combination vaccine DTaP-HIB-inactivated polio vaccine (IPV), PCV, hepatitis B vaccine (HBV) in children at 6, 7, and 8 months after HCT followed by a booster dose of each vaccine at 18 months after HCT. Immunization against meningococcus, hepatitis A, and the human papilloma virus are optional. MMR and the live varicella vaccine may be given at ≥24 months after HCT provided that the patient is "immunocompetent." The inactivated influenza vaccine is advised in all patients who are at least 4–6 months after HCT at the start of the influenza season.

The immunogenicity of vaccines administered according to almost all vaccine guidelines remains unknown as most of the vaccine literature has not assessed patients immunized according to published guidelines. An exception is the study by Patel et al., who vaccinated 38 pediatric transplant recipients following an autologous (n = 10), HLA-matched related (*n* = 80) or unrelated (*n* = 200) HCT according to the recommendation of the 2002 Royal College of Paediatrics and Child Health [41]. These guidelines recommend immunization of autologous and HLA-matched transplant recipients starting at 12 months after HCT and unrelated HCT recipients starting at 18 months, due to concerns about delayed immune reconstitution in the latter group. Patients received 3-monthly doses of DTaP, IPV, HIB, and PCV7. Using this schedule, >90% of children responded to polio, tetanus, *H. influenzae* type B (HIB), and measles. Over 80% of patients responded to all seven serotypes included in PCV7 (threshold for response >0.35 μg/mL). Although this study did not correlate in vitro immunologic parameters and vaccine response, the proportion of responders was higher than that observed in most pediatric studies, other than reports in which patients were immunized following acquisition of preset immune milestones, such as CD4 cell count >200 cells/μL [42,43].

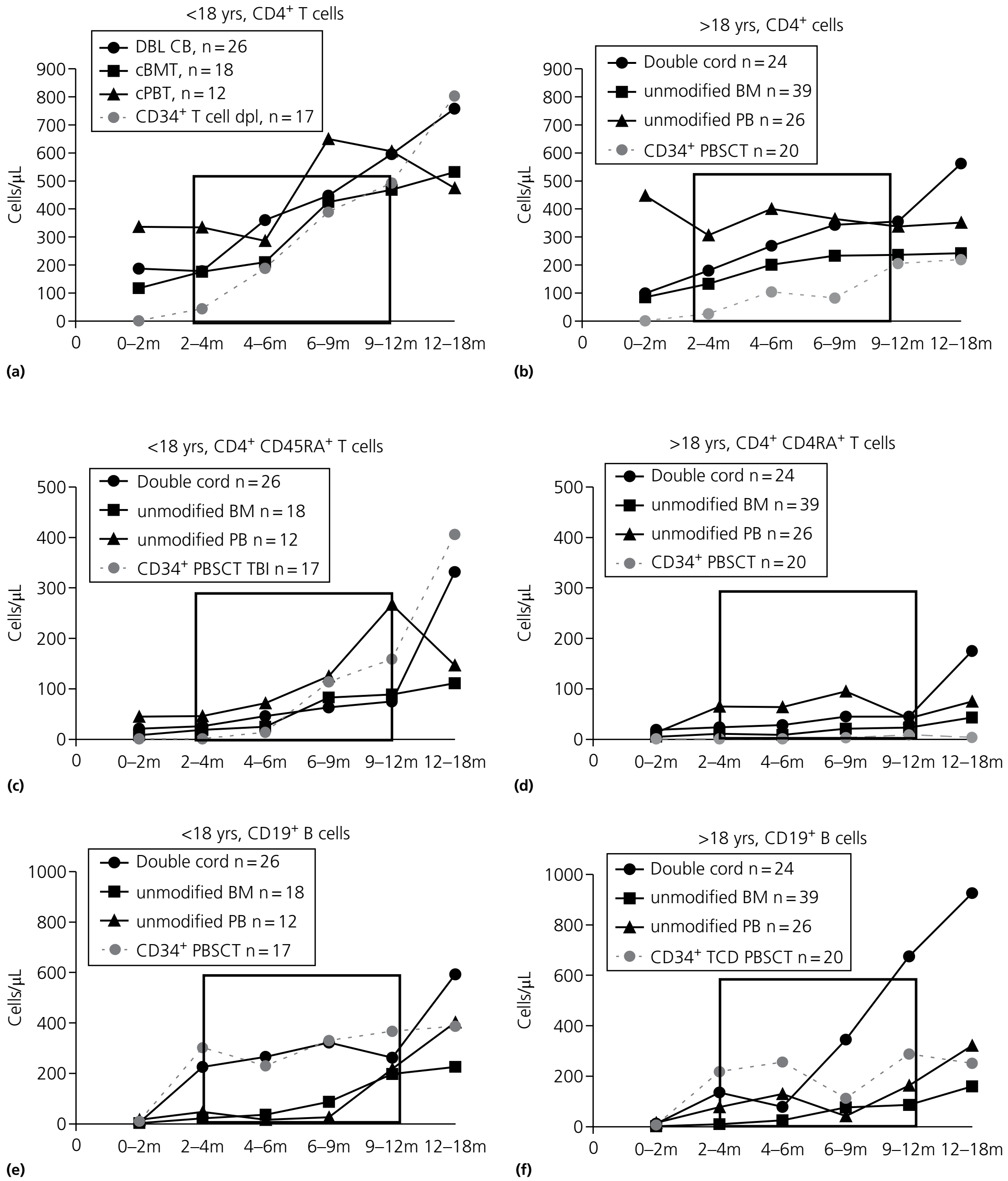

Figure 106.1 Comparison of CD4, $CD4^+$ CD45RA, and CD19 cells in patients <18 years (left) or >18 years (right) of age following double-cord, T-replete BM/PBSCT or $CD34^+$ T-cell-depleted unrelated PBSCT. Rectangle emphasize the differences in patient groups at 4–12 months after HCT, intervals during which revaccination is recommended.

There remain limited data assessing whether vaccination of all patients at fixed times after HCT is effective in patient groups whose immune recovery differs from that of an HLA-matched sibling. Figure 106.1 demonstrates the kinetics of recovery of median numbers of $CD4^+$ T cells (a, b), $CD4^+CD45RA^+$ T cells (1b, c), and $CD19^+$ cells (c, d) in patients younger or older than 18 years of age following an unrelated unmodified bone marrow or PBSC, double cord blood, or $CD34^+$ T-cell-depleted (TCD) HCT. At 3–12 months after HCT, a time when many guidelines recommend initiation of vaccines, there are clear differences in the median levels of populations required for T-cell-dependent B-cell responses. A significant number of patients will likely have absent or limiting populations of helper T cells and B cells required for vaccine response. Although vaccination based on fixed times after HCT may be sufficient for highly immunogenic vaccines, such as tetanus, polio, and the *H. influenzae* conjugate vaccine (reviewed in [12,13]), some studies suggest that response to recombinant hepatitis B vaccine (rHBV) and the pneumococcal conjugate vaccines require critical levels of T- and B-cell subsets [42–44]. For example, response to pneumococcal conjugate [43,44] and influenza vaccines [45] has been correlated with higher levels of naïve $CD4^+CD45RA^+$ and activated $CD4^+CD154^+$ T cells, respectively. Impaired B-cell responses are related to deficits in $CD27^+$ memory B cells capable of isotype switching. This subset can take years to recover after HCT [46,47], particularly in patients with chronic GVHD [48] and recipients of rituximab [3]. Roll et al. showed a significant correlation between levels of naïve $CD27^-$ B cells and response to the H1N1 vaccine [20]. Following PCV13, one study showed impaired response in patients lacking memory CD27$^+$ B cells capable of isotype switching [44].

Immunogenicity of vaccines after HCT

Current vaccine guidelines recommend three doses of tetanus/diphtheria toxoid, IPV, and the protein-conjugated *H. influenzae* and pneumococcal vaccines due to multiple studies demonstrating a higher proportion of responders following three doses of each vaccine compared with one or two doses (reviewed in [4]). Although patients who receive transplants from donors immunized against tetanus, diphtheria, and *H. influenzae* prior to stem-cell harvest develop protective antibody titers earlier after HCT than patients whose donors were not immunized prior to bone marrow (BM) harvest [49,50], this approach is only feasible in recipients of a related HCT. Donor immunization with PCV7, but not PPV23, has been shown to enhance recipient response to PPV23 after HCT [51].

Following three doses of tetanus, diphtheria, polio, and/or HIB, at least 50% of patients will seroconvert or mount a 3–4-fold rise in titer depending on when immunization is started (reviewed in [2,4]). Differences in the proportion of responders in published papers generally reflects differences in the age of the immunized patients, the proportion of patients requiring treatment for moderate to severe GVHD, the vaccine schedule utilized, and the threshold for response. For example, in a study by Parkkali et al. [52], over 95% of HLA-matched sibling bone marrow transplant (BM)T recipients immunized with IPV at 3, 6, and 12 months responded to all three polio serotypes (seroconversion or >4-fold response) compared with only a 50% response in the study by Ljungman et al. in 29 HLA-matched sibling bone marrow transplant recipients immunized at 12, 13, and 14 months [53]. In a study by Jaffe et al., 96% of 219 patients following an HLA-matched related ($n = 162$), HLA-mismatched ($n = 24$), or unrelated ($n = 69$) HCT developed a threefold or greater rise in titer against all three polio serotypes after receipt of three IPV vaccinations given at 2-month intervals [42]. Younger age at transplantation ($p = 0.01$) was the only factor associated with improved response to IPV vaccination [42].

Pertussis

In the last decade, pertussis has risen steadily in adolescents and adults, with an estimated incidence of 800,000 to 3.3 million cases/year (reviewed in [9]). In an effort to decrease the growing number of individuals with this disease, in 2005 two vaccines containing acellular pertussis were licensed for use in adolescents and adults for the first time in the United States [9]. The two vaccines (Tdap) contain similar amounts of tetanus and diphtheria toxoid, but differ in the amount of pertussis toxoid (PT). Adacel (Sanofi Pasteur, Toronto, Canada) contains 2.5 μg/dose PT compared with 8 μg / dose in Boostrix (GlaxoSmithKline Biologicals, Rixensart, Belgium) [9]. In 2006, the Advisory Committee on Immunization Practices (ACIP) recommended that all adolescents (aged 11–18 years) and adults (aged 19–64 years) receive a single dose of Tdap to replace their scheduled Td booster [9].

Following a single vaccine, autologous and allogeneic HCT recipients respond poorly to PT, despite an adequate response to tetanus and diphtheria toxoid. Of 41 adults and children immunized with one vaccine, only seven of 41 patients responded to PT, six of whom were <18 years of age when immunized [54]. Following an autologous HCT, two of 28 adult patient responded to a single Tdap [55]. This limited pertussis response is likely due to the small amount of PT contained in either vaccine [2.5 vs. 8 μg/dose) compared with 25 μg /dose in DTap, the vaccine used for primary immunization of infants and children under 7 years of age [9]. In view of this poor response, the 2009 CIBMTR guidelines recommend three doses of the 8 μg/dose Tdap in all HCT recipients, other than latex-allergic patients, who should receive Adacel [4]. To date, 99 patients, median age 49 years, have received three immunizations with Tdap containing 8 μg PT/dose at the Memorial Sloan-Kettering Cancer Center. Stem cells were derived from an HLA-matched related ($n = 36$) or alternative ($n = 63$) donor. There were no significant site or other adverse reactions. Overall response was 56%, including 11 of 13 patients <21 years and 46 of 89 patients >21 years of age ($p = 0.02$). In view of the morbidity and mortality of pertussis [18], recent data from California suggesting that vaccine efficacy decreases even in healthy children following the fifth dose of DTaP [56], and approval of Tdap in all individuals aged 7 years or older [57,58], it seems prudent to administer three doses of an age-appropriate pertussis vaccine, followed by a booster 6–12 months later to all HCT recipients, and to consider measuring pre- and post-vaccine titers to document response, particularly in adults caring for infants or patients with infant siblings.

Haemophilus influenzae and pneumococcal vaccines

Multiple studies have shown the poor response of HCT recipients to T-cell-independent B-cell antigens (reviewed in [2,4,11]). The polysaccharide pneumococcal vaccine PPV23 is poorly immunogenic in HCT recipients, particularly when given <1 year after HCT or at any time to patients with chronic GVHD (reviewed in [2,4,11,59,60]). Prospective studies demonstrating responses of 60–74% in patients given three doses of PCV7 following an autologous [61] or allogeneic HCT [51,63] are the basis for their recommended use in the 2009 CIBMTR guidelines [4]. These guidelines were the first to recommend a pneumococcal conjugate vaccine for any adult population. In 2011, the US Food and Drug Administration (FDA) approved a single dose of PCV13 for the prevention of pneumonia and IPD in adults over 50 years of age [64]. In 2012, a single dose of PCV13 was approved for patients over 19 years of age with an immunocompromising condition [65]. Whether a single dose of this vaccine will provide durable immunity in the latter populations needs to be evaluated. Three doses of PCV13 and consideration of a fourth dose in patients with chronic GVHD are currently recommended following HCT [4].

The best response to PCV7 (74%) was noted in a study by Meisel et al., which evaluated patients under 16 years of age [62]. It should be noted that in this study all patients were immunized at 6–9 months after HCT. Patients on immunosuppressive therapy (IST) at the time of immunization had a poorer response than those in whom IST was no longer required (42.9% vs. 80.6%, $p = 0.06$) [63]. In a retrospective study by Pao et al. [43], response was also superior in children compared with adults (88% vs. 45%, $p < 0.001$), although these children were not on IST. Although the PCV7 response in this study was adversely affected by older age ($p < 0.001$), individuals over 50 years old responded significantly better if vaccinated following acquisition of a circulating CD4 cell count ≥200 cells/μL and a PHA T-cell proliferative response of >75% lower limit of normal (11 of 19 vs. 0 of 8, $p < 0.006$). A similar trend was observed in patients with limited chronic GVHD. In all patients, higher levels of circulating CD4$^+$CD45RA$^+$ cells correlated with improved PCV7 response [43]. Meerveld-Eggink et al. [66] assessed PCV7 response in 26 patients (median age 56.5 years, range 44–67 years) following reduced-intensity conditioning. Patients were eligible for immunization if they were >12 months post-HCT and off IST. Owing to persistent GVHD, only 35% met the vaccination criteria at 12 months. The median time to first vaccination was 15 months, range 12–36 months. Following two doses of PCV7 administered 6 weeks apart, 73 and 62% of patients achieved a titer of 0.35 μg/mL or >1 μg/mL, respectively.

The current CIBMTR guidelines recommend three doses of PCV13 starting at 3–6 months after HCT. This is based on results of a multicenter randomized, prospective trial by Cordonnier et al. testing non-inferiority of PCV7 response initiated early (3 months) or late (9 months) after HCT [67]. Patients aged 5–65 years were randomized to three monthly doses of PCV7 starting at 3 ($n = 75$) or 9 ($n = 83$) months after HCT, followed 8 months later by PPV23. All patients received myeloablative conditioning and most received an HLA-matched related transplant. Grade II–III GVHD was seen in 22 and 18% of patients randomized to early or late vaccination, respectively. No GVHD, limited GVHD, or extensive chronic GVHD was seen in 57, 35, and 8% of patients vaccinated early and in and 55, 31, and 13% of patients immunized later. When a threshold titer >0.15 μg/mL against all seven vaccine strains measured 1 month after the third PCV7 (primary end-point) was utilized, the percentage of responders was similar in both groups (79% vs. 82%, $p = 0.64$). Ad hoc analysis using a threshold of 0.5 μg/mL resulted in a similar but lower proportion of responders in the early and late vaccine groups (56% vs. 54%, $p = 0.84$). The percentage of patients who retained a titer of >0.15 μg/mL against all seven serotypes 24 months after HCT was significantly lower in patients who started vaccination at 3 months compared with 9 months after HCT (59% vs. 83%, $p = 0.013$]. At 24 months after vaccination, 34% vs. 55% of patients retained a titer of >0.5 μg/mL ($p = 0.54$). Factors associated with poor response were presence of chronic GVHD and older donor age. One month following immunization with PPV23, 80% vs. 87% of patients responded to PPV23 specific serotypes 1 and 5 (threshold response: 0.15 μg/mL) compared with 42% vs. 60% when a threshold response of 0.5 μg/mL was considered positive ($p = 0.08$) [68]. In a subset of patients ($n = 28$), functional antibody measured by an opsonophagocytic assay was compared with immunoglobulin G (IgG) levels measured by enzyme-linked immunosorbent assay (ELISA). The study showed a significant correlation between the two assays, demonstrating that the conjugate vaccine induced functional antibody [69].

Until results of the recently completed multicenter international trial of PCV3 followed by PPV23 in allogeneic HCT recipients is published, there are limited data on the immunogenicity of this vaccine following HCT. A report of 59 patients (median age 39 years) who received three doses of PCV13 administered 1–3 months apart was presented at the American Society of Hematology Annual Meeting in December 2011 [44]. Patients in this study were immunized upon acquisition of minimal milestones of immune competence (CD4 cell count >200 cells/μL and IgG level >500 mg/dL at least 6 weeks off γ-globulin replacement therapy). The median time to vaccination was 452 days (range 143–1187 days) after HCT. Time to vaccination was significantly shorter in patients <21 versus >21 years of age (median 318 vs. 498 days, $p = 0.006$) and significantly longer in patients who received rituximab after HCT (median 610 vs. 405 days, $p = <0.001$). Response was defined as seroconversion (>1 μg/mL) or a threefold rise in titer against serotypes 1, 3, 4, 14, 19, 23, 26, 51, and 56. The overall response was 73%. The response was 87% in patients younger than 21 years compared with 67% in older patients. Poorer responses were observed in recipients of rituximab after HCT despite a median CD19 cell count of 342/μL at the time of vaccination. Response following PCV13 correlated with higher levels of circulating $CD4^{+}CD45RA^{+}$ T cells/ul ($p = 0.03$) and $CD27^{+}$, IgM^{-} (memory isotype switched) B cells/μL ($p = 0.03$). In this limited sample, PCV13 was found to be very immunogenic when given upon acquisition of preset immune milestones. The results of the international PCV13 trial will determine whether immunization at earlier times after HCT will yield similar results.

Meningococcus

Despite rapid and appropriate treatment for meningococcal disease, 10–14% of infected patients die and up to 20% of survivors experience significant morbidity, including vasculitis, suppurative arthritis, myocarditis, and deafness [70]. In 2005 [71], the first protein-conjugated tetravalent meningococcal vaccine was licensed in the United States, followed by a second vaccine in 2010 [72]. Both vaccines contain antigens derived from meningococcal serogroups A, C, Y, and W-135. Parkkali et al. demonstrated that although allogeneic HCT recipients including those with GVHD are capable of responding to the meningococcal polysaccharide vaccine, a rapid decrease in antibody titers was observed 6 months after vaccination [73]. Of 46 pediatric patients immunized with the tetravalent meningococcal conjugate vaccine following an HLA-matched sibling ($n = 17$) or an alternative donor ($n = 29$), only 15% responded to all four serogroups and 35% responded to none [74]. Although receipt of a T-cell-depleted graft was associated with a poorer response ($p = 0.044$), response was not affected by patient age, diagnosis, time to vaccination, or prior history of GVHD [75]. Eight of 14 patients who did not respond to the first vaccine had a complete response to a second dose. These data suggest that the response to MCV4 is suboptimal in HCT recipients and that pre- and post-vaccine titers, particularly in patients entering college or traveling to endemic areas, should be assessed to ensure adequate protection. Administration of a two-dose series, as currently recommended for patients with asplenia, complement deficiency, and HIV infection, should be considered in this patient population [75].

Influenza

Influenza, particularly if acquired early after HCT, can be associated with significant morbidity and mortality (reviewed in [76]). Although immunization with the inactivated influenza vaccine has been shown by Machado et al. to decrease the risk of influenza when administered >6 months after HCT (two of 19 vaccinated compared with 12 of 24 unvaccinated) [77], the majority of serious infections occur earlier after HCT when vaccine response is poor (reviewed in [76]). Engelhard et al. showed that none of the 10 patients immunized <6 months after an autologous or T-cell-depleted allogeneic HCT developed protective hemagglutination inhibition (HI) titers (>1:40) compared with nine of 14 patients immunized >2 years after HCT [78]. Receipt of a second immunization 4 weeks later did not significantly increase the proportion of responders.

Several studies have evaluated the response of HCT recipients following immunization with the 2009 pandemic influenza A/H1N1 vaccine. Gouérin-El Khourouju et al. compared T- and B-cell responses between 14 vaccinated and 14 unvaccinated pediatric HCT recipients; 10 of 15 vaccinated patients received an HLA-matched sibling HCT. The median time to vaccination was 5.7 months after HCT. Immunized children had higher T-cell proliferative responses, higher antibody levels, and a predominance of

IL-2, rather than γ-interferon intracellular cytokine production [79]. Of 82 allogeneic HCT recipients immunized with the H1N1 vaccine at a median of 19 months after HCT [80], 51% had seroprotective titers (≥1:40 hemagglutination level) against the H1N1 antigens. Seroconversion could not be measured in this study owing to a lack of pre-vaccine titers. Chronic GHVD and type of conditioning did not affect whether titers ≥1:40 were detected after vaccination. Seroprotection was affected by the interval between immunization and HCT and prior rituximab treatment. Only one of 11 patients who received rituximab the year before vaccination had seroprotective levels following immunization [80]. Gueller et al. evaluated seroconversion in 14 patients immunized with an AS03-adjuvanted H1N1 vaccine at a median of 19.7 months (range 4.7–49.3 months) following an allogeneic ($n = 14$) or autologous ($n = 3$) HCT [81]. The rate of seroconversion was 41.2% after the first vaccine, and notably 81.8% in 11 patients who received a second dose. Engelhard et al. evaluated antibody response following an ASO3-adjuvanted A/California/7/2009 (H1N1) virus vaccine in 55 allogeneic and 23 autologous HCT recipients [82]. The median patient age was 50 years. The median time to immunization was 27 months (range 1–290 months). Approximately 50% of allogeneic transplant recipients received a reduced-intensity conditioning regimen. Prior to immunization, 17.9% had protective titers HI titers ≥1:40. Logistic regression analysis demonstrated that development of HI titers ≥1:40 was significantly associated with higher lymphocyte counts and higher baseline titers. Although responses were observed, patients at high risk of complications from influenza had the poorest response.

Owing to the variable response to the seasonal and pandemic influenza strains, current vaccine guidelines recommend yearly inactivated influenza vaccination in patients >6 months after HCT, strongly advocate immunization of household members and caretakers, and stress prompt evaluation of patients with respiratory symptoms to allow timely administration of anti-viral therapy in polymerase chain reaction (PCR)-positive patients [11].

Hepatitis B

Current ACIP guidelines recommend universal rHBV immunization for all individuals less than 18 years of age [83]. Proof of vaccination is often mandatory for school entry and certain jobs. Machado et al. [84] reported a 100% seroconversion rate in 50 HCT recipients immunized >1 year after transplant. Despite this initial response, 60% of patients became seronegative 1 year after immunization. Jaffe et al. evaluated the rate of seroconversion in 267 seronegative patients immunized with three doses of rHBV, vaccinated on the basis of preset immune milestones (circulating CD4 cell count ≥200 cells/μL, IgG level ≥500 mg/dL and PHA response≥75% of the lower limit of normal) [42]; 65% of patients received an HLA-matched related HCT, 9% an HLA-mismatched related HCT, and 25% an unrelated donor HCT. A total of 64% of patients seroconverted [58], including 73% of children and 59% of adults ($p = 0.02$). In multivariate analyses, seroconversion was adversely affected by age >18 years ($p < 0.01$) and prior chronic GVHD ($p < 0.000$), but not by receipt of a T-cell-depleted or alternative donor graft [42]. Serial titers were evaluated in 98 patients, 80 of whom remained immune for more than 5 years following immunization. The higher percentage of patients with sustained immunity in this study compared with the study of Machado et al. [84] suggests qualitative and/or quantitative differences in the circulating memory T and/or B cells present at the time of vaccination.

Hepatitis A

Current ACIP guidelines recommend universal routine immunization of all children 12–23 months against hepatitis A virus (HAV) (reviewed in [85]). Hepatitis A vaccination is also recommended for persons who are at increased risk for infection, such as healthcare workers, for persons who are at increased risk for complications from hepatitis A, and recently for household and close personal contacts of children adopted from countries with high or intermediate rates of hepatitis A [85]. Only three studies have evaluated the prevalence and persistence of hepatitis A titers before and after HCT, respectively, and there are no published reports on the immunogenicity of the HAV vaccination post-HCT. Dignani et al. evaluated HAV titers in 36 adult patients, 88% of whom received an autologous HCT. At a median follow-up of 12 months, 14% of seropositive patients became seronegative [86]. In 71 adult patients, median age 26 years, 91.6% of whom received an autologous HCT, loss of seroprotection was reported in 11.2% [87]. Unal Ince et al. studied HAV titers in 81 pediatric recipients of an allogeneic HCT performed in a single institution from 1997 to 2006 [88]. Of the 49 patients with documented hepatitis A serology prior to HCT, 23 were seropositive. Ten of 23 patients became seronegative at a median of 12 months after HCT (range 12–32 months). Loss of protective antibody was higher in recipients of grafts from HAV-seronegative donors (75% vs. 26%). Of the 39 HAV-seronegative patients immunized at the Memorial Sloan-Kettering Cancer Center, seroconversion was observed in 64% of patients given two vaccines, including 10 of 18 (55%) patients transplanted from an unrelated or HLA-mismatched donor (T.N. Small, unpublished data).

Measles, mumps, rubella, varicella zoster (VZ)

Despite recent outbreaks of mumps and measles [25,26], there are limited data on the immunogenicity and durability of response following immunization with MMR, particularly in recipients of a TCD or alternative donor HCT or any adult HCT population (reviewed in [89]). Most studies have been performed in children and young adults at least 24 months following an autologous or allogeneic HCT. The majority of vaccinated allogeneic transplant recipients had no evidence of GVHD and were not on IST. In these patient populations, at least 60% of patients seroconverted to measles and/or mumps (reviewed in [90]). During a large outbreak of measles in Brazil, Machado et al. evaluated the response of 51 patients following an autologous ($n = 10$) or allogeneic ($n = 41$) HCT vaccinated earlier post-HCT, i.e. at 9–18 months [90]. Approximately half of the patients in this study were actually on IST, the majority due to extensive chronic GVHD. Despite this, no patient had a serious adverse event. Seven of nine patients with negative titers (<100 IU/mL) seroconverted and the majority of patients with pre-existing titers mounted a threefold response.

Varicella/herpes zoster

To date, four studies have evaluated the live attenuated varicella vaccine (LAVV) following an allogeneic or autologous transplantation (reviewed in [89]). These studies include a total of 34 autologous and 96 allogeneic HCT recipients all under the age 20 years [89]. The vaccine was immunogenic in at least 50% of patients and appears to be safe when given according to preset levels of immune competence, generally defined as a circulating CD4 cell count of >200 cells/μL and response to at least one non-live vaccine administered after HCT. In the two largest series reported to date, response following the LAVV was 55 and 64%, respectively, with additional patients seroconverting following a second dose [91,92]. As two doses of the LAVV are currently recommended for healthy VZV-seronegative individuals, studies prospectively assessing the immunogenicity of a two-dose schedule in eligible HCT are needed [93].

The LAVV Zostavax [7] is currently approved for the prevention of herpes zoster in VZ-*seropositive immunocompetent* individuals over 50 years of age. This vaccine should not be confused with Varivax, the live vaccine approved to prevent primary varicella in VZ-*seronegative* individuals (reviewed in [89]). Zostavax contains approximately 14 times more plaque-forming units (reviewed in [7]) than Varivax, and is currently not approved in immunocompromised patients. Since 2007, the University of California at San Francisco has immunized primarily autologous HCT recipients with Zostavax, at the discretion of the treating physician, in patients felt to be immunocompetent and in whom the potential benefit was felt to outweigh potential risks [94]. Naidus et al. retrospectively assessed this experience in 31 patients following an autologous ($n = 26$), HLA-matched related ($n = 3$), or unrelated ($n = 2$) HCT [94]. The median patient age at vaccination was 55 years (range 31–80 years) and the median time from HCT to vaccination was 482 days in autologous HCT recipients and 1323 days in the five allogeneic HCT recipients. At a median follow-up of 268.5 days, only one patient had developed shingles, which was limited. This occurred in a patient with multiple myeloma following an autologous HCT [94]. There is a great need for a prospective study assessing this vaccine following autologous and allogeneic transplantation. A report of a 76-year-old woman receiving chemotherapy for recurrent breast cancer who developed disseminated varicella following Zostavax administration emphasizes the risk of this vaccine in patients whose immune system is compromised [95]. Until studied in the context of a trial, Zostavax is currently contraindicated in the 2009 CIBMTR vaccine guidelines [4]. Hata et al. evaluated the use of a heat-inactivated varicella zoster vaccine in patients following autologous HCT [96] in a randomized controlled trial which showed that 13% of patients who received the heat-inactivated vaccine developed herpes zoster within 12 months of HCT compared with 33% in the control group. Although inactivated vaccines to prevent herpes zoster are currently undergoing testing in HCT recipients, they are not commercially available.

Other live vaccines

Although additional live attenuated vaccines are licensed for use in immunocompetent individuals, most are contraindicated in HCT recipients. These vaccines include the live attenuated intranasal influenza vaccine, the live attenuated rotavirus vaccine, and the yellow fever vaccine (reviewed in [89]).

The live attenuated intranasal influenza vaccine has been evaluated in pediatric patients with asymptomatic or mildly symptomatic HIV infection and in oncology patients under 21 years of age (reviewed in [89,97]). Although the vaccine was well tolerated and did not result in prolonged viral shedding, antibody responses following this vaccine were inferior to those observed following the inactivated trivalent influenza vaccine and remain contraindicated given the availability of the inactivated alternative.

The live rotavirus vaccine is contraindicated post-HCT owing to a lack of safety information and documented cases of rotavirus infection following administration to infants with severe combined immunodeficiency have been published [11,98].

The yellow fever vaccine can cause life-threatening complications, even in healthy individuals [99]. There are, however, case reports of successful vaccination of HCT patients who were >2 years following HCT, off IST, without GVHD (reviewed in [89]). Nevertheless, owing to the limited safety data, this vaccine is currently contraindicated in all patients <24 months post-HCT and in those with chronic GVHD or on IST at any time post-HCT. For patients living in or traveling to endemic areas who do not have these risk factors, the risk–benefit ratio in individual patients is advised [4].

Administration of live vaccines and isolation of household contacts given live vaccines

Immunocompetent family members and household contacts of HCT recipients *should not* receive the oral polio virus, smallpox vaccine, or live influenza vaccine owing to concerns regarding transmission of vaccine virus to an immunocompromised host. The 2009 CIBMTR guidelines recommend that MMR be given to household contacts who are more than 12 months old and are not pregnant or immunocompromised as no evidence exists that live attenuated vaccine strain viruses in MMR vaccine are transmitted from person to person [11]. The guidelines also recommend that Varivax be given to susceptible, eligible household contacts owing to the greater risk and morbidity from acquisition of wild-type varicella from a VZ-seronegative family member or household contact compared with transmission and complications from the attenuated vaccine strain [11]. If a post-vaccination rash develops within 42 days of vaccination, the vaccinee should avoid contact with HCT recipients until the rash has totally crusted over.

Conclusion

All vaccine guidelines, including those published recently, acknowledge in their introduction the variable kinetics of immune reconstitution of patients undergoing transplantation today. Although

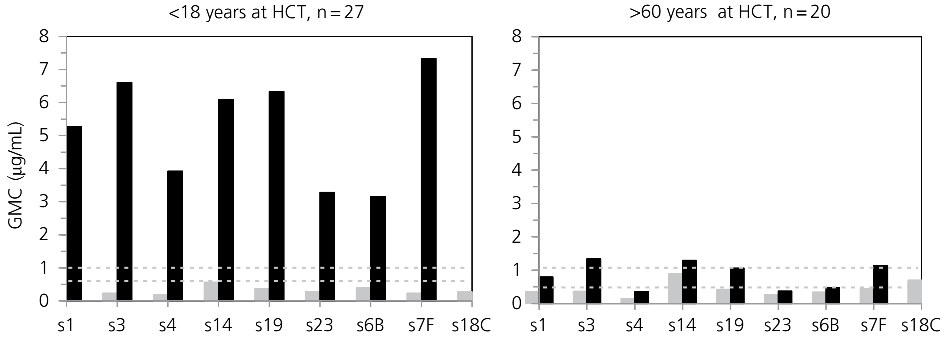

Figure 106.2 Geometric mean antibody concentration (GMC) in patients <18 or >50 years of age before (gray boxes) and after (black boxes) vaccination with three doses of PCV13.

immunization of patients using a standard timetable without documentation of pre- and post-vaccine titers is a less complicated and more cost-effective intervention, the protection afforded by this strategy may be suboptimal for patients who historically have responded poorly to vaccination, and ultimately more costly. Studies reported in this chapter have shown the effect of age on vaccine response, particularly following the pneumococcal conjugate vaccine, rHBV, measles, and mumps. The number of transplants performed in patients over the age of 60 years reported to the CIBMTR continues to increase [100]. Even immunocompetent individuals of this age respond poorly to many vaccines. Figure 106.2 demonstrates the difference in the magnitude of responses following three doses of PCV13 in 47 patients, 27 below the age of 18 years, compared with the response of 20 patients above the age of 60 years. Although responses were seen in both groups, the magnitude of response was different. The duration of seroprotection in these two populations will likely differ and studies addressing this are needed, as are prospective, multicenter trials testing current guidelines in groups who historically are poor responders, such as patients on IST, and those underrepresented in the vaccine literature, such as cord blood HCT recipients. The latter population may respond significantly differently than other populations owing to the complete lack of transfer of any donor memory cells. Ideally, future trials will include in vitro studies of immune reconstitution in order to identify surrogate markers of vaccine success or failure. In vitro correlates of vaccine responses might allow earlier revaccination of patients with the requisite T- and B-cell populations and prevent premature vaccination and/or risk in patients unable to respond. For patient groups with poor response, additional trials assessing vaccine adjuvants should be undertaken [101].

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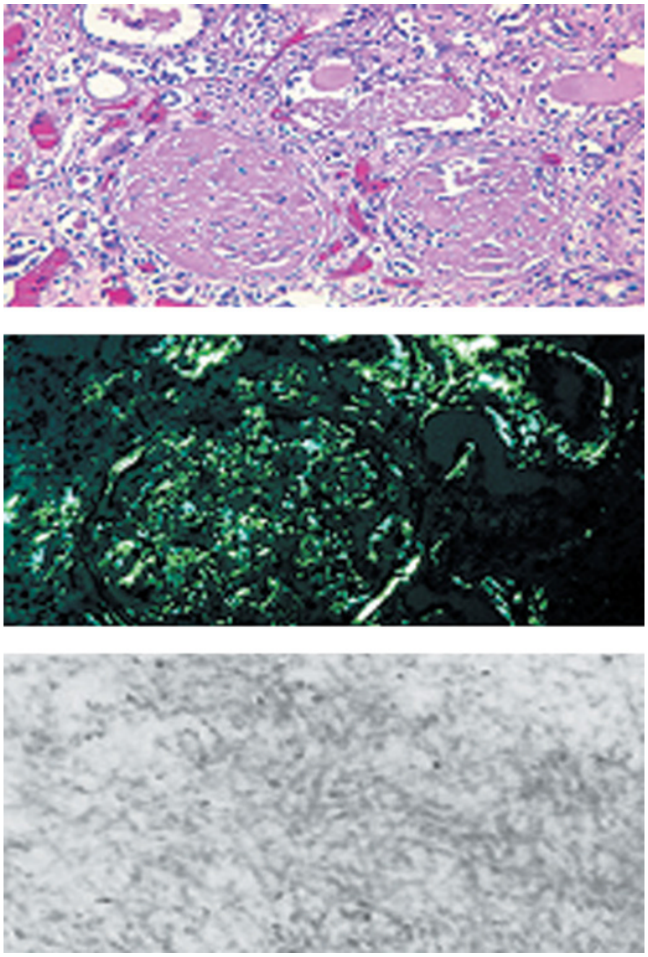

Figure 60.1 Renal biopsy showing amyloidosis. These images show three views of a renal biopsy: with periodic acid–Schiff (PAS) staining showing amorphous material in glomeruli (top), Congo Red staining showing apple-green birefringence in polarized light (middle), and electron microscopy showing non-branched linear fibrils about 10 nm in length.

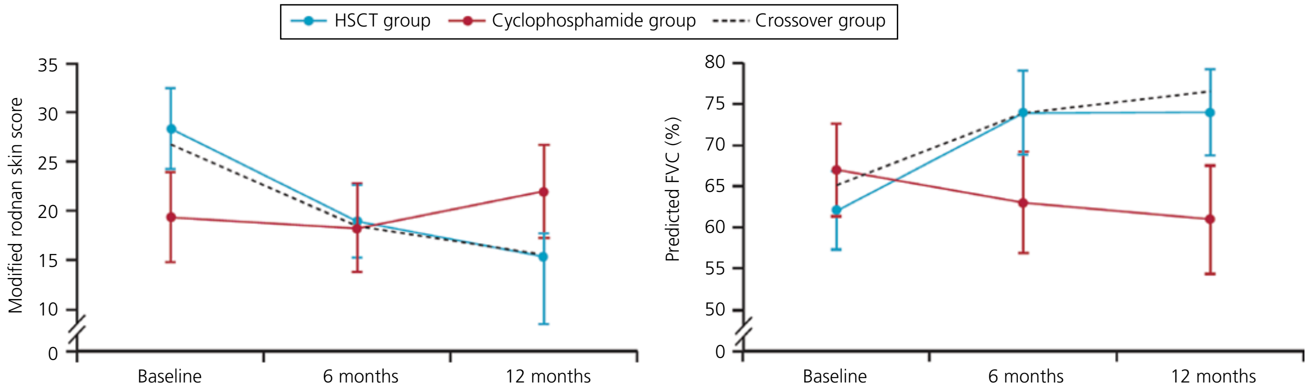

Figure 66.6 Improvement in skin score and lung function after HDIT and autologous HCT in comparison with conventional immunosuppressive therapy. All 10 patients randomly allocated to receive HSCT improved at or before 12 months' follow-up, compared with none of nine allocated to cyclophosphamide (odds ratio 110, 95% CI 14.04–∞; $p = 0.00001$). Eight of nine controls had disease progression (without interval improvement) compared with no patients treated by HSCT ($p = 0.001$), and seven patients switched to HSCT. Compared with baseline, data for 11 patients with follow-up to 2 years after HSCT suggested that improvements in mRSS ($p < 0.001$) and forced vital capacity ($p < 0.3$) persisted. Source: Burt et al. 2011 [81]. Reproduced with permission of Elsevier.

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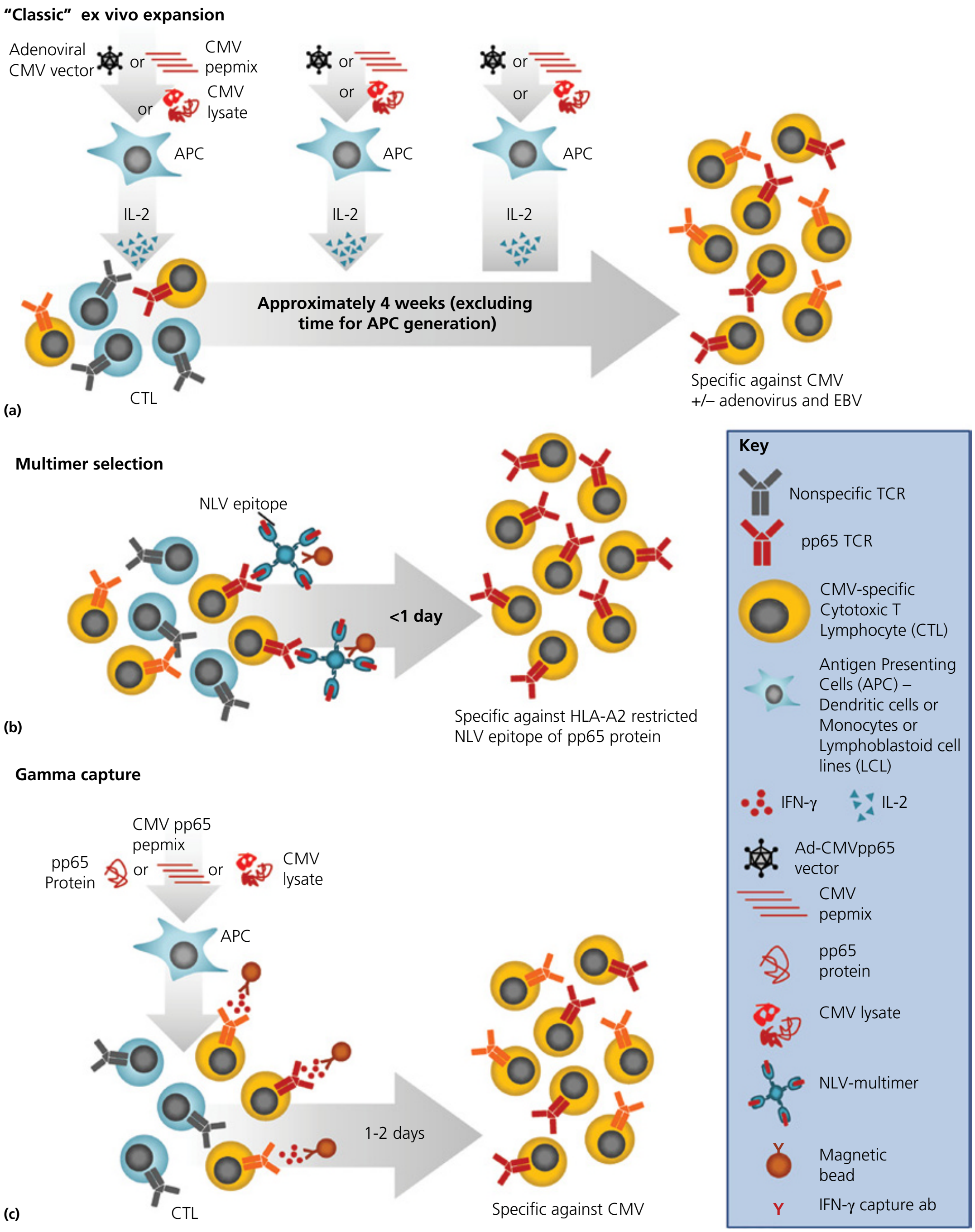

"Classic" ex vivo expansion
Adenoviral CMV vector
or
CMV pepmix
or
CMV lysate
APC
IL-2
CTL
Approximately 4 weeks (excluding time for APC generation)
Specific against CMV +/– adenovirus and EBV
(a)
Multimer selection
NLV epitope
<1 day
Specific against HLA-A2 restricted NLV epitope of pp65 protein
(b)
Gamma capture
CMV pp65 pepmix
pp65 Protein
CMV lysate
APC
CTL
1-2 days
Specific against CMV
(c)
Key
Nonspecific TCR
pp65 TCR
CMV-specific Cytotoxic T Lymphocyte (CTL)
Antigen Presenting Cells (APC) – Dendritic cells or Monocytes or Lymphoblastoid cell lines (LCL)
IFN-γ
IL-2
Ad-CMVpp65 vector
CMV pepmix
pp65 protein
CMV lysate
NLV-multimer
Magnetic bead
IFN-γ capture ab

Figure 68.1 GMP compliant approaches for the generation of CMV-specific T cells. (a) "Classic" ex vivo expansion method of CMV-CTL manufacture where antigen-presenting cells (APCs) are prepared using either dendritic cells or monocytes or EBV-transformed B lymphoblastoid cell lines (LCLs). The APCs are then either pulsed with CMV peptides or protein or infected with CMV lysate or transduced with an adenoviral vector expressing the CMV pp65 transgene. The APCs are then irradiated and used to stimulate peripheral blood mononuclear cells (PBMCs) from the same donor. Approximately 9–12 days later, and weekly thereafter, the T cells are restimulated with the APCs usually in the presence of IL-2. At the end of the CTL manufacturing process (approximately 4 weeks), the CTLs are frozen and released. Functional testing is performed prior to infusion into the patient. (b) This shows how CMV-specific T cells can be selected using multimer selection. In the example shown, T cells recognizing the HLA A2-restricted CMV pp65 epitope NLV are selected directly from the donor PBMCs by first incubating with the HLA–peptide tetramer and then selecting the tetramer-positive T cells with magnetic beads. This process generally takes less then 1 day of processing. (c) This shows an alternative method of rapidly generating a CMV-specific T cell line using the gamma capture technology. Donor PBMCs are stimulated with either recombinant CMV protein or CMV lysate or GMP grade-selected HLA-restricted CMV peptides or overlapping peptides spanning entire CMV antigens such as pp65. After overnight stimulation, IFN-γ-secreting cells are selected using a Cytokine Secretion System and a CliniMACS immunomagnetic separation device (both from Miltenyi Biotech) which "capture" the IFN-γ-secreting CMV-specific T cells. CMV-specific T cells selected using this methodology can either be infused immediately after the isolation procedure or cryopreserved for future use. The manufacturing time for this procedure is 1–2 days.

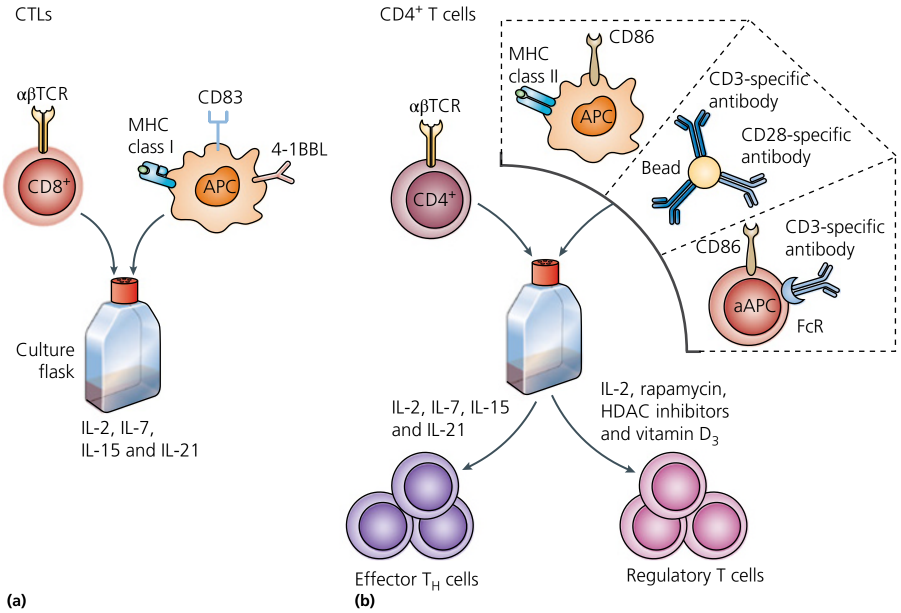

CTLs
αβTCR
CD8$^+$
CD83
MHC class I
APC
4-1BBL
Culture flask
IL-2, IL-7, IL-15 and IL-21
(a)
CD4$^+$ T cells
αβTCR
CD4$^+$
MHC class II
CD86
APC
CD3-specific antibody
Bead
CD28-specific antibody
CD86
CD3-specific antibody
aAPC
FcR
IL-2, IL-7, IL-15 and IL-21
IL-2, rapamycin, HDAC inhibitors and vitamin D$_3$
Effector T$_H$ cells
Regulatory T cells
(b)

Figure 69.1 Cell culture approaches for adoptive transfer of lymphocyte subsets. Culture conditions required for distinct lymphocyte subsets vary, depending on the activation and costimulatory requirements. CTLs express αβ TCRs and are stimulated by APCs that express MHC class I. CTLs require 4-1BB stimulation for expansion, and have been expanded by the addition of feeder cells or artificial APCs (aAPCs) that express the costimulatory ligands. CD4 T cells are stimulated by APCs that express peptide-loaded MHC class II. The dominant costimulatory molecule for CD4 cells is CD28. Effector CD4 cells can be stimulated by beads or cell-based aAPCs in the presence of various cytokines. Regulatory CD4 T cells (Treg cells) require culture in IL-2, and the addition of number of reagents to the culture may enhance the suppressive functions of ex vivo expanded Treg cells.

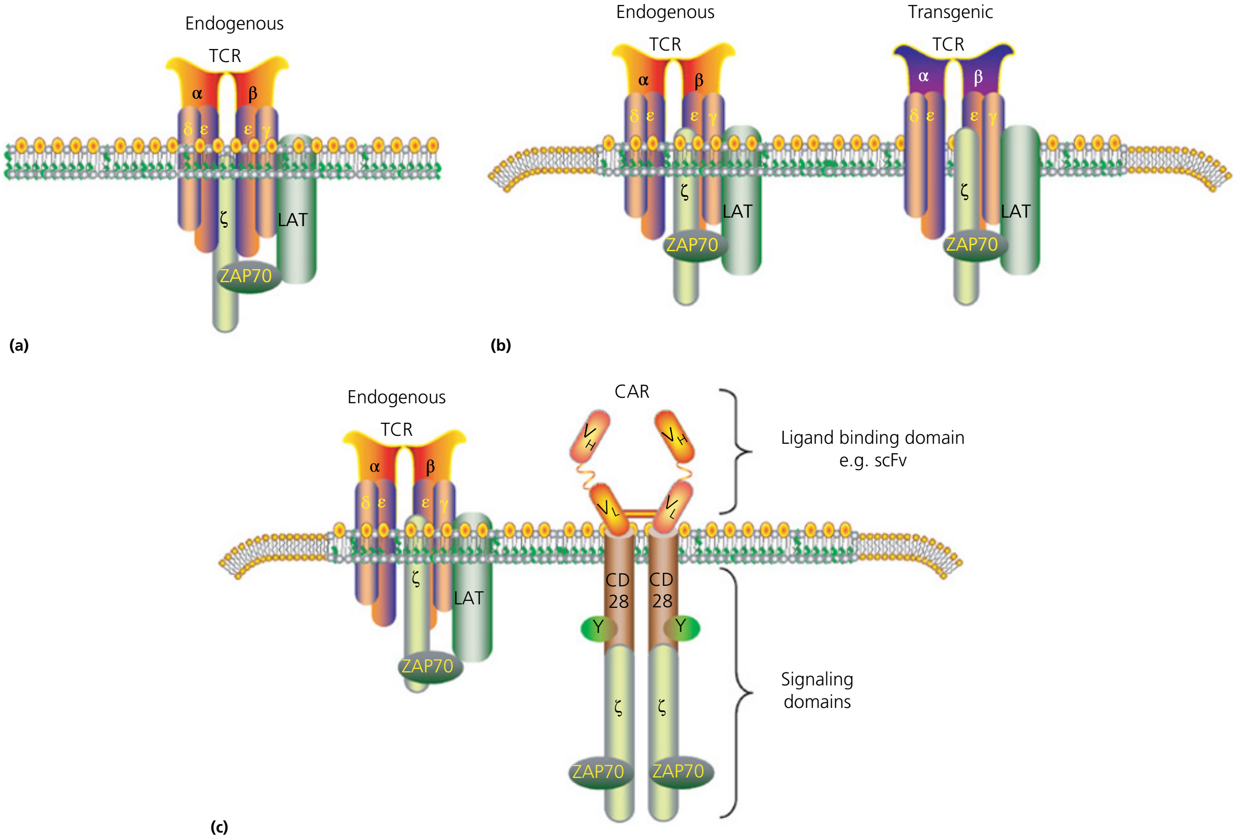

Endogenous
TCR
α
β
δ ε
ε γ
ζ
LAT
ZAP70
(a)
Endogenous
TCR
Transgenic
TCR
(b)
Endogenous
TCR
CAR
V$_H$
V$_L$
CD 28
Y
ζ
ZAP70
Ligand binding domain e.g. scFv
Signaling domains
(c)

Figure 69.2 T cells can be engineered to have retargeted specificity for tumors. (a) Endogenous T cells express a TCR with a single specificity. Bispecific T cells are created by the introduction of genes that encode proteins that recognize antigens expressed by target tumor cells. (b) These genes can encode natural TCRs that function in the same MHC-restricted manner as endogenous TCRs but have tumor-antigen specificity. (c) Alternatively, these genes can encode chimeric tumor antigen–specific receptors (CARs) that target surface antigens in an MHC-independent fashion. T bodies express an extracellular ligand generally derived from an antibody and intracellular signaling modules derived from T-cell signaling proteins.

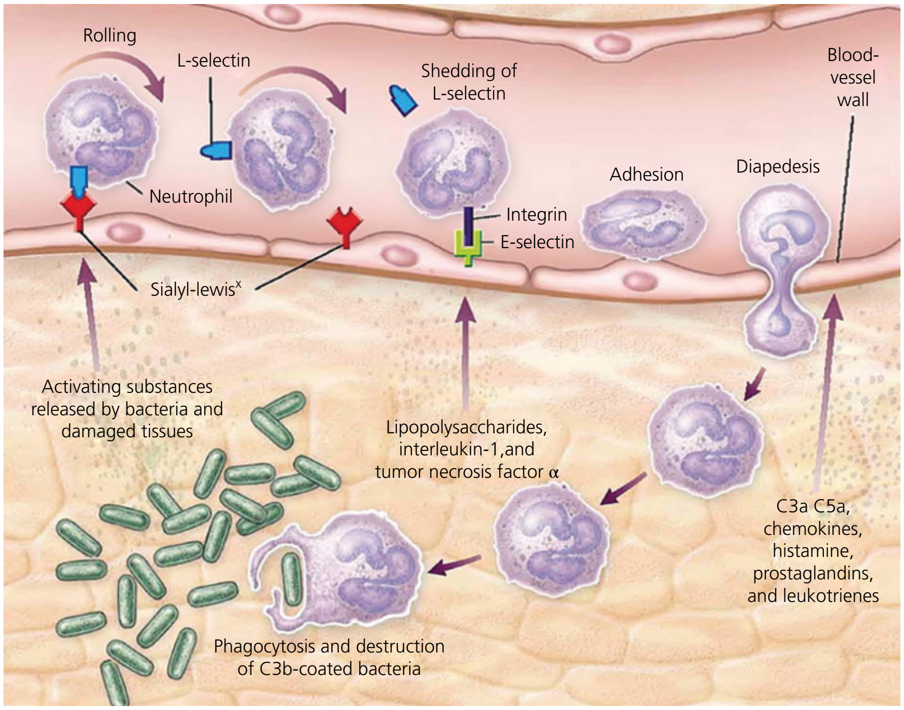

Rolling
L-selectin
Shedding of L-selectin
Blood-vessel wall
Neutrophil
Adhesion
Diapedesis
Integrin
E-selectin
Sialyl-lewis^x
Activating substances released by bacteria and damaged tissues
Lipopolysaccharides, interleukin-1,and tumor necrosis factor α
C3a C5a, chemokines, histamine, prostaglandins, and leukotrienes
Phagocytosis and destruction of C3b-coated bacteria

Figure 75.1 The acute inflammatory response. Neutrophils are among the first cells to arrive at the scene of an infection and are important contributors to the acute inflammatory response. As the neutrophil rolls along the blood-vessel wall, the L-selectin on its surface binds to carbohydrate structures such as sialyl-LewisX on the adhesion molecules on the vascular endothelium, and its progress is eventually halted. As the neutrophil becomes activated, it replaces L-selectin with other cell-surface adhesion molecules, such as integrins. These molecules bind E-selectin, which is present on the blood-vessel wall as a result of the influence of inflammatory mediators such as bacterial lipopolysaccharides and the cytokines interleukin-1 and TNFα. The activated neutrophil then enters the tissues, where it is attracted to the infection site by a number of chemoattractants. The neutrophil can then phagocytose and destroy the C3b-coated bacteria. Source: Delves PJ, Roitt IM. The immune system. *N Engl J Med* 2000; **343**(1): 37–49. Reproduced with permission of the Massachusetts Medical Society.

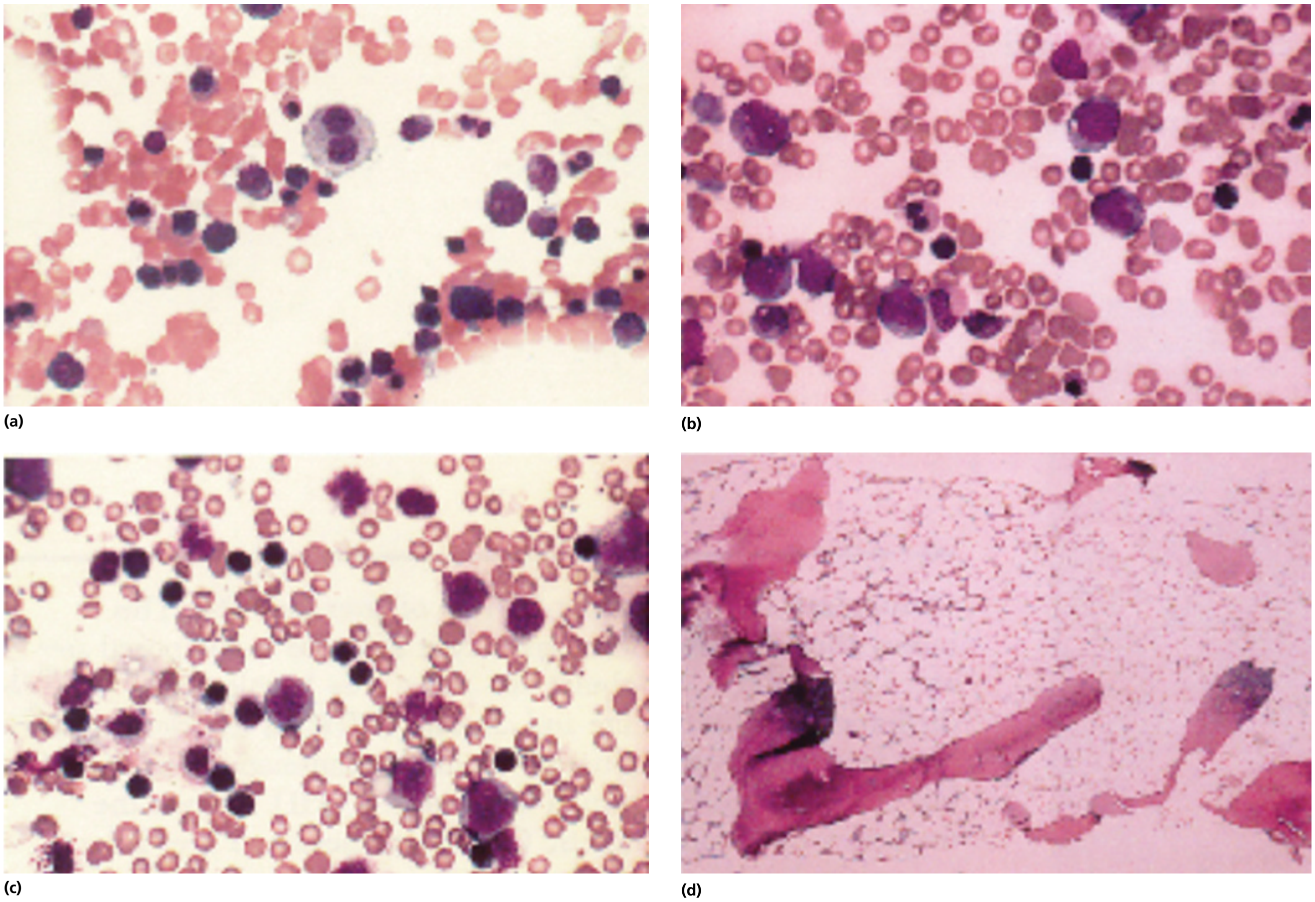
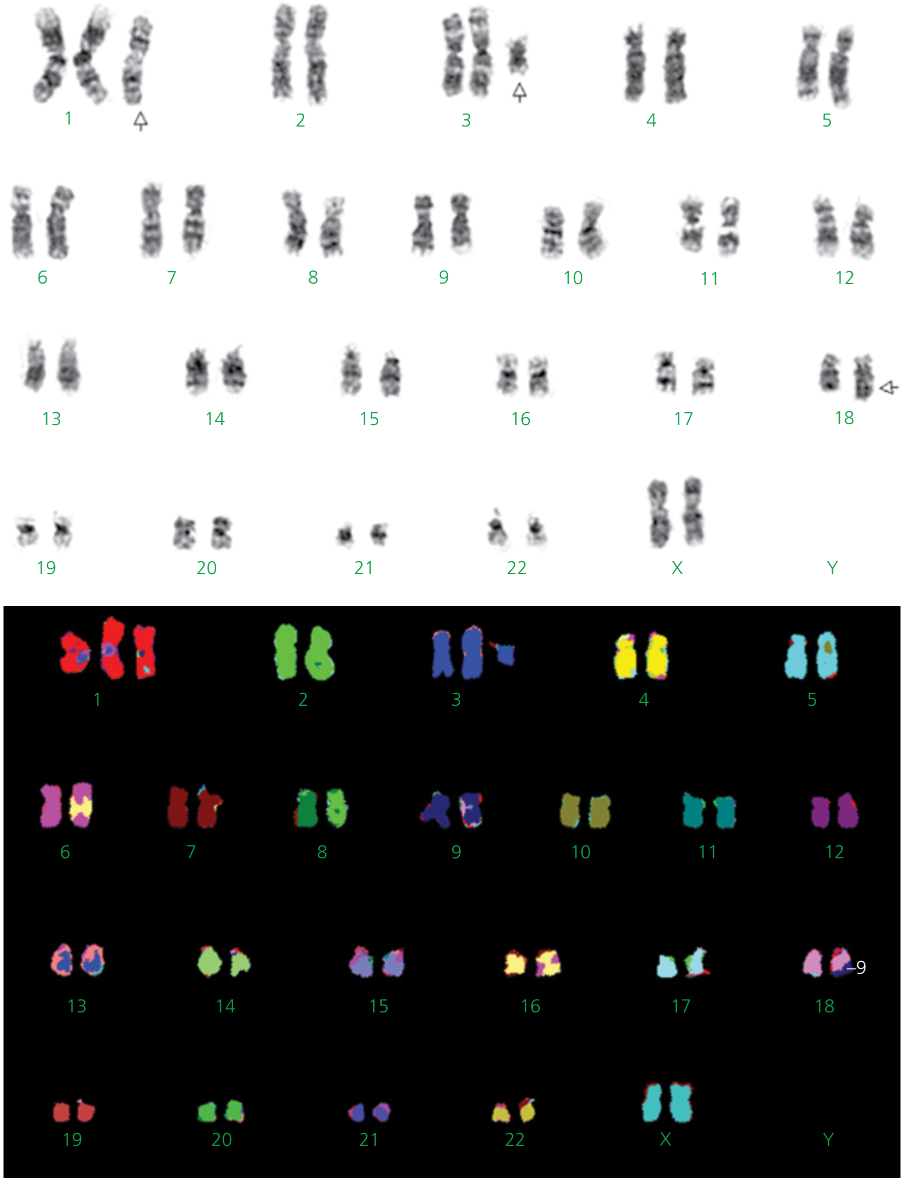


Figure 76.3 Progression of dysplasia to refractory anemia with excess blasts in a single patient with FA over a 9-month period. (a) Marrow aspirate on 02-21-96 demonstrating pancytopenia and early dysplastic changes in neutrophils; (b) marrow aspirate on 07-03-96 demonstrating progressive dysplasia and 3% blasts; (c) marrow aspirate on 09-24-96 demonstrating progressive dysplasia and 12% blasts; (d) marrow biopsy on 09-24-96 demonstrating severe aplasia.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11
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21
22
X
Y

Figure 76.4 Upper panel: G-banded karyotypes from an abnormal clone in the marrow of an FA patient. This is a hyperdiploid metaphase cell with 48 chromosomes including two extra derivative chromosomes (designated by arrows); one derivative chromosome is composed of two copies of the long arm of chromosome 1 and the other contains two copies of a small portion of the long arm of chromosome 3. This cell contains a total of four copies of portions of chromosomes 1 and 3. Lower panel: A karyotype of the same abnormal clone as above, as characterized by fluorescent in situ hybridization (FISH) using a multicolor paint probe (Vysis spectral vision M-FISH). M-FISH confirmed that the two derivative chromosomes detected by G-banding represented extra copies of portions of chromosomes 1 and 3. Source: Courtesy Betsey Hirsch PhD.

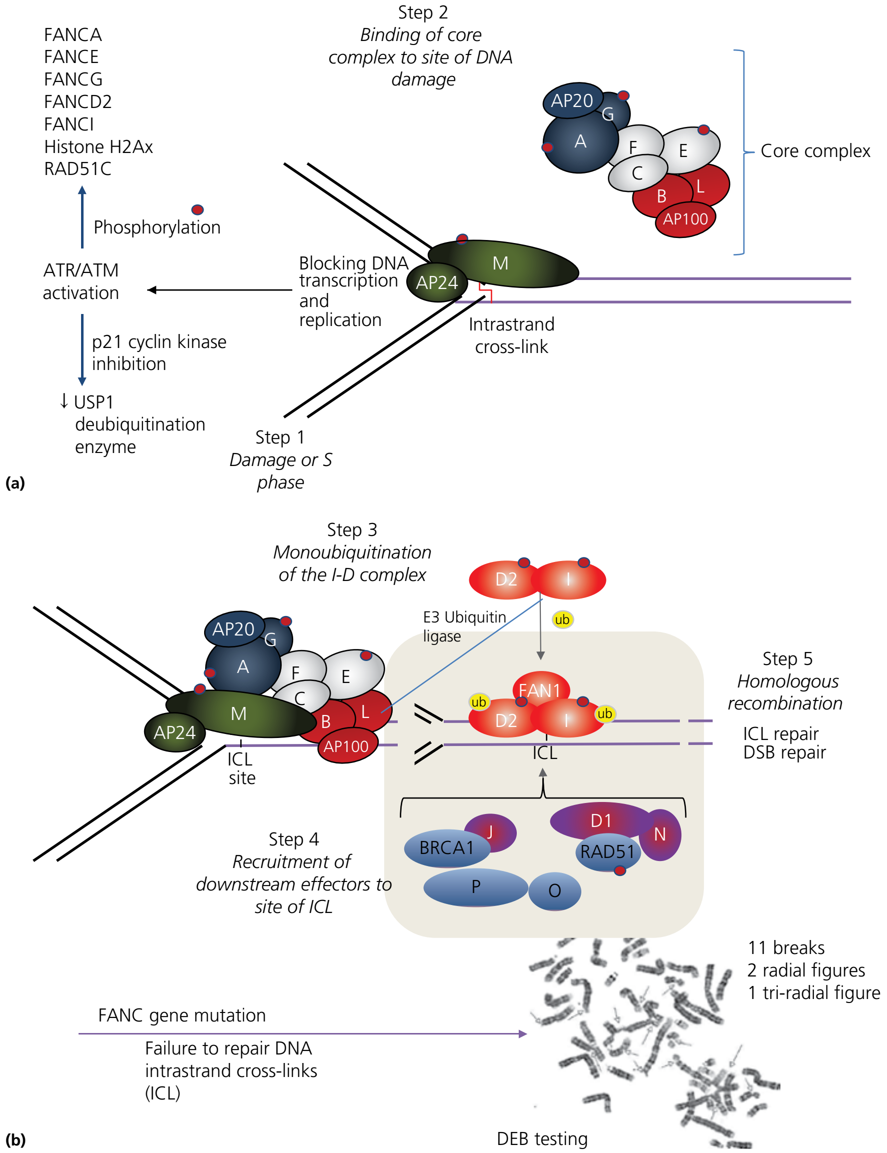

FANCA
FANCE
FANCG
FANCD2
FANCI
Histone H2Ax
RAD51C
Phosphorylation
ATR/ATM
activation
p21 cyclin kinase
inhibition
↓ USP1
deubiquitination
enzyme
Step 2
Binding of core
complex to site of DNA
damage
AP20
G
A
F
E
C
L
B
AP100
Core complex
Blocking DNA
transcription
and
replication
M
AP24
Intrastrand
cross-link
Step 1
Damage or S
phase
(a)
Step 3
Monoubiquitination
of the I-D complex
D2
I
E3 Ubiquitin
ligase
ub
AP20
G
A
F
E
C
M
B
L
AP24
AP100
ICL
site
FAN1
ub
D2
I
ub
ICL
Step 5
Homologous
recombination
ICL repair
DSB repair
J
BRCA1
D1
N
RAD51
P
O
Step 4
Recruitment of
downstream effectors to
site of ICL
11 breaks
2 radial figures
1 tri-radial figure
FANC gene mutation
Failure to repair DNA
intrastrand cross-links
(ICL)
(b)
DEB testing

Figure 76.7 Model of the FA pathway in DNA repair compiling the most recent data. (a) Step 1: DNA damage and stalled replication fork signal ATR results in phosphorylation of the FANCA, E and G of the core complex, and FANC D2 and I of the I-D complex, RAD51C and histone H2AX as well as activation of p21 which downregulates USP1 deubiquitination enzyme and in turn permits ubiquitination of FANC D2 and I. Step 2: Upon recognition of the intrastrand DNA cross-link (ICL), FANCM and FAAP24 and FANCM-associated histone fold 1/2 bind near the site of the intrastrand cross-link and recruit the 8-member core complex which assembles the core nuclear ubiquitin ligase complex which monoubiquinates FANCD2 and FANCI. (b) Step 3: The modified I-D protein complex associates with chromatin and recruits FAN1 critical to excision of the ICL and formation of 3′ flaps of ssDNA for repair. Step 4: Downstream proteins FANCN, D1, J, O and P, and BRCA1 assemble and nucleate the single strand DNA with RAD51C. Step 5: This enables homologous recombination and ICL/double-strand break (DSB) repair. Mutations any of the FANC genes leads to inability to repair ICL efficiently. Loss of the FA pathway leads to chromosomal aberrations upon exposure to cross-linking agents such as diepoxybutane (DEB), leading to an infrequency of chromosomal breaks and radial figures as shown in the side panel. Source: Courtesy of Betsey Hirsch PhD. Model based on a compilation of data [29–52].

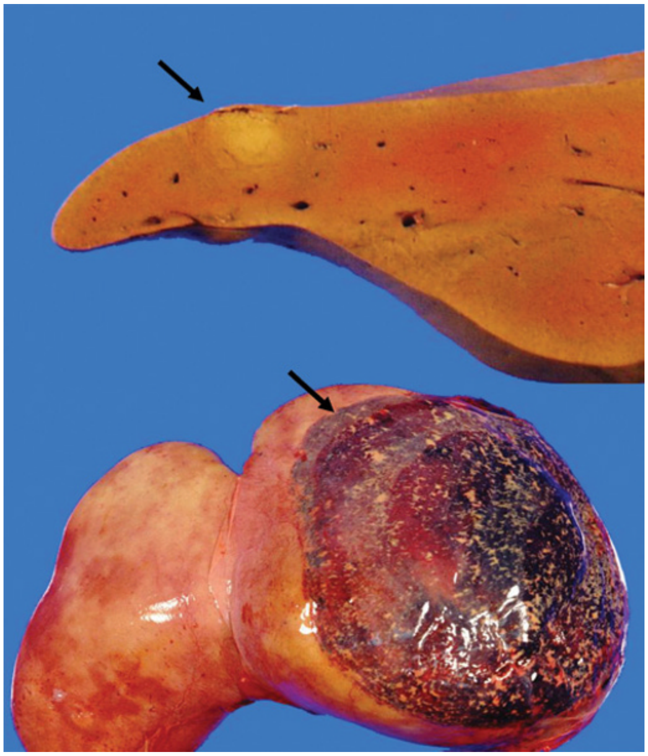


Figure 76.11 Hepatic adenoma. Pathological specimen obtained at autopsy from a patient with FA with multiple adenomata not revealed by ultrasound prior to HCT. Lower panel shows area of massive intrahepatic hemorrhage in one previously unidentified adenoma. Source: Kumar et al. 2004 [89]. Reproduced with permission of Lippincott Williams & Wilkins.

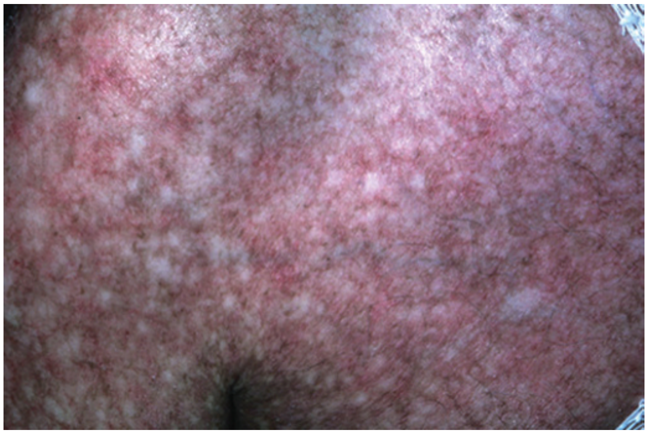


Figure 84.5 Poikiloderma, a diagnostic manifestation of cGVHD, which includes both atrophic and pigmentary changes. Note the combination of reticulated pattern, epidermal atrophy (cigarette-paper wrinkling of the skin surface), erythema, and brown pigment forming the borders of skincolored or depigmented skin.

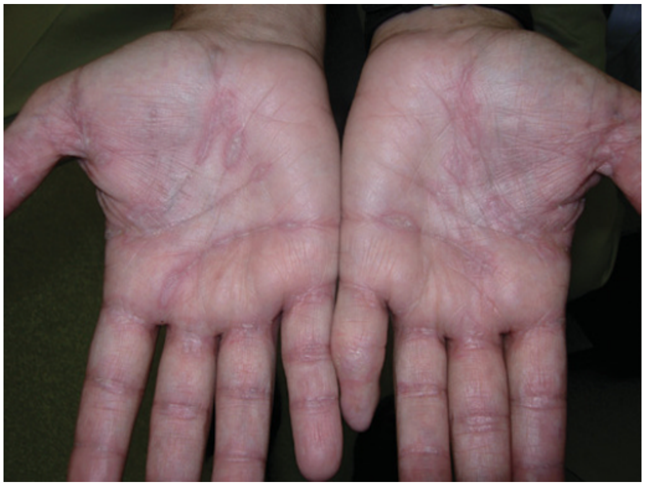


Figure 84.6 Lichen planus-like eruption involving the palmar surfaces, a diagnostic manifestation of cGVHD. Note the erythematous/violaceous flat-topped papules or plaques with or without surface reticulations or a silvery or shiny appearance on direct light.

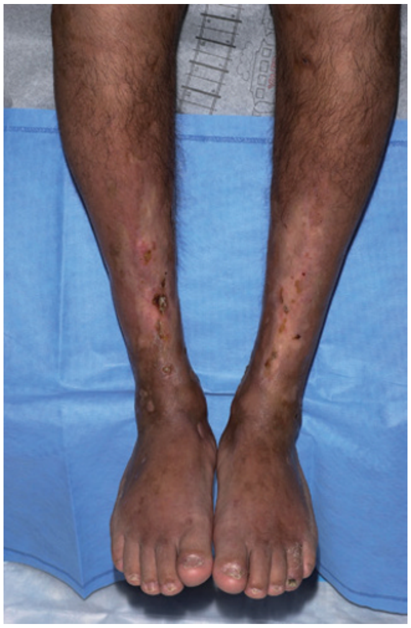


Figure 84.7 Diffuse deep sclerotic features involving the lower extremities, a diagnostic feature of cGVHD. Note the non-movable/hidebound subcutaneous tissues, erosions, and ulcerations, loss of hair follicles, and toenail dystrophy.

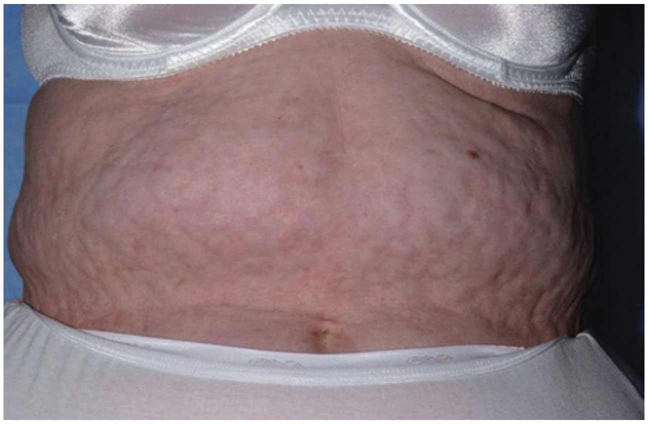


Figure 84.8 Alternative manifestation of deep sclerosis common in areas of excess subcutaneous tissue such as the abdomen, upper arms, and buttocks. Tissue is not hidebound but demonstrates prominent rippling, dimpling, or a cellulite-like appearance.

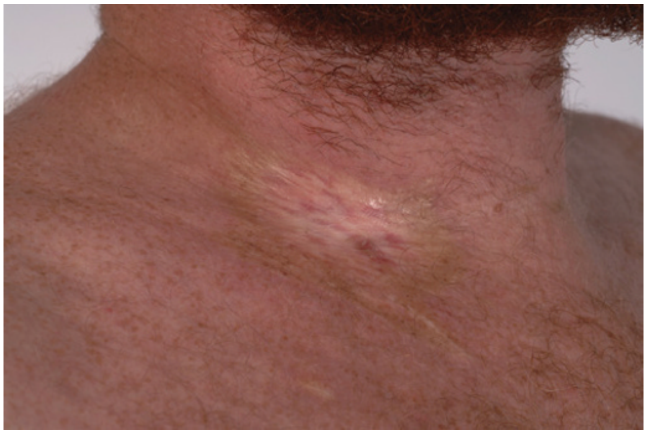
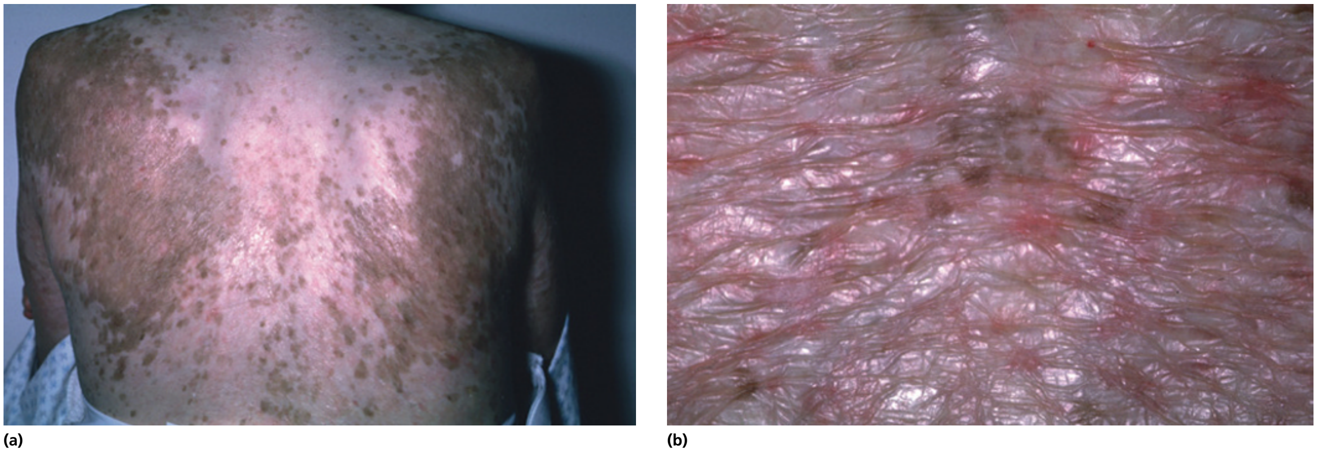


Figure 84.9 Morphea-like superficial sclerotic skin changes, a diagnostic feature of cGVHD. Note the localized, patchy areas of movable smooth or shiny skin with a leathery-like consistency, often with dyspigmentation. In this picture, there is a fibrotic, hypopigmented area in the center of the plaque with a slightly hyperpigmented border.



(a) (b)

Figure 84.10 Lichen sclerosis-like lesion, a diagnostic manifestation of cGVHD. Note the discrete to coalescent gray to white movable papules or plaques, with a shiny appearance and leathery consistency (a) or cigarette paper-like textural changes (b).

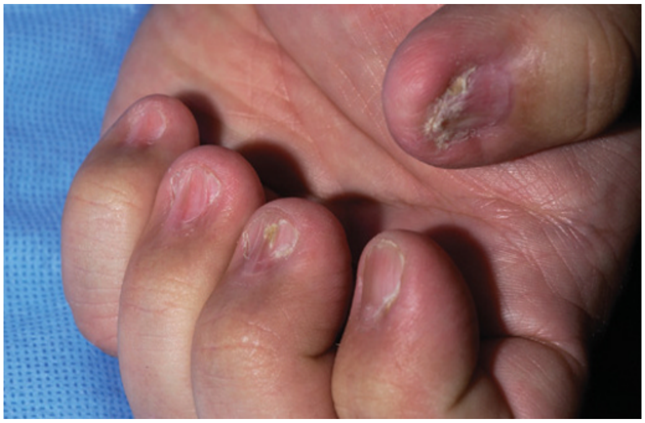


Figure 84.11 Nail dystrophy involving multiple digits is a distinctive feature of cGVHD, although it is insufficient alone to establish the diagnosis. Note the longitudinal ridging, splitting, and brittle nail features, and also periungual erythema.

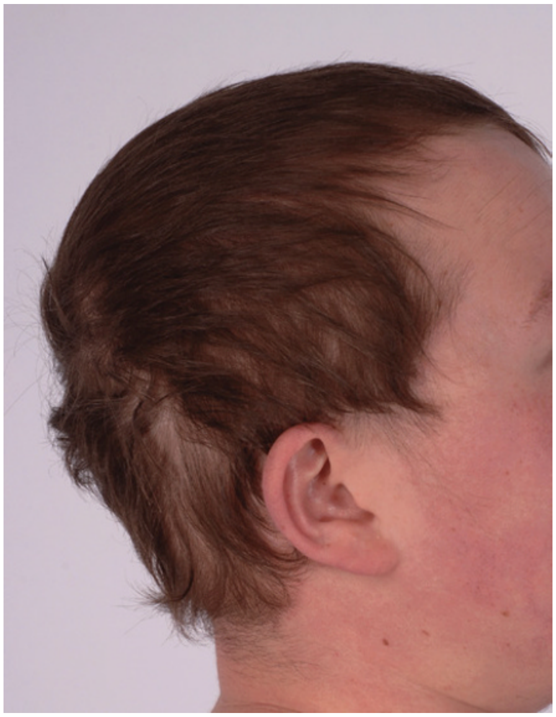


Figure 84.12 New onset of patchy alopecia after recovery from chemoradiotherapy is another distinctive manifestation of cGVHD (but is insufficient alone to establish the diagnosis). Patchy alopecia may involve loss of scalp or body hair.

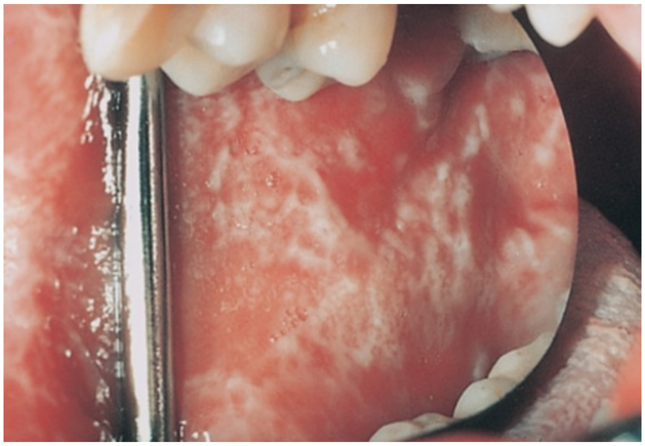


Figure 84.13 Lichenoid lesions of oral cGVHD showing crythema, white striae, plaque, and ulcer formation, day 394.

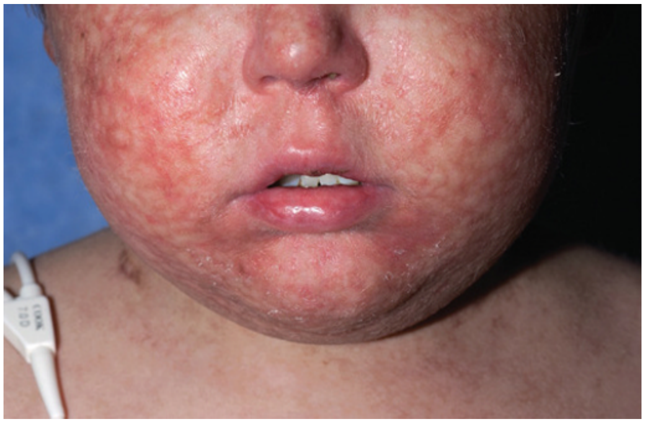


Figure 84.14 Decreased oral range of motion and microstomia in a patient with sclerotic features of cGVHD. This is a diagnostic manifestation. Note the erythema, edema, and atrophy of the vermillion lip and labial mucosa.

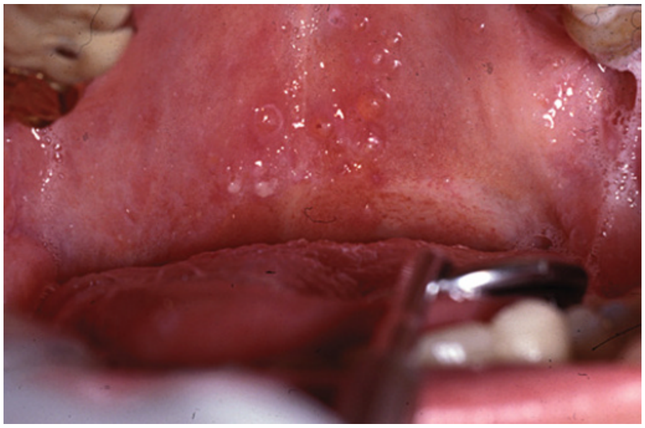


Figure 84.15 Numerous vesicle-like mucoceles (a distinct manifestation of cGVHD) are seen along the center of the soft palate. Patchy white lichenoid hyperkeratosis (diagnostic of cGVHD) and interspersed moderate erythematous changes are also evident across the soft palate. Additionally, note the foamy mucus saliva consistent with GVHD salivary gland dysfunction.

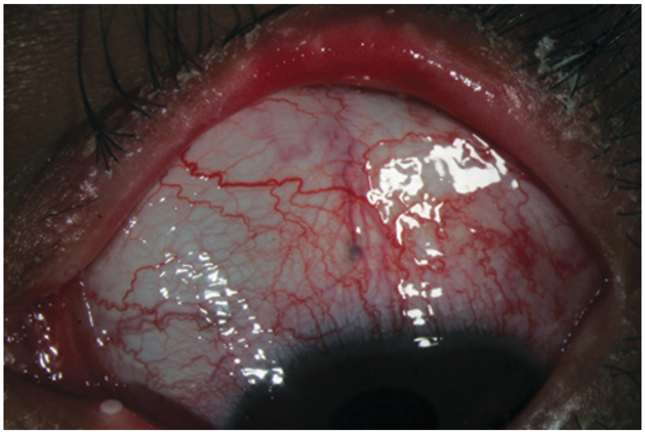


Figure 84.16 Keratoconjunctivitis sicca with blepharitis. The eyelid margins are thickened, edematous, and erythematous. Also note plugging of the meibomian gland orifices (along the eyelid margin) and significant conjunctival hyperemia/injection. A punctal plug can be seen at the lower left margin of the image.

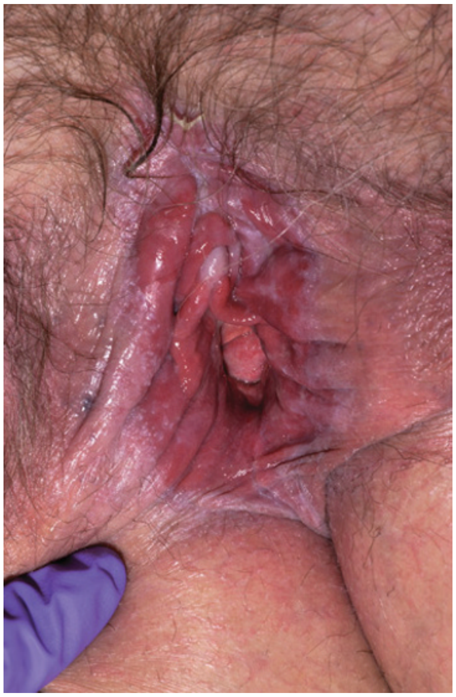


Figure 84.17 cGVHD involving the vulvovaginal tissues. Note lichen planus-like manifestations, which are diagnostic of cGVHD. Also seen are erythema and agglutination of the labial tissues.

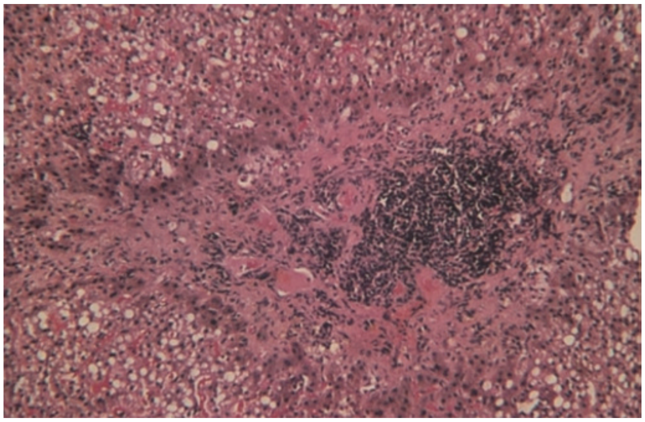


Figure 84.18 cGVHD of the liver, day 166. This biopsy shows a prominent plasmacytic infiltrate in the portal area and a paucity of interlobular bile ducts. The histological dichotomy between acute GVHD and cGVHD is less clear in the liver than in skin. However, increased portal inflammation and loss of bile ducts are more common in cGVHD. Despite the frequency of chronic hepatic GVHD, cirrhosis and liver failure are rare and may reflect superinfection with hepatitis C virus. (See also Chapter 23.)

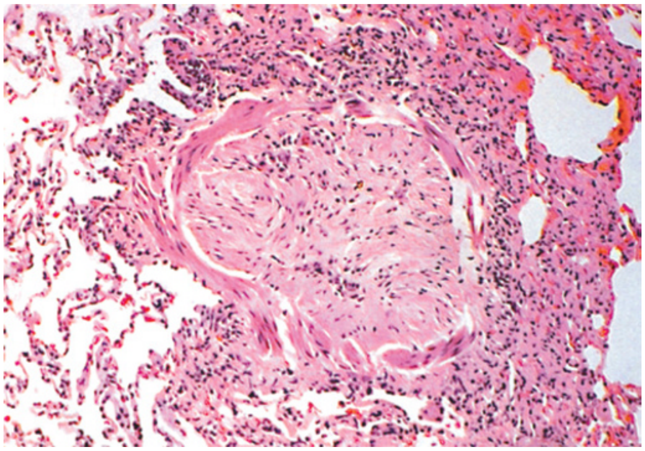


Figure 84.19 Bronchiolitis obliterans in a lung biopsy, day 396. The patient had cGVHD with severe obstructive pulmonary disease. Similar small airway inflammation with fibrosis is seen in lung allograft rejection. (See also Chapter 23 and Chapter 47.)

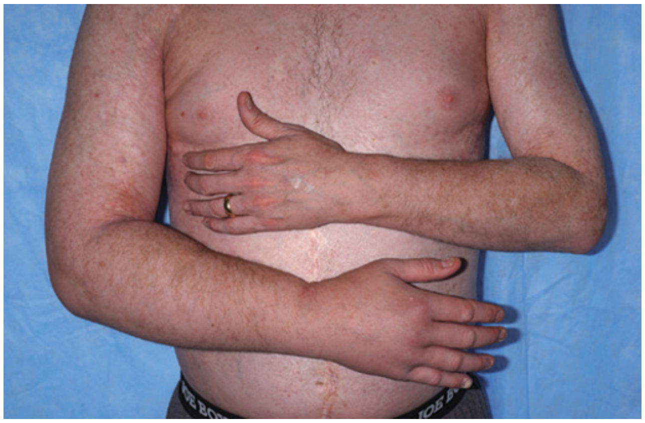
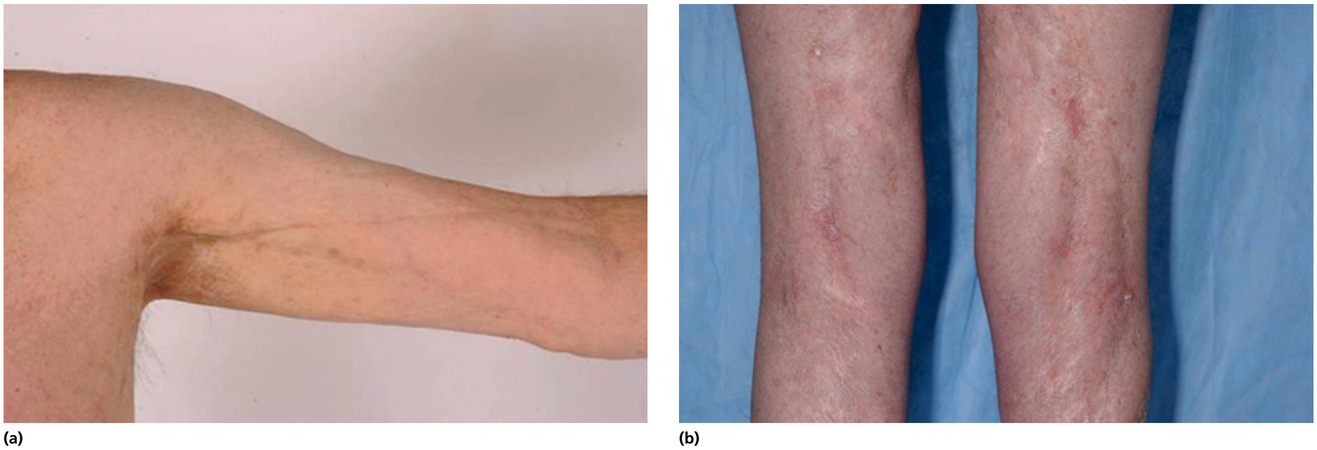


Figure 84.20 Edema in the extremities can be bilateral or, as shown, unilateral. Edema may be an early sign of evolving subcutaneous sclerosis and fasciitis.



(a)

(b)

Figure 84.21 Manifestations suggestive of fascial involvement by cGVHD (a diagnostic feature) can include rippling or the groove sign.

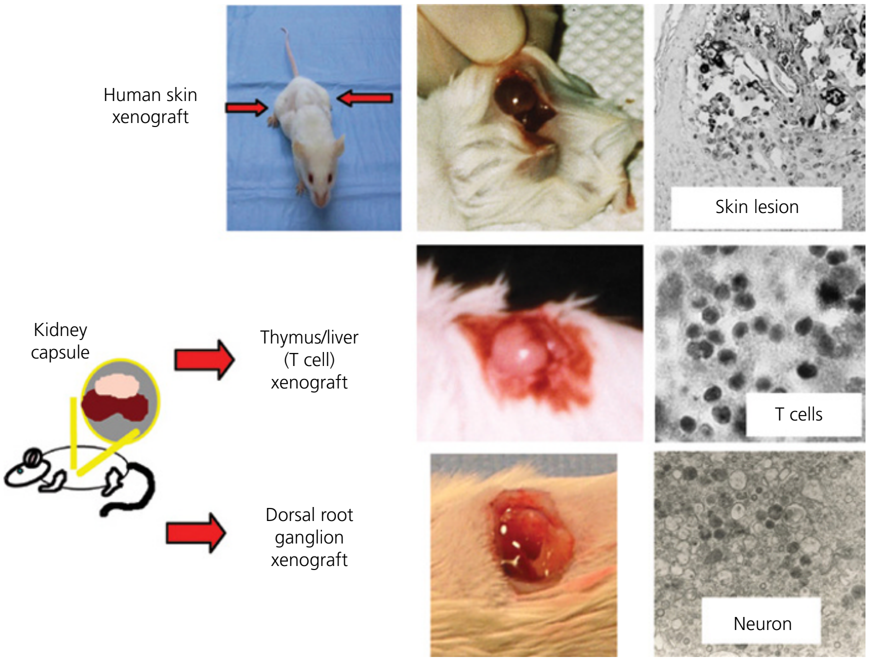

Human skin xenograft
Skin lesion
Kidney capsule
Thymus/liver (T cell) xenograft
T cells
Dorsal root ganglion xenograft
Neuron

Figure 89.4 Modeling varicella zoster virus (VZV) pathogenesis in human tissue xenografts in vivo. Human tissue xenografts are maintained in mice with severe combined immunodeficiency. VZV infection of cells within their tissue microenvironments can be evaluated in mice with skin, T-cell (thymus/liver) or dorsal root ganglia xenografts.

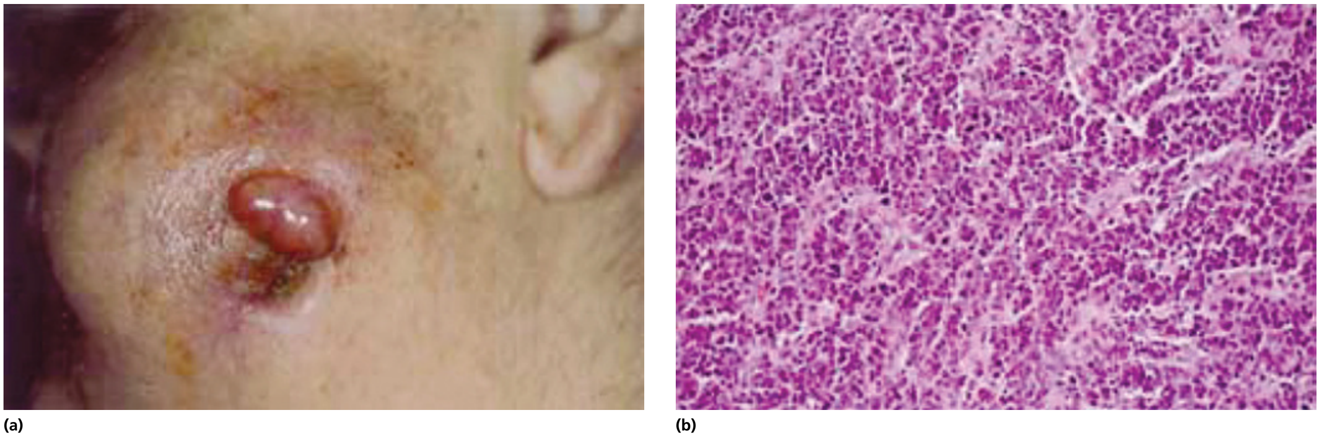

(a) (b)

Figure 93.4 Merkel cell carcinoma: clinical and pathologic appearance. Merkel cell carcinoma in an HCT patient on left cheek (a) showing nests and cords of small cells with small cytoplasm in trabecular pattern on H&E staining (b). Source: Gilaberte et al. 2000 [134]. Reproduced with permission of Karger Publishers.

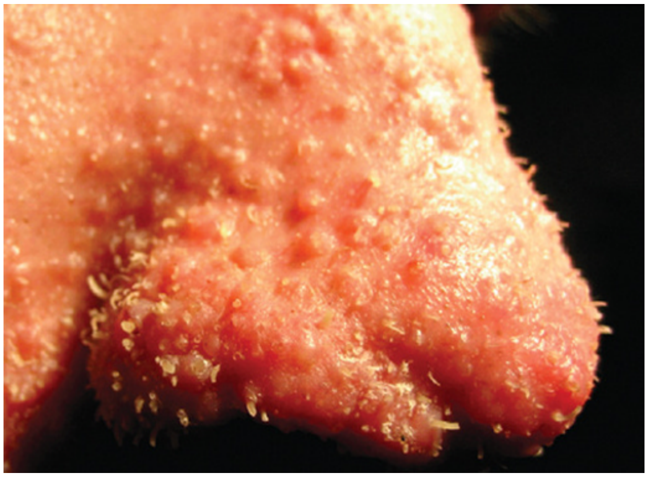


Figure 93.5 Trichodysplasia: clinical appearance. Close-up image of spiculae on the nose of an immunosuppressed patient. Source: van der Meijden et al 2010 [137]. Reproduced with permission of PLoS in accordance with the Creative Commons Attribution Licence.

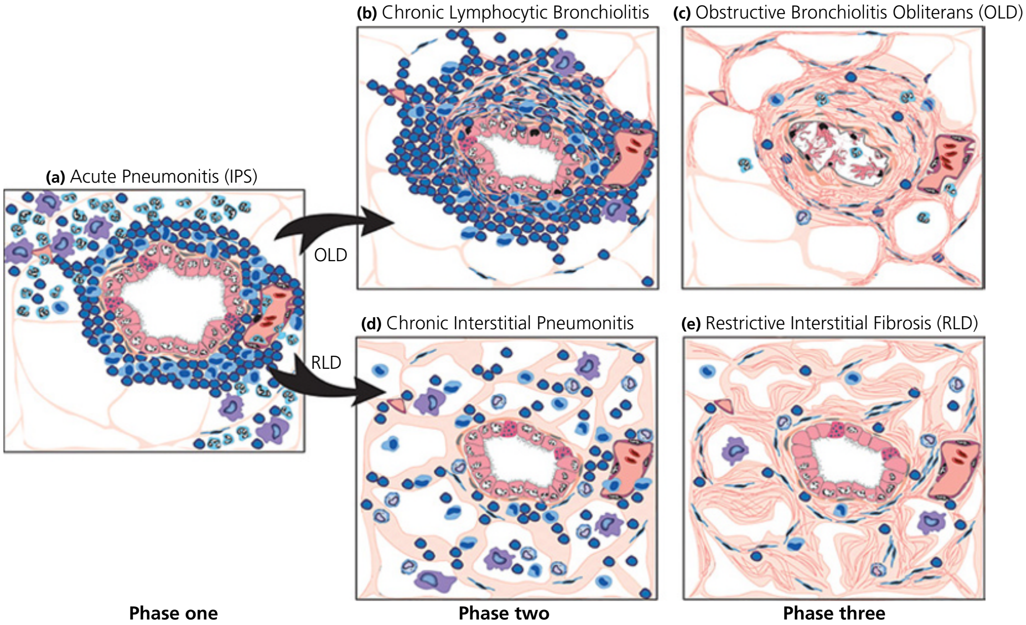

(b) Chronic Lymphocytic Bronchiolitis
(c) Obstructive Bronchiolitis Obliterans (OLD)
(a) Acute Pneumonitis (IPS)
OLD
RLD
(d) Chronic Interstitial Pneumonitis
(e) Restrictive Interstitial Fibrosis (RLD)
Phase one
Phase two
Phase three

Figure 95.5 A triphasic model of OLD and RLD following allogeneic HCT. Immune-mediated injury to the lung after allogeneic SCT can be conceptualized in three phases. In phase one, acute lung injury develops as a consequence of an allogeneic immune response and results in the sequential influx of lymphocytes, macrophages and neutrophils into an inflamed pulmonary parenchyma (a). Persistence of an inflammatory signal in the setting of dysregulated repair mechanisms promotes the transition from acute to chronic injury in phase two (b and d). As chronic inflammation proceeds to phase three, lung fibroblasts increase dramatically in number and contribute to the enhanced deposition of collagen and granulation tissue in and around bronchial structures, ultimately resulting in complete obliteration of small airways and fixed OLD (c). If, by contrast, the principal target of early damage is the alveolar epithelium, fibroblast proliferation and intraseptal collagen deposition ultimately result in interstitial fibrosis and RLD (e).

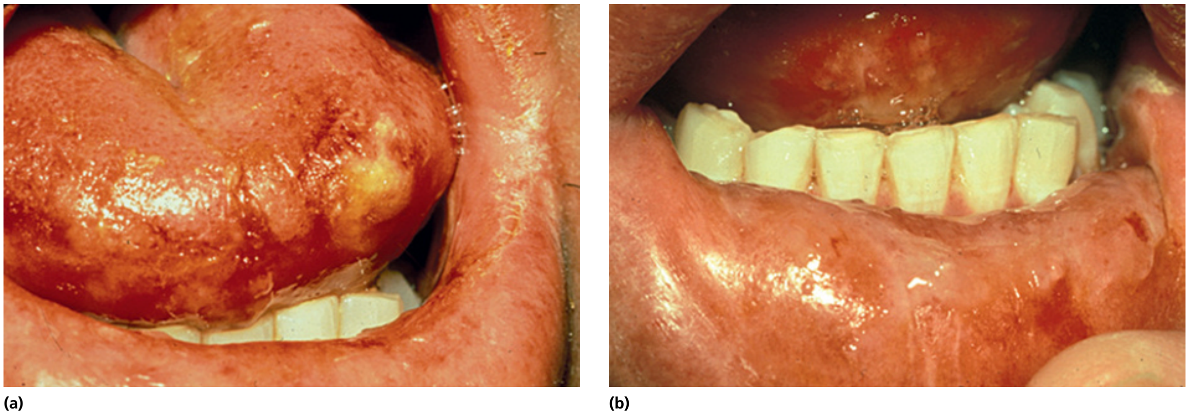

(a) (b)

Figure 101.2 Severe oral mucositis occurring 8 days after hematopoietic cell transplantation following conditioning with VP-16, cyclophosphamide, and total body irradiation (World Health Organization mucositis score of 4). Bleeding ulcerations are noted on the anterior ventral area of the tongue (a) in this patient, with simultaneous pseudomembranous fibrin exudate-covered ulcers evident on the lower labial mucosa (b).

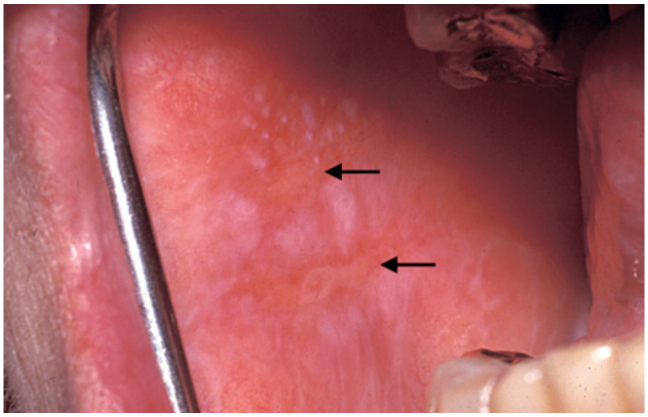


Figure 101.3 Oral chronic graft-versus-host disease in a patient 1 year post-HCT, with lichenoid hyperkeratotic changes (stria-, papule-, and plaque-like changes) associated with generalized atrophic and erythematous mucosal changes involving the right buccal mucosa. Small initial pseudomembranous fibrin exudate-covered ulcers are indicated by arrows.

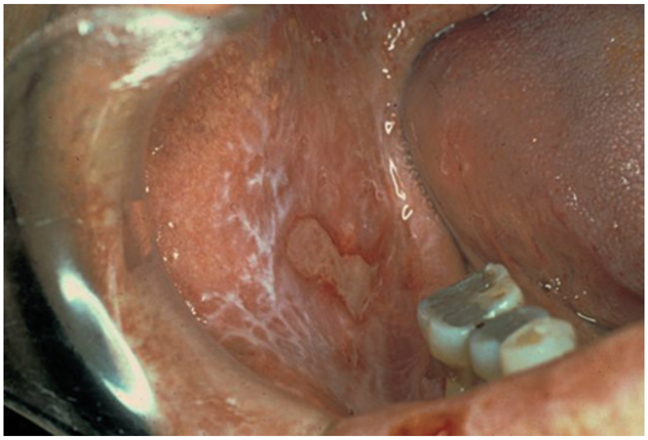


Figure 101.4 Severe oral chronic graft-versus-host disease showing distinct hyperkeratotic lichenoid striae and a pseudomembranous fibrin exudatecovered ulceration with intense surrounding inflammation in a patient approximately 18 months post-HCT.

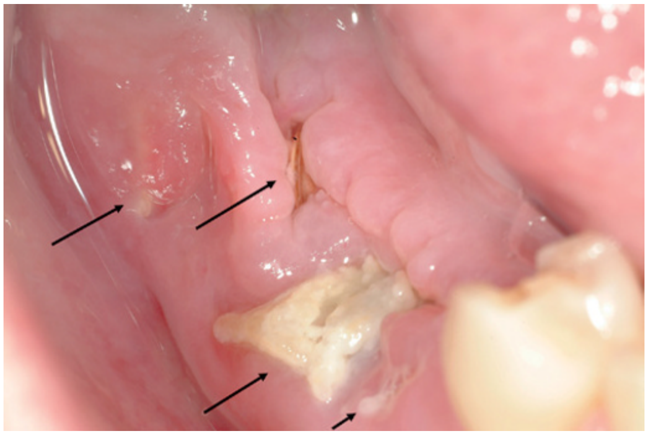


Figure 101.5 A 45-year-old male with a history of multiple myeloma who had been treated with intravenous zoledronate for 2 years prior to having two lower right molars extracted. He presented with three areas of bisphosphonate-associated osteonecrosis of the lower right mandible 6 months following the extractions. Lesions were non-painful and stable. Arrows indicate areas of bone exposure.

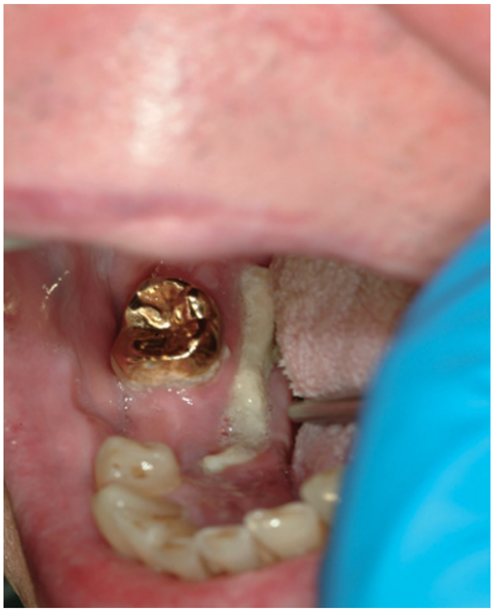


Figure 101.6 Bisphosphonate-associated osteonecrosis of the jaw involving the lower right lingual ridge in a patient with multiple myeloma treated with intravenous zoledronate monthly for 20 months. The patient's second bicuspid and first molar had been extracted 3 months earlier due to what was thought to be severe peridontal disease; the primary extraction site healed, but an extensive area of osteonecrosis developed along the mandibular lingual torus that was present lingual to the original extraction site.

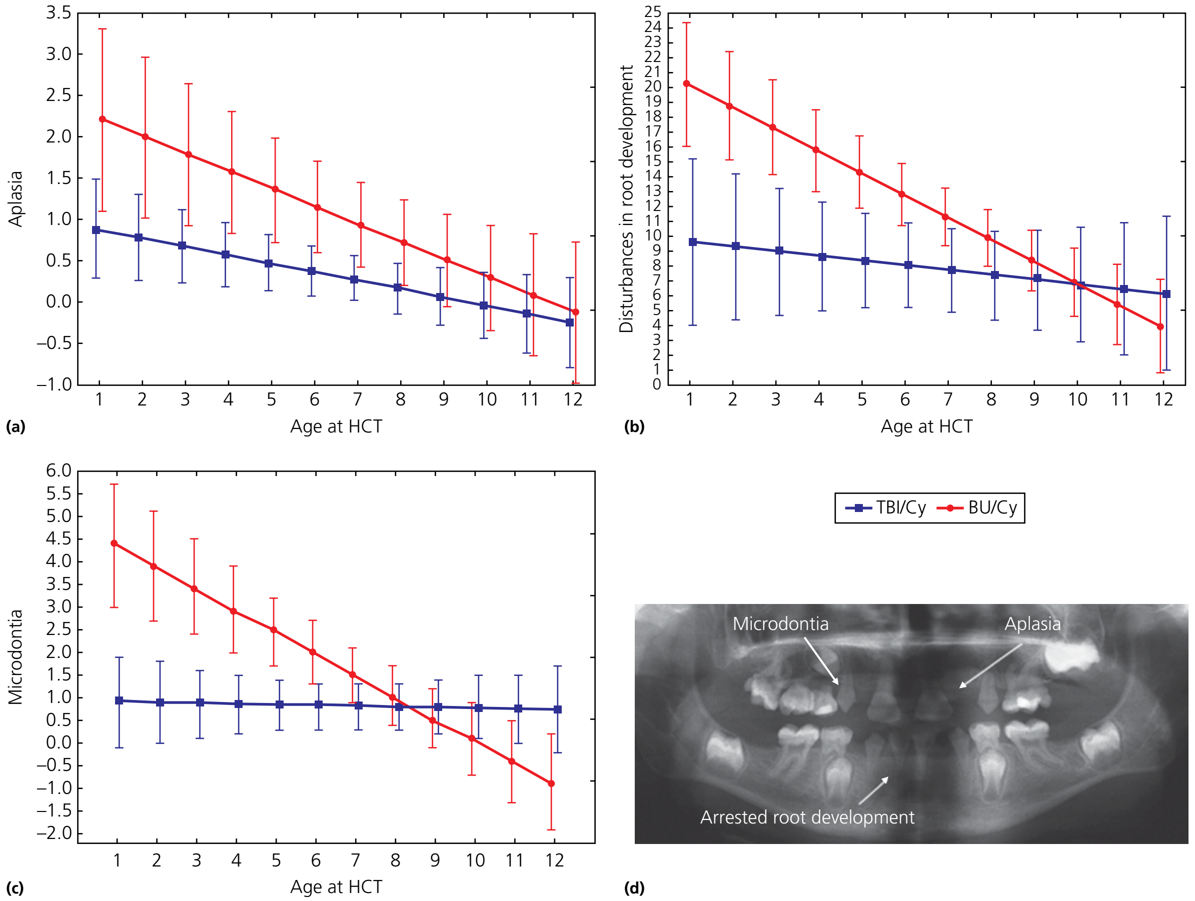


FIGURE 101.7 Disturbances in dental growth and development in long-term survivors of HCT. Conditioning regimes for HCT can cause severe disturbances in dental growth and development for pediatric HCT patients with TBI-containing regimes causing more problems than chemotherapy-only regimes. The younger the age at the time of transplant, the more significant the disturbance in dental developmental. (a) Number of missing teeth in children subjected to HCT by age. (b) Number of teeth with disturbances in root development by age. (c) Number of teeth with microdontia by age. (d) Panoramic radiograph showing arrested root development, microdontia, and aplasia of teeth in a pediatric long-term survivor post-HCT. Source: reproduced with permission of G. Dahllöf, K. Garming Legert, M. Remberger, and O. Ringdén Support Care Cancer (2012) 20 (Suppl 1):S226; 945.

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Abbreviations used: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CMML, juvenile (chronic) myelomonocytic leukemia *and* chronic myelomonocytic leukemia; GI, gastrointestinal; GVHD, graft-versus-host disease; GVL, graft-versus-leukemia effect; GVT response, graft-versus-tumor response; HC, hematopoietic cell; HD, Hodgkin disease; HCT, hematopoietic cell transplantation; HPC, hematopoietic progenitor cells; HSC, hematopoietic stem cell; HSPC; hematopoietic stem and progenitor cell; JMML, juvenile myelomonocytic leukemia; mAb, monoclonal antibody; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms; MSC, mesenchymal stem cell; NHL, non-Hodgkin lymphoma; PBHC, peripheral blood hematopoietic cells; PBHCT, peripheral blood hematopoietic cell transplantation; PBPC, peripheral blood progenitor cells; PBSC, peripheral blood stem cells; PTLD, post-transplant lymphoproliferative disorder; QoL, quality of life; TBI, total body irradiation.

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